OVARIAN RESERVE IN PATIENTS WITH HYPERRESPONSE FOLLOWING GONADOTROPHIN STIMULATION

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OVARIAN RESERVE IN PATIENTS WITH HYPERRESPONSE FOLLOWING GONADOTROPHIN STIMULATION

GRADUATION THESIS

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The graduation thesis was graded on 25.06.2024 in Rijeka, before the Committee composed of the following members:

1. Associate Professor Tea Štimac, MD, PhD (President of the Committee)
2. Assistant Professor Aleks Finderle, MD, PhD
3. Assistant Professor Marko Klarić, MD, PhD

The graduation thesis contains 24 pages, 6 figures, 2 tables, 33 references.

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List of abbreviations and acronyms

AIT - Autoimmune Thyroiditis

AMH - Anti-Müllerian Hormone

ART - Assisted Reproductive Technology

E2 - Estradiol

FSH - Follicle Stimulating Hormone

FSH/LH - Follicle Stimulating Hormone to Luteinizing Hormone ratio

GnRH - Gonadotropin-Releasing Hormone

hCG - Human Chorionic Gonadotropin

IVF - *In Vitro* Fertilization

LH - Luteinizing Hormone

OHSS - Ovarian Hyperstimulation Syndrome

PCOS - Polycystic Ovary Syndrome

T - Testosterone

VEGF - Vascular Endothelial Growth Factor

1. INTRODUCTION

1.1. *In vitro* fertilization

In vitro fertilization (IVF) is an assisted reproductive technology used to overcome various causes of infertility and assist in achieving live birth (1). It can be conducted in a natural cycle, which relies on the natural maturation of a single oocyte during a woman's menstrual cycle with minimal or no hormonal stimulation. Another approach is the conventional stimulated cycle, which involves the use of hormonal drugs to induce the development of multiple follicles (oocytes) in one treatment cycle (2). Natural cycle IVF is less invasive and involves lower risk of complications such as ovarian hyperstimulation syndrome (OHSS), but results in lower pregnancy rates compared to stimulated IVF (3). Conversely, conventional IVF involves stimulating the ovaries with a combination of fertility drugs, including gonadotrophins to stimulate follicle development and gonadotropin-releasing hormone analogue to prevent premature ovulation during follicle development (4). Human Chorionic Gonadotropin (hCG) is used in both treatments to initiate the final maturation of oocytes prior to their retrieval (5). Transvaginal ultrasound-guided oocyte retrieval is then performed 35-37 hours after administering hCG. Further steps include fertilization of these oocytes in the laboratory and development of embryos ("*in vitro*"). On days 3 to 5 post fertilization, the developed embryo is transferred into the uterine cavity (6). Supernumerary embryos are cryopreserved and stored for future treatment. These steps typically occur over a two to three week interval, known as an IVF cycle.

1.2. *In vitro* fertilization and ovarian hyperresponse

While both natural and conventional IVF cycles aim to enhance fertility outcomes, the use of gonadotrophins in conventional stimulated IVF increases the risk of ovarian hyperresponse or other serious complications such as thrombosis, ovarian torsion or urgent surgery (7). Ovarian hyperresponse involves an enhanced and abnormal reaction of the ovaries to fertility drugs, leading to the development of a high number of follicles (8). Although a higher number of follicles may increase the likelihood for more oocytes to be retrieved, it also elevates the risk of OHSS, a serious complication whose severe form is characterized by enlarged ovaries, ascites, acute respiratory distress syndrome and thromboembolic disease (9,10). The

predisposition for developing OHSS is a significant concern, not only because of severe health risks but it may also require cancellation of the embryo transfer, thus affecting the overall success rates of the IVF cycle (11). Furthermore, the assessment of ovarian reserve prior to IVF, which includes measuring AMH concentration or antral follicle counting, often identifies women with unexpectedly high AMH and significant risk. Such elevated AMH concentration is frequently observed in individuals with polycystic ovary syndrome (PCOS), and indicates a large number of antral follicles. This characteristic is particularly relevant, as it not only shows the diagnostic value of AMH in identifying PCOS but also highlights the increased risk of ovarian hyperresponse and OHSS in these patients, guiding the customization of their treatment protocol to reduce these risks (12).

1.3. Estimation of ovarian reserve and prediction of ovarian response in IVF stimulated cycle

Predicting ovarian response prior to stimulation is crucial for optimizing treatment protocols and increasing the likelihood of successful outcomes. Ovarian reserve estimation is central in assessing a woman's reproductive potential and her response to ovarian stimulation in IVF treatments (13). Several markers were routinely used to predict ovarian reserve and subsequently, response to IVF treatment. They include age, anti-Müllerian hormone (AMH) concentration, follicle-stimulating hormone (FSH), FSH to LH ratio, estradiol concentration in early follicular phase and antral follicle count (14). Age is a crucial predictor, as it influences both the quantity and quality of oocytes. As women age, ongoing atresia of nondominant follicles occurs, and the risks of genetic abnormalities and mitochondrial deletions in the remaining oocytes substantially increase, further impacting fertility potential (15). Moreover, AMH that is expressed by small (<8mm) preantral and early antral follicles is the most useful biochemical marker since it represents the size of primordial follicle pool (16). Lower AMH suggests a diminished ovarian reserve, which influences the approach to fertility treatment (17). On the other hand, high AMH, often but not exclusively seen in PCOS, indicates a large number of available follicles but can also suggest challenges such as incresed risk of OHSS during fertility treatments (18). Measurement of FSH concentration is also considered as a simple and sensitive predictor of ovarian reserve. With decreasing ovarian function, granulosa and lutheal cells secrete less inhibin, a peptide hormone responsible for inhibiting FSH secretion by anterior pituitary. This reduction in inhibin results in elevated FSH concentration in early follicular phase and increased FSH/LH ratio (19). Serum estradiol concentration is measured

together with FSH to decrease the incidence of false-negative results. Even though there is depletion of ovarian follicles in older women, estradiol is elevated due to increased stimulation of ovarian steroidogenesis by high FSH. Elevated estradiol can in turn provide negative feedback on pituitary gland and lower FSH levels which can falsely suggest a better ovarian function. An additional marker of ovarian response is antral follicle count which involves sonographic evaluation of small antral follicles which are between 2 and 10 mm in size. If the count is lower than 10 for both ovaries this predicts a poor ovarian response (20).

1.4. Endocrinopathies and ovarian response in IVF

Endocrinopathies such as PCOS, autoimmune thyroiditis and hyperprolactinemia significantly influence reproductive potential in women undergoing IVF. Polycystic ovary syndrome is a multifactorial and heterogeneous syndrome, characterized by chronic oligo- or anovulation, hyperandrogenism and presence of polycystic ovaries observed with transvaginal ultrasonography. Based on different combinations of these clinical manifestations, four phenotypes of PCOS have been identified (21). It is also associated with metabolic disturbances, including predisposition to obesity, insulin resistance and dyslipidemia (22). In addition to elevated AMH levels mentioned previously, many women with PCOS exhibit abnormal gonadotrophin dynamics with elevated LH concentrations and disrupted follicular development. Follicles become arrested at much smaller diameters (5-8 mm) than those predisposed to ovulate and show abnormal hormone production with low estradiol and high androgen concentrations. This hormonal imbalance is exacerbated by elevated AMH concentration, which is 2-3 times higher than in healthy women. Elevated AMH reduces the sensitivity of follicles to FSH, important for their growth and development. Additionally, it inhibits aromatase, an enzyme important for the conversion of androgens to estrogens (23). This is a crucial part for anovulation and subfertility seen in these patients. Finally, the complex relationship between hormonal imbalances, follicular dynamics and metabolic factors leads to infertility and necessitates *in vitro* fertilization treatments. Another endocrinopathy related to female reproductive potential is autoimmune thyroiditis (AIT), a condition caused by cell- and antibody-mediated destruction of thyroid tissue. It is prevalent among women of reproductive age. Autoimmune thyroiditis can lead to reduced gonadotropin-releasing hormone (GnRH) release from the hypothalamus, diminished LH and FSH production by the pituitary gland, and subsequently impaired follicular development and ovulation, leading to infertility.

Additionally, thyroid hormones directly influence the metabolism and growth of ovarian follicles and are vital for the synthesis of ovarian steroids, particularly estrogen. The overall hormonal balance maintained by thyroid hormones is crucial for regular menstrual cycles, and disruptions can lead to conditions such as amenorrhea or oligomenorrhea (24). Moreover, demand for increased thyroid hormone production, which rises by approximately 50% during pregnancy, highlights the additional challenges in ART scenarios, particularly since ovarian stimulation in ART can strain thyroid function and potentially lead to hypothyroidism in women with thyroid autoimmunity or those already treated with levothyroxine (25). Women suffering from autoimmune thyroiditis very often experience repeated spontaneous miscarriages or premature delivery, regardless of how they conceived (naturally or through assisted reproduction) (26). Therefore, preparing the patient for ovarian stimulation is the foundation of a successful IVF cycle that leads to a full term, healthy pregnancy. Another common endocrine disorder related to infertility is hyperprolactinemia, characterized by abnormally high concentration of prolactin in the blood. This condition often accompanies hypothyroidism. Hyperprolactinemia influences the hypothalamic-pituitary-ovarian axis as well. Increased concentration of prolactin inhibits the release of GnRH from hypothalamus, which in turn reduces pituitary gland's production of FSH and LH. This can result in irregular menstrual cycles and anovulation. Transient increases in prolactin levels during IVF cycles, possibly triggered by the stress of the procedure or the use of exogenous gonadotrophins, can further complicate fertility treatments by affecting follicular development and oocyte maturation (27).

1.5. Ovarian hyperstimulation syndrome

Ovarian hyperstimulation syndrome (OHSS) is an important iatrogenic complication of ovarian stimulation for IVF, characterized by cystically enlarged ovaries and a rapid fluid shift from the intravascular space to the transcellular space, resulting in ascites, hypercoagulability and electrolyte imbalances. It occurs when ovaries stimulated by exogenous gonadotrophins are subsequently exposed to hCG. A single high dose of hCG used to initiate final maturation of oocytes can lead to early onset OHSS, which occurs within 10 days following oocyte retrieval. Late OHSS, on the other hand, manifests after this period due to the additional production of endogenous hCG in early pregnancy (28). Exogenous hCG, which has a stronger biological activity compared to endogenous LH, induces intensive luteinization of granulosa cells causing

them to overexpress and release vascular endothelial growth factor (VEGF) into the follicular fluid. The abundance of VEGF leads to exaggerated perifollicular neovascularization, where newly formed blood vessels exhibit increased permeability. As a result, a substantial fluid shift occurs from the intravascular compartment into the third space. This can result in intravascular hypovolemia and development of edema, ascites, hydrothorax, pericardial effusion and reduced renal blood flow (29). Ovarian hyperstimulation syndrome can be classified into three stages based on severity. Mild OHSS is characterized by bilateral ovarian enlargement (<8 cm) with multiple follicular and corpus luteum cysts, abdominal distension, nausea vomiting and diarrhea. There are typically no biochemical abnormalities at this stage, and patient surveillance is sufficient. Moderate OHSS is characterized by ultrasound findings of ascites and ovarian enlargement of up to 12 cm. Abdominal discomfort and gastrointestinal symptoms are more pronounced than in mild OHSS. Additionally, laboratory findings such as elevated hematocrit (>41%) and white blood cell count (>15,000/mL), alongside hypoproteinemia are evident. Severe OHSS presents with significant ascites with severe abdominal pain, and possible pleural effusion, leading to hypoxia. Patients may gain 15-20 kg over 5-10 days, exhibit progressive leukocytosis, and complications such as hypovolemia, oliguria or anuria, and severe gastrointestinal disturbances. Critical OHSS involves potential life-threatening events such as severe thromboembolic complications, disseminated intravascular coagulation, liver failure, acute kidney injury, sepsis, acute respiratory distress syndrome, and cerebral complications. In the most severe cases, ovarian torsion and ovarian rupture with hemorrhage may require urgent surgical intervention (30, 31).

2. AIMS AND OBJECTIVES

The objective of this study is to investigate the relationship between markers of ovarian reserve, associated endocrinopathies and increased ovarian response in patients undergoing gonadotrophin stimulation in IVF procedures. This research aims to identify factors that significantly correlate with ovarian hyperresponse, which could help in customizing treatment plans to minimize risks and improve clinical outcomes.

3. PARTICIPANTS AND STUDY DESIGN

The study consists of retrospective analysis of medical records from infertile female patients treated at the Department for Human Reproduction, Clinical Hospital Center Rijeka from 2018 to 2023. Data were collected from the medical database of the Department of Human Reproduction and the hospital data base IBIS. In total, 74 stimulated *in vitro* fertilization cycles in 72 patients were analyzed, with some patients undergoing multiple treatment cycles within the study period. The inclusion criteria for the study were preserved or high ovarian reserve and 10 or more oocytes retrieved in a single IVF cycle.

The goals of the study were:

1. To investigate the relationship between excessive ovarian response to gonadotrophin stimulation and serum AMH concentration.
2. To determine the circumstances that lead to an excessive response of the ovaries.
3. To establish the incidence of polycystic ovary syndrome (PCOS) among the studied group.

The following data were collected and compared to fulfill the objectives of the study:

1. Total number of participants and IVF cycles.
2. Age of the participants.
3. Number of oocytes retrieved.
4. Concentration of Follicle Stimulating Hormone (FSH).
5. Concentration of Luteinizing Hormone (LH).
6. Concentration of Estradiol (E2).
7. Concentration of Testosterone (T).
8. Concentration of Anti-Müllerian Hormone (AMH).
9. Incidence of study participants with endocrinopathies.

Due to variation in laboratory standards and normal reference values for Anti-Müllerian Hormone concentrations across different laboratories, we standardized the analysis methods by categorizing AMH concentrations into five distinct categories. This categorization was achieved by dividing each laboratory's reference range into 5 equal segments, and then classifying them as follows:

- A: Below the minimal value of the reference range (severe diminished ovarian reserve)
- B: Below the mean value of the reference range (diminished ovarian reserve)
- C: Middle of the reference range (appropriate ovarian reserve)
- D: Above the mean value of the reference range (preserved ovarian reserve)
- E: Above the maximal value of the reference range (high ovarian reserve – OHSS risk)

This categorization aids in achieving consistency in data interpretation and enables a more accurate comparison of AMH's role across the study group.

4. RESULTS

4.1. Distribution of participants by age group

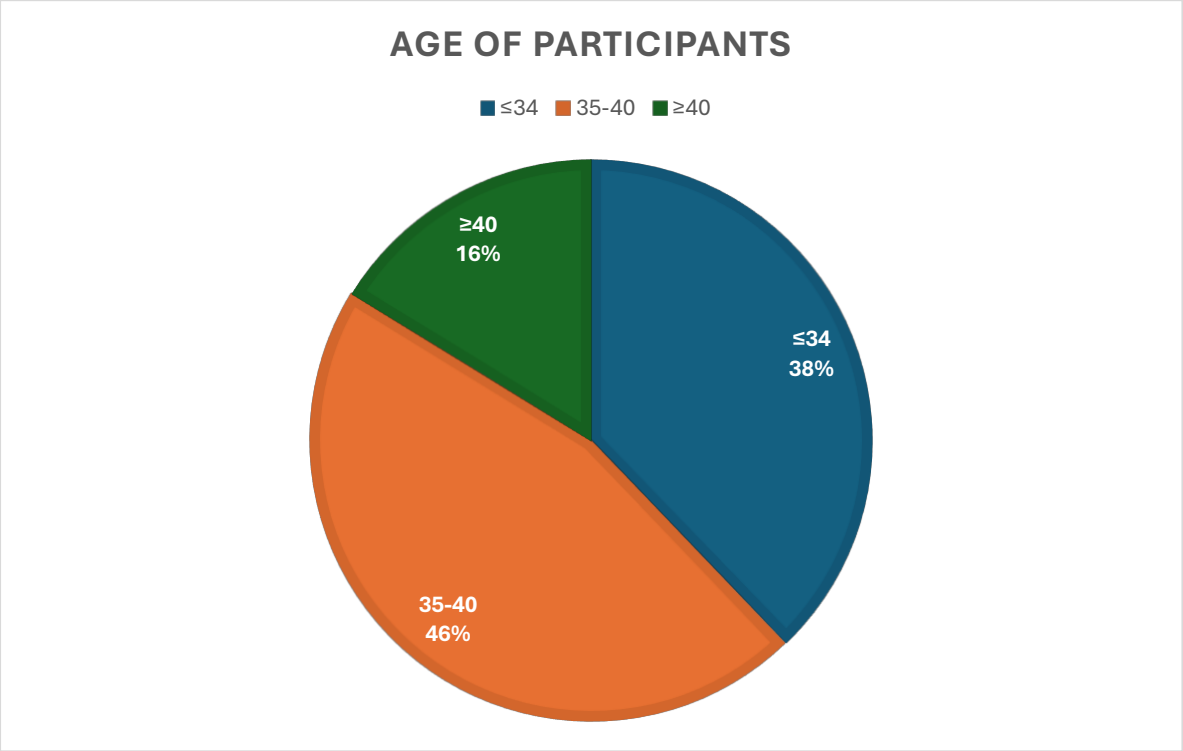

Figure 1. Distribution of participants by age (N=74)

The mean age of the participants with gonadotrophin stimulation for *in vitro* fertilization cycles was 35.5 years (±4.58), indicating a central tendency towards the mid-reproductive years. Two thirds of the participants (62%) were treated at an older reproductive age (after 35 years). The youngest participant was 19 years old, and the oldest was 48 years old. Notably, the largest proportion of participants was observed within the 35-40 age group, accounting for 46% of the study participants.

4.2. Impact of age on oocyte number after ovarian stimulation and retrieval

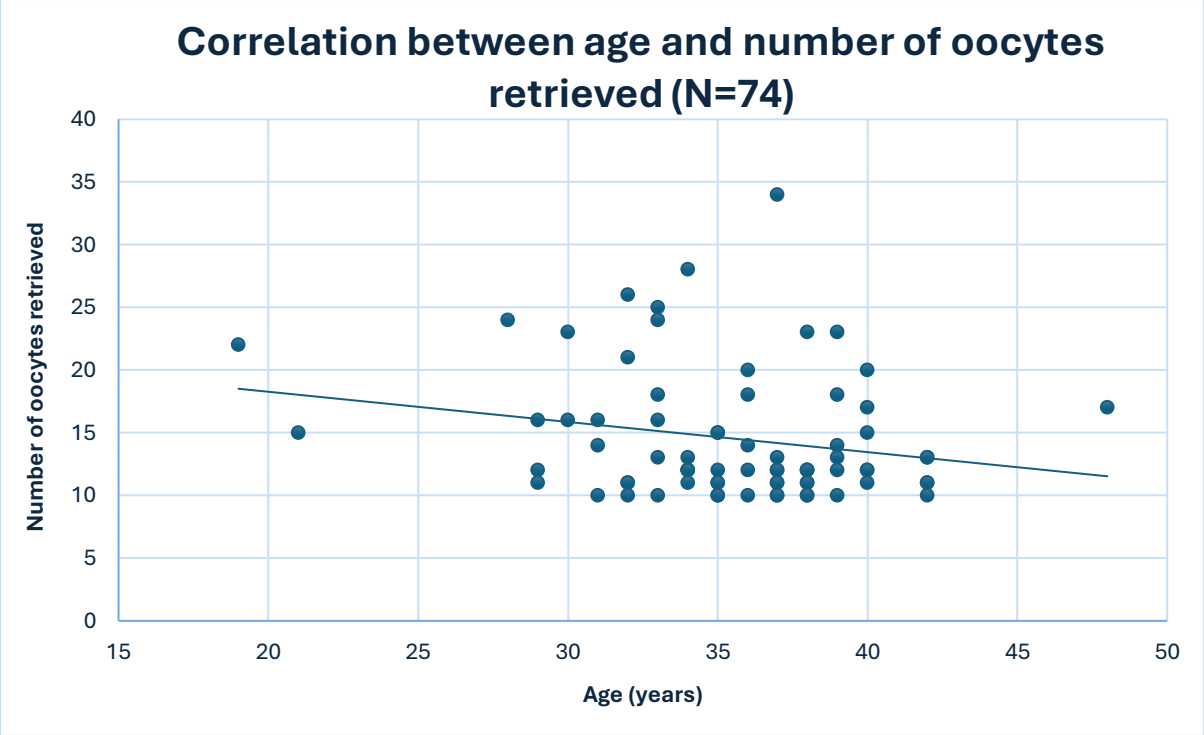

Figure 2. Correlation between age and number of oocytes retrieved (N=74)

The downward trend line indicates that in older participants, the quantity of oocytes retrieved during stimulated *in vitro* fertilization cycles tends to decrease, instead of a good ovarian response to stimulation. Specifically, the Fig. 2 shows variability in the number of oocytes retrieved across different ages, but the overall direction of the trend is negative with ageing which is in accordance with generally known facts about ovarian ageing.

4.3. Analysis of oocyte number across AMH categories

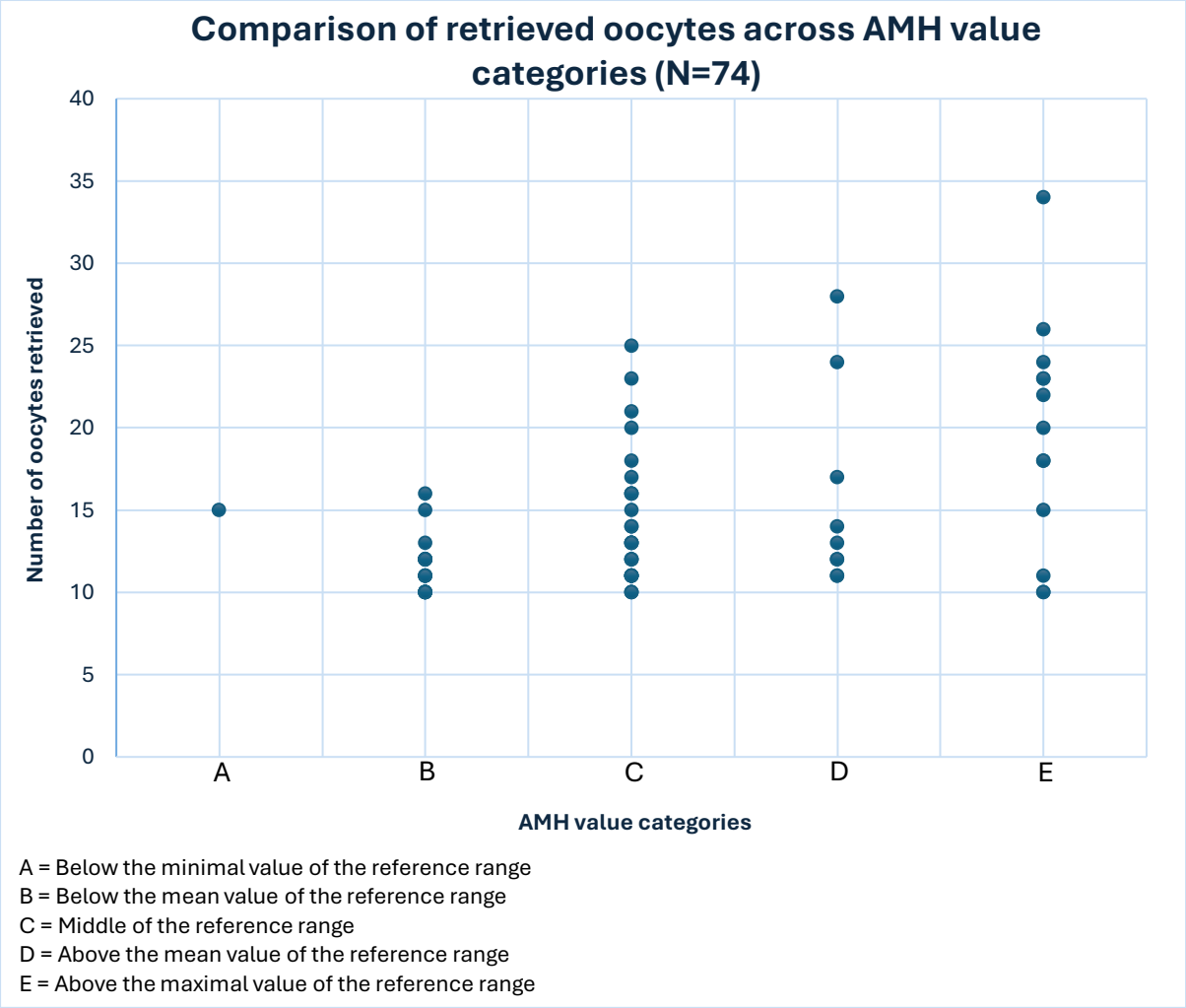

Figure 3. Comparison of retrieved oocytes across AMH value categories (N=74)

Each AMH category, except "Below the minimal value of the reference range" which only includes data from one study participant, shows a broad distribution of oocyte number post-retrieval. This indicates significant variability within most categories. We observed a greater dispersion of the number of oocytes in the categories with higher AMH values. Additionally, OHSS was identified in 3 participants (4%) in the middle AMH category, which contrasts with the general understanding of ovarian reserve and high AMH concentrations.

4.4. Age distribution across different AMH value categories

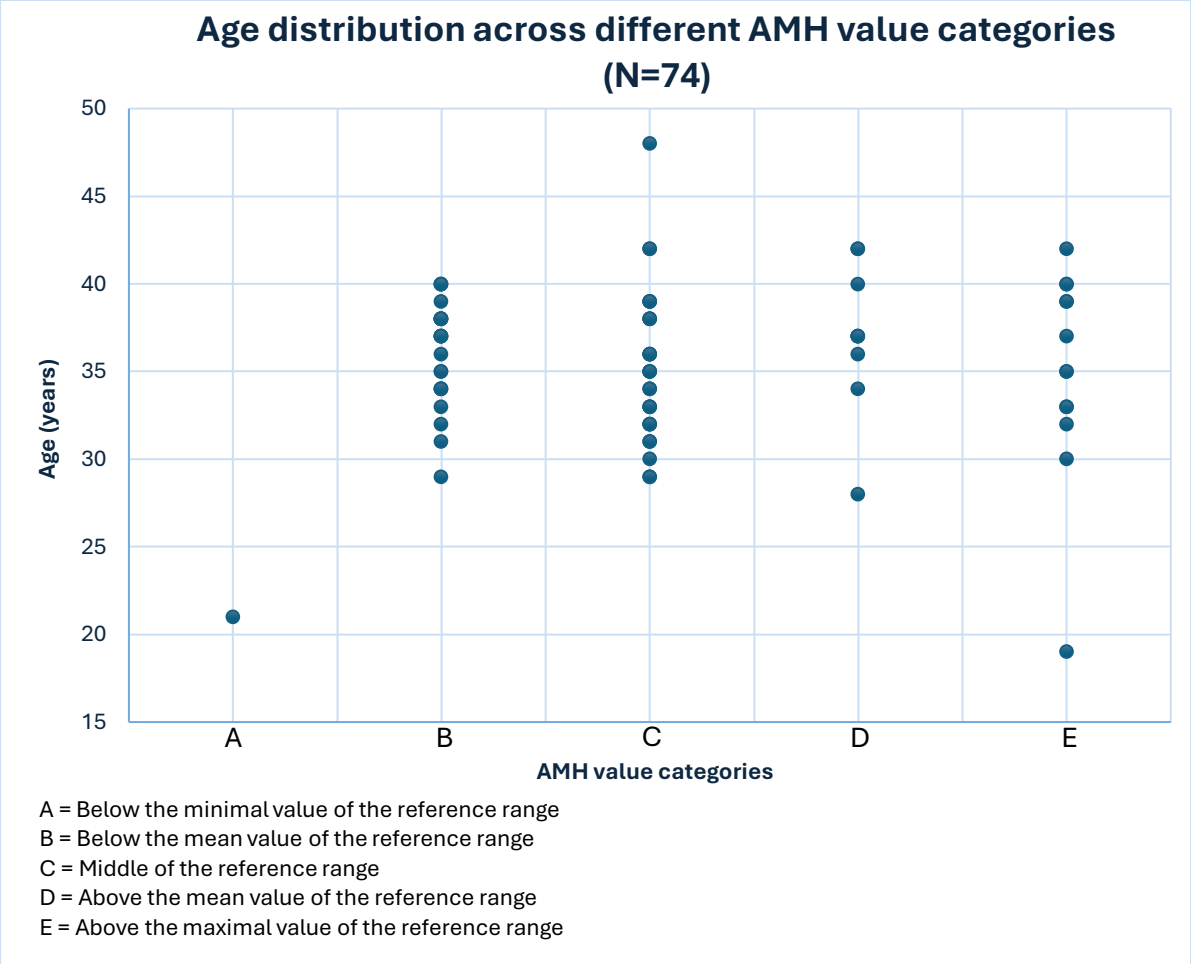

Figure 4. Age distribution across different AMH value categories (N=74)

There is no clear age-related trend in AMH categories in this study group. We emphasize that the study group consists of participants with a good ovarian response to gonadotrophin stimulation. Results showed that age of participants varies widely across all AMH categories, as each category includes both young and old participants. The youngest participant, aged 19, is represented in the "Above the maximal value of the reference range" group, however, the second youngest participant, aged 21, is in the "Below the minimal value of the reference range"group. Older participants over the age of 40 are presented in middle and high AMH value categories. This observation is contradictory with known facts about AMH and ageing.

4.5. Anti-Müllerian Hormone concentration and reference ranges across different laboratory units

Table 1. Distribution of AMH concentration across different laboratory reference ranges (N=74)

Range of reference values	Number of patients	Mean AMH	Range of measured values	Above the reference range (N)	Below the reference range (N)
0,059 - 4,44 µg/L	7	3,66	1,58 - 6,63	2	0
0,41 - 6,96 µg/L	32	3,66	0,9 - 10,27	3	0
0,67 - 7,55 µg/L	2	2,86	2,06 - 3,66	0	0
0,71 - 7,59 µg/L	14	5,07	2,03 - 11,33	3	0
0,78 - 5,24 µg/L	1	5,8	5,8	1	0
0,8 - 5,2 µg/L	1	6,7	6,7	1	0
1,05 - 53,5 µg/L	3	36,93	10,2 - 73,5	1	0
1,18 - 9,16 µg/L	1	4,13	4,13	0	0
1,2 - 9,05 µg/L	4	5,69	2,68 - 8,59	0	0
1,52 - 9,95 µg/L	1	1,19	1,19	0	1
1,65 - 9,15 µg/L	1	2	2	0	0
2,9 - 49,7 µg/L	1	12,9	12,9	0	0
4,8 - 53,9 µg/L	1	22,2	22,2	0	0
4,11 - 58 µg/L	2	43,6	18,1 - 69,1	1	0
6,75 - 47,84 µg/L	1	23,5	23,5	0	0
8,1 - 18,3 µg/L	1	50,7	50,7	1	0
8,4 - 65,4 µg/L	1	34	34	0	0

Table 1. summarizes AMH concentration across various reference ranges in different laboratory units. For 74 stimulated IVF cycles we observed 17 different reference range values. This fact significantly complicated the analysis of collected data on AMH concentrations in the participants. There was a significant variability in AMH concentration within each category. Notably, 13 patients (17%) had AMH levels exceeding the upper limits of their reference ranges. There is one noted exception where a patient's level falls below the expected range.

4.6. Overview of measured basal hormone concentrations (3rd-5th day of cycle) in study participants

Table 2. Hormonal concentration of study participants (N=74)

Measured concentration of sex hormones	Mean ± SD	Range of measured concentrations	Reference range
Follicle-stimulating hormone (IU/L)	7 ± 1,35	6,2 - 10	Follicular phase: 2,4 - 12,6
Luteinizing hormone (IU/L)	6,54 ± 2,77	0,4 - 15,8	Follicular phase: 3,5 - 12,5
Estradiol (pmol/L)	229,64 ± 215,25	65,7 - 1547	Follicular phase: 45 - 854
Testosterone (nmol/L)	0,97 ± 0,43	0,1 - 2,4	0,29 - 1,67

Follicle-stimulating hormone has a narrow range, while luteinizing hormone shows a broader range of measured concentrations. Estradiol concentration differs notably, with an extensive range. On the other hand, testosterone concentration is stable among the group. The mean concentration of all sex hormones at the beginning of the menstrual cycle were measured within the reference range.

4.7. Distribution of endocrinopathies

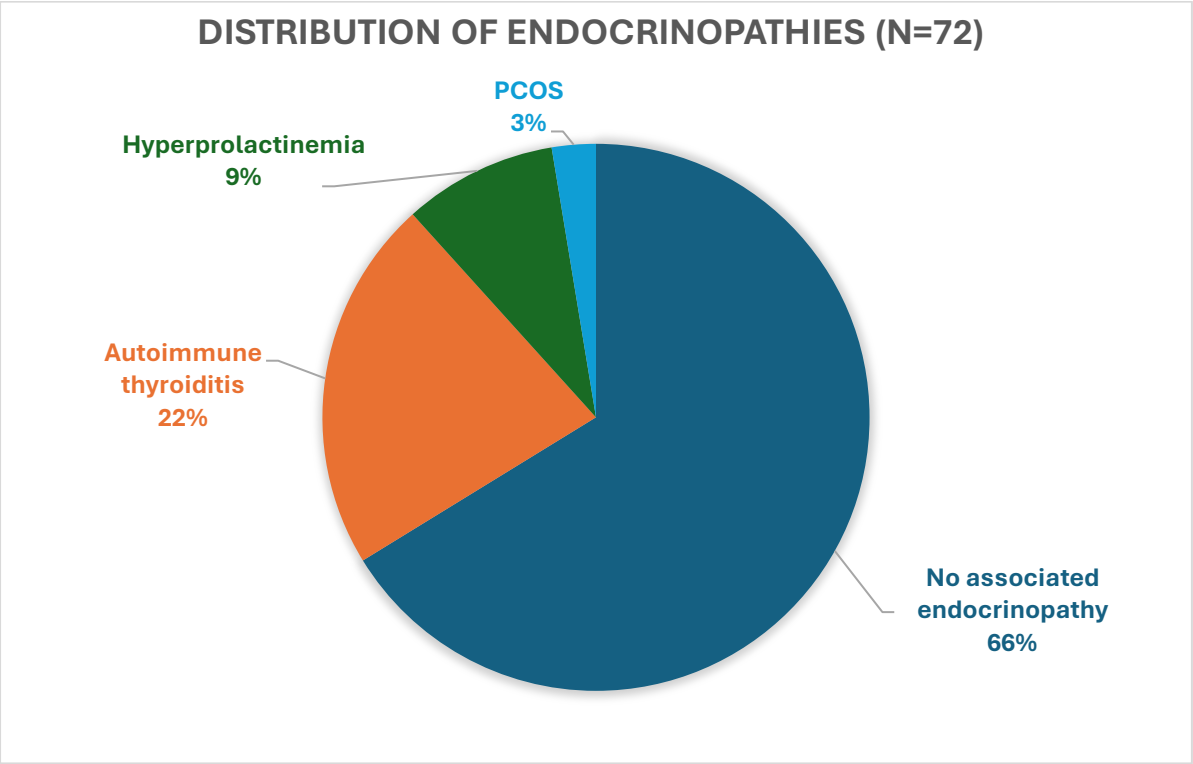

Figure 5. Distribution of endocrinopathies (N=72)

The largest proportion of study participants (66%) did not have an associated endocrinopathy. Autoimmune thyroiditis was present in 22% of the participants, making it the most common endocrinopathy in the group of study participants with good/high ovarian response. Hyperprolactinemia affected 9% of the participants, while PCOS was observed in only 3% of the study group. Ovarian hyperstimulation syndrome was identified in 3 participants (4%) in the middle AMH category, each with distinct endocrinopathies: first participant had autoimmune thyroiditis and hyperprolactinemia, second had no accompanying endocrinopathies, and third had hyperprolactinemia.

4.8. Incidence of endocrinopathies by AMH category

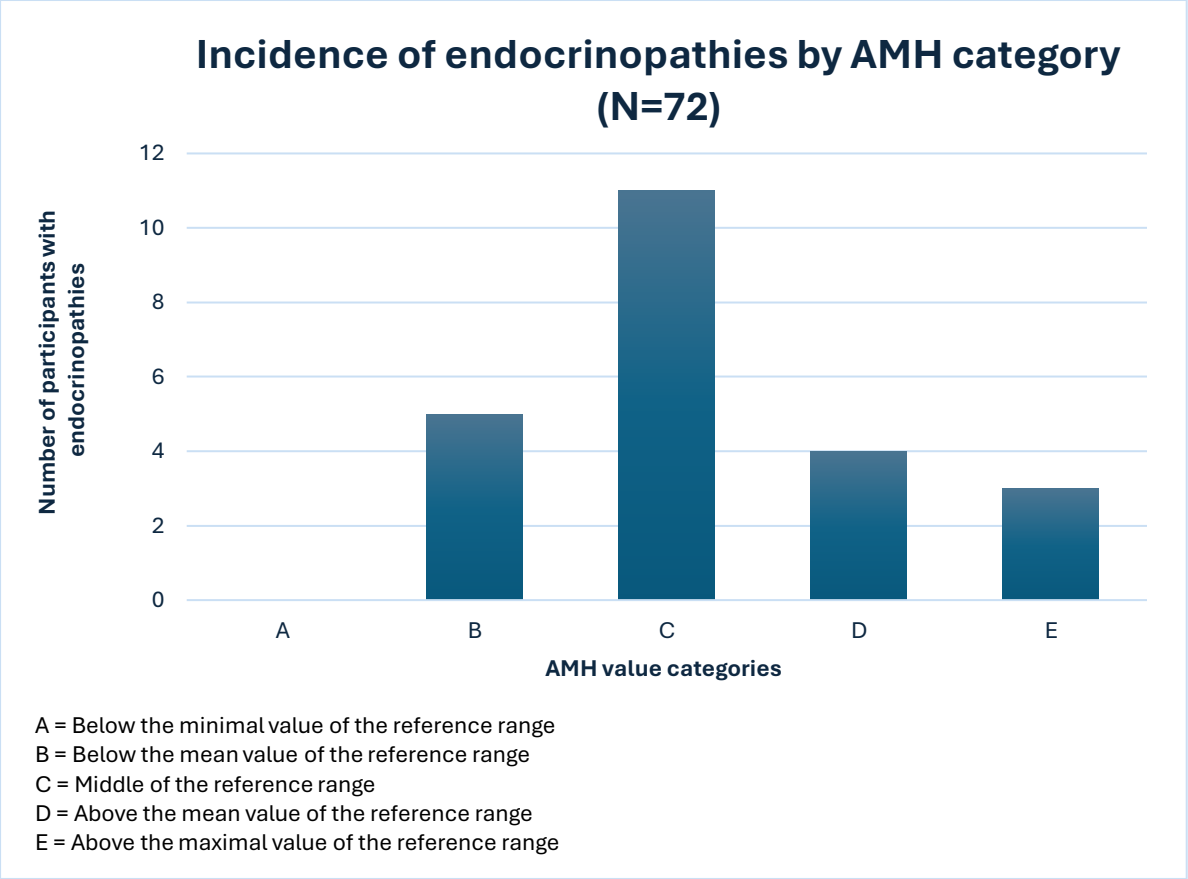

Figure 6. Incidence of endocrinopathies by AMH category (N=72)

Figure 6. demonstrates the incidence of endocrinopathies across defined categories of AMH concentration, including PCOS, autoimmune thyroiditis and hyperprolactinemia. The highest incidence of all endocrinopathies (22%) was observed in participants with AMH concentration in the categories of „middle and below the mean value of the reference range" (B+C). Lower incidence (9,7%) was noted in categories with high AMH concentrations (D+E). Two participants with PCOS (2,7%) were identified in the category „middle of the reference range" and another case in the category „above the maximal AMH value of the reference range". Autoimmune thyroiditis was present in all AMH categories except the lowest one (23,6% of study participants), and hyperprolactinemia was observed in AMH categories B-D (9,7% of study participants).

5. DISCUSSION

Results of this research paper demonstrate the relationship between various factors influencing and prediciting ovarian reserve and the response to gonadotrophin stimulation in infertile female patients with preserved ovarian reserve and good response to ovarian stimulation. Despite the previously mentioned challenges associated with ovarian ageing, results showed that among study participants, that had preserved/high ovarian reserve, a significant proportion was in the older reproductive age group at the time of their infertility treatment. In addition, it was observed that age distribution varied significantly across all defined AMH value categories, with participants ranging from young adults in their twenties to those in their forties. Peculiarly, middle and high AMH value categories had the widest range with participants over the age of forty. These observations are inconsistent with general expectations about AMH concentration declining steadily with age and suggests that factors other than age might influence AMH concentration. Supporting this, a longitudinal study by de Kat et al. (2016.) revealed that AMH decline does not follow a consistent pattern and varies considerably among individuals. The study found that the rate of AMH decline depends on the initial levels and varies with age and proximity to menopause, suggesting a complex interaction between chronological age, biological aging, and ovarian reserve. Particularly, the study suggested that women do not uniformly experience reductions in AMH as they age, and even within similar age groups, there can be significant disparities in AMH concentration (32). This could explain why in this research, some of the older participants still exhibited high AMH concentration, contradicting common expectations. In this study group it was also noticed that in different age groups, patients exhibited high or extremely high AMH concentrations, putting them at an increased risk for OHSS. However, mild OHSS was identified in three participants within the middle AMH concentration category which demonstrates that these values of AMH do not exempt patients from the risk of developing OHSS and that AMH concentration is not the sole predictor of this complication. It is interesting that a greater dispersion in the number of oocytes retrieved in the categories with higher AMH values was observed. Even though higher AMH concentration is generally associated with a substantial ovarian reserve, the variability in oocyte yield highlights that AMH alone may not provide a precise prediction of ovarian response to stimulation. Moreover, in this study group the relationship between other endocrinopathies and ovarian reserve was established. Autoimmune thyroiditis was the predominant endocrinopathy among the study participants. Notably, here the incidence of associated endocrinopathies was inversely related to ovarian reserve. In the group of participants with preserved/high ovarian

reserve incidence of endocrinopathies was lower. Particularly, the incidence was highest among those with mid-range and lower concentrations of AMH, suggesting a possible impact of other endocrinopathies on ovarian reserve. These results align with the study by Öztürk Ünsal et al. (2021.) in which women with Hashimoto's thyroiditis tend to have lower AMH concentrations compared to age-matched healthy women. This highlights the impact of thyroid autoimmunity on ovarian reserve. Furthermore, the study emphasized that despite the absence of overt thyroid dysfunction, thyroid autoimmunity could subtly impair follicular development, potentially through autoimmune processes that affect the ovarian tissue directly or disrupt the ovarian microenvironment (33). This supports our observations of a higher incidence of endocrinopathies, particularly autoimmune thyroiditis, in participants with lower AMH concentration, indicating a potential impact of thyroid abnormalities on ovarian reserve. Additionally, in our study participants were selected based on their high ovarian response to stimulation, and it was anticipated that high AMH concentation would correlate with a greater prevalence of PCOS. However, AMH varied widely among participants, and the incidence of PCOS was low, with only two cases accounting for 3% of the study group. One case occurred in the middle of the reference range and another case above the maximal value. This finding underscores that while high AMH concentrations can rise suspicion of PCOS, it is not a definitive indicator of the condition.

6. CONCLUSIONS

1. Most participants in the study group with preserved/high ovarian reserve were in the older reproductive age group (62%) at the time of *in vitro* fertilization treatment, which is contrary to data on ovarian aging from the general population.
2. There is a general negative correlation between the age of female participants and number of oocytes retrieved during conventional IVF cycles. As age increases, there is also a trend towards fewer oocytes being retrieved in females with a preserved/high ovarian reserve.
3. There is a wide range of retrieved oocytes within all five AMH categories. The widest range in the number of aspired oocytes was observed in the category with highest AMH values.
4. There is a wide age distribution within each AMH category. Therefore, AMH levels can significantly vary irrespective of age, reflecting broad differences in individuals with preserved/high ovarian reserve.
5. The most common endocrinopathy in the study group was autoimmune thyroiditis (22%).
6. Associated endocrinopathies are less frequent in participants with preserved/high ovarian reserve. The highest incidence of endocrinopathies was observed in the categories with middle or lower concentrations.
7. Only two cases of PCOS have been recorded: one in the medium and another in the very high AMH category.
8. Ovarian hyperstimulation syndrome was rarely noted in this study group (4%), with all cases occurring in the middle AMH category. Additionally, two out of the three cases had associated endocrinopathies.
9. Other factors, such as age, associated endocrinopathies, and specific fertility treatment protocols, also play critical roles in the outcomes of oocyte retrieval.

7. SUMMARY

Aim: To investigate the relationship between markers of ovarian reserve, associated endocrinopathies and increased ovarian response in patients undergoing gonadotrophin stimulation during *in vitro* fertilization procedures.

Methods: A retrospective analysis of medical records from infertile female patients with preserved or high ovarian reserve and 10 or more oocytes retrieved in a single IVF cycle was done. Analysed data included ovarian reserve markers and associated endocrinopathies.

Results: The mean age of participants was 35.5 years (±4.58), with 62% treated after the age of 35. AMH values showed broad distribution in oocyte number, with OHSS identified in 3 participants in the middle AMH category. No clear age-related trend in AMH concentration was observed. PCOS prevalence was low (3%) among the study group, while autoimmune thyroiditis (22%) and hyperprolactinemia (9%) were more common. Endocrinopathies were most frequent in the middle AMH category.

Conclusion: This research indicates that a significant proportion of stimulated IVF patients with preserved/high ovarian reserve are of older reproductive age. A negative correlation between age and the number of oocytes retrieved was observed. Concentrations of AMH varied significantly, suggesting they are not strictly age dependent. Autoimmune thyroiditis was the most common endocrinopathy, while PCOS was rare. These results highlight the complexity of factors influencing IVF outcomes and indicate the need for further research.

Keywords: Anti-Müllerian Hormone, endocrinopathy, *in vitro* fertilization, ovarian reserve

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9. CURRICULUM VITAE

Ivana Šteta was born on July 15, 2000, in Dubrovnik, Croatia. In the academic year 2018/2019, she enrolled in the Integrated Undergraduate and Graduate study of medicine in English at the Faculty of Medicine, Rijeka. During her studies, she participated in a summer Erasmus+ professional practice at the departments of Anesthesiology, Dermatology and Ophthalmology in Coimbra, Portugal. She also took part in the Erasmus+ Blended Intensive Programme "Diagnostics in Gynaecology" in Maribor, Slovenia. Additionally, she participated as an active participant in four different scientific conferences. She attended the workshop "How to Write a Good Case Report?" and the 6th School of Interventional Radiology. She is fluent in English and German.

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Ethics Committee

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„Ovarian reserve in patients with hyperresponse following gonadotrophin stimulation"

Researcher: Ivana Šteta

Mentor: prof. Neda Smiljan Severinski, MD, PhD

Location: Clinical Hospital Center Rijeka, Department of Obstetrics and Gynaecology

Documents submitted with the Ethics Committee and documents inspected during the Meeting of the Ethics Committee:

1. Application form
2. Letter of approval - Head of Department of Obstetrics and Gynaecology
3. Research protocol
4. Mentor's approval
5. Data accession statement (IBIS)

The Ethics Committee has evaluated all documents named above in respect of their compliance with the ethical standards and valid international agreements on carrying out clinical research and trials, and has issued the following:

DECISION

The Ethics Committee of the Clinical Hospital Center Rijeka has assessed the application in accordance with the Code of Ethics and related laws and regulations.

The proposed study and proposed methodology of research is unambiguous in respect to ethical principles and standards in research, and we therefore **support its realisation.**

Decision passed on: 19.04.2024.

Class: 003-05/24-1/49
Reg.No.: 2170-29-02/1-24-2

Ethics Committee
prof. Ivan Bubić, MD, PhD