

ROLE OF AGE AS A MAJOR DETERMINANT OF ASSISTED REPRODUCTIVE TECHNOLOGIES OUTCOME

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Abstract

The aim of the present study was to determine the influence of age on the prediction of the outcome of assisted reproductive technologies. The study included data of 1992 women who had performed ovarian stimulation according to a protocol with a gonadoliberein antagonist or a long protocol and underwent a follicular puncture. Patients were divided into four age groups, where numbers of oocytes collected, number of atretic oocytes and percentage of clinical pregnancies were evaluated. We found significantly reduced average number of oocytes collected with increase of age. In patients with poor ovarian reserve the average age is higher than in patients with good response, i.e. normal ovarian reserve. We have found clear tendency of decreased clinical pregnancy rate with the increase of women's age. In conclusion, our data show convincingly that age is a strong prognostic marker of ovarian reserve in both quantitative and qualitative terms, estimated by the clinical pregnancies achieved.

Key words: age, ovarian reserve, prognostic value

Introduction. Female age is traditionally recognized as a major determinant of the outcome of assisted reproductive technologies (ART) factor [1–3]. It can also be treated as a marker for assessing ovarian reserve (OR). The decline in female fertility with age has long been well studied and documented [4,5]. One third of

the women who postpone pregnancy for over 35 years of age and more than half of those who postpone it for over 40 years have difficulty conceiving. In the context of ART, a reduced ovarian response to ovarian stimulation is expected with age [6–8] and this is associated with a poor ART outcome. The relationship between age and the unsatisfactory ovarian response to controlled ovarian hyperstimulation (COH) was thoroughly analysed in a study involving 3825 women, starting the first cycle of ART in S.I.S.Me.R. Department, Italy and the IVF Department of the University Hospital of Modena and treated in the period between January 2004 and December 2009. All patients underwent conventional COH with different starting doses of FSH/HMG, according to the age. As expected, the incidence of poor ovarian response (POR) increases with age, occurring above 50% over the age of 40. Naturally, some elderly women can still produce more follicles and oocytes, while there are also young women with POR [9].

Materials and methods. This was a retrospective, single-centre cohort study. Approval to retrieve and analyse the data was provided by the Ethics Committee of the Specialized Obstetrics and Gynaecology Hospital “Dr. Shterev”, Sofia. Regarding the influence of the age factor on OR and its correctness as its marker, 1992 women were examined which had performed conventional COH according to a protocol with a gonadoliberein antagonist or a long protocol and underwent a follicular puncture for the period January 2017 – December 2019 at the Medical Complex “Dr. Shterev”, Sofia. Ovulation triggering was performed with 10 000 IU human chorionic gonadotropin (HCG) at 34–36 hours before follicular puncture. Embryo transfer was performed on day 3 or day 5 depending on the number and quality of embryos and characteristics of the particular patient. After embryo-transfer, luteal support is assigned. The choice of stimulation protocol and gonadotropins doses as well as ART procedure – IVF/ICSI/IMSI and the application of additional techniques such as assisted hatching, co-cultivation, local endometrial lesion, MACS (elimination of spermatozooids with fragmented DNA after passing through a magnetic field), etc. is consistent with the type, prescription and aetiology of infertility in the individual couples and is subject to strict medical indications and standard operating procedures.

Patients were divided into four age groups according to the criteria adopted by the IVF monitoring group of the European Association for Human Reproduction and Embryology: group 1 ≤ 30 years ($n = 394$), group 2 – 31–34 years ($n = 473$), group 3 – 35–39 years ($n = 695$), group 4 ≥ 40 years ($n = 430$).

To assess the ability of a woman’s age to predict the response to COH in quantitative terms, the average number of oocytes retrieved by follicular puncture was studied. In order to confirm the strength of age to predict the ovarian response to COH, it was studied as a marker in different types of ovarian response to conventional COH – poor response with less than 5 retrieved oocytes, satisfactory response with between 5 and 10 retrieved oocytes, and good response with over 10 retrieved oocytes.

To assess the ability of age to predict the ovarian potential in qualitative terms, the average number of atretic oocytes retrieved and their percentage of the total number of the obtained oocytes were studied.

And most importantly, the ability of age alone to predict the outcome of ART was studied, taking into account the percentage of clinical pregnancies achieved after ART in the different age groups.

Statistical analysis. The statistical analysis was performed using the software package SPSS Statistics 17.0. For all quantitative markers of ovarian reserve, the main statistical indicators are calculated and presented in tables: sample size (n), minimum value (min), maximum value (max), arithmetic average ($\bar{x}$), standard error of arithmetic average ($s_{\bar{x}}$), standard deviation (SD) and median (Me). The Mann–Whitney U test was used to compare the two independent extracts. The accepted significance level (α) is 0.05. Differences where the value of $p \leq \alpha$ are considered reliable. The effect of smoking on ovarian reserve markers was investigated by nonparametric rank analysis of variance (Kruskal–Wallis ANOVA on ranks, H test). The Dunn method was applied to the multiple comparisons of all group pairs. The distribution of the frequencies of pregnancies for embryo transfer in the separate subgroups of patients was analyzed. The results are summarized (in number and %) in frequency tables of the type 2×2 . The Pearson test χ^2 was used to prove differences in the percentage of pregnancies.

Results. From the performed analysis, the Kruskal–Wallis test proved statistically significant differences between age groups in regard to the average number of the retrieved oocytes ($H = 361.8$, $p < 0.001$). There is a marked decrease in the average number of oocytes retrieved after conventional COH with the age (Fig. 1). The definiteness of this inverse correlation between age and the number of oocytes retrieved is confirmed by the detection of a statistically significant difference between each of the studied groups – multiple comparisons by Dunn’s method proved differences between all pairs of age groups ($p < 0.05$).

The study of the type of ovarian response to COH and its relationship with the age confirms the data on the inverse correlation between the average number of oocytes retrieved and the age. In patients with a POR, the average age is expected

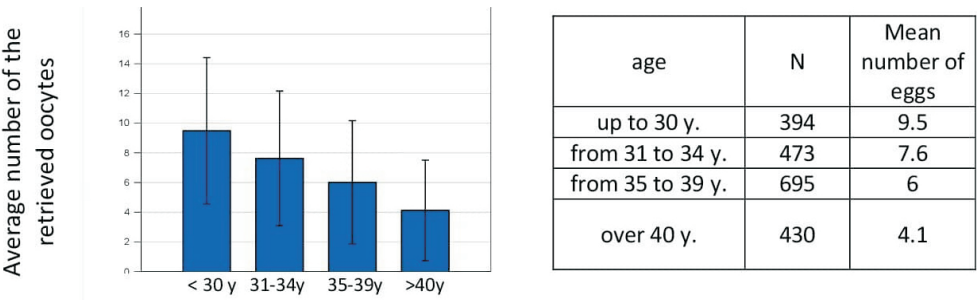


Fig. 1. Comparison between the age groups on the average number of the retrieved oocytes

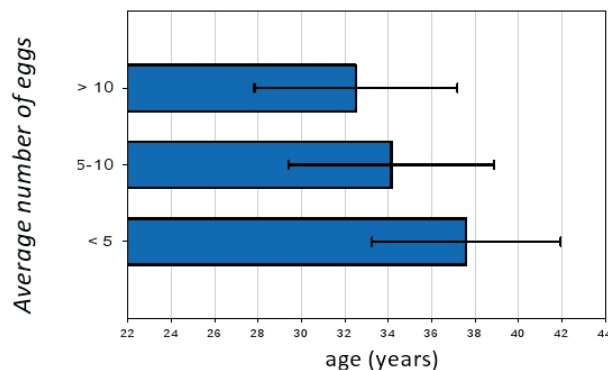


Fig. 2. Comparison of the age of patients with different response to stimulation

to be the highest – 37.6 years, in the group with a satisfactory response to COH is borderline and in accordance to other authors in 34 years and the youngest (mean 32.5 years) is a group with a very good response to stimulation and retrieved more than 10 oocytes. The groups of patients with different response to stimulation differed significantly in the average age ($H = 335$, $p < 0.001$) (Fig. 2).

The relationship between age and the proportion of atretic oocytes from the total number of oocytes retrieved was studied in 1949 women. The analysis of the distribution of atretic oocytes also shows a decrease in their average number with advancing age of the woman, reciprocal to the general trend of decrease in the total number of the retrieved oocytes, represented by a dotted line in Fig. 3. However, the nonparametric dispersion analysis showed significant differences in the number of atretic oocytes between the groups ($H = 44.4$, $p < 0.001$). As a proportional distribution of the atretic oocytes in the different age groups, how-

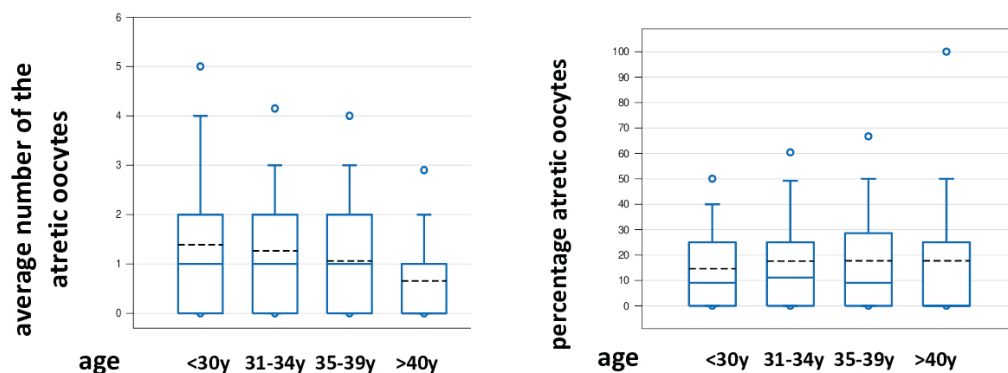


Fig. 3. Comparison between the age groups in the number and the proportion of the total number of the atretic oocytes

ever, no significant difference was observed, i.e. their proportion in the different age groups remains the same – the differences between the age groups in the percentage of atretic oocytes are untrustworthy ($H = 4.8$, $p = 0.185$).

As expected, the analysis of the data on the achieved clinical pregnancies in the different age groups shows a clear tendency of their decrease with the increase of the age of the women. The percentage of the achieved clinical pregnancies in the two age groups up to 35 years is almost the same, without statistically proven difference, 43.7% and 42.5%, respectively. In the group over 35 years of age there is still a very good result for ART – 34.7%, but it decreases significantly compared to the first two groups. In the age group over 40 years the ART outcome also differs significantly from the other groups and is very unsatisfactory – only 14.9% ($\chi^2 = 69.5$, $p < 0.001$) (Fig. 4).

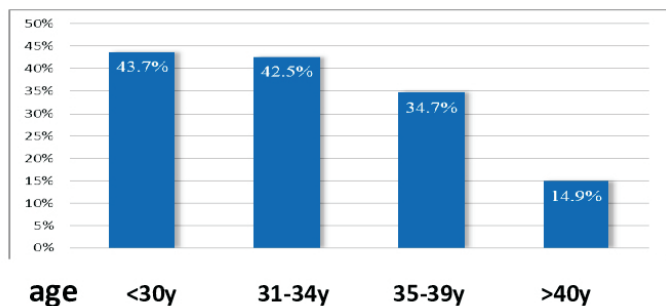


Fig. 4. Comparison between the age groups by percentage of pregnancies

Discussion. The results of the study clearly confirm the strong and indisputable predictive value of a woman's age in regard to quantitative ovarian reserve – the response to COH as number of oocytes. There is a convincing inverse relationship between the number of the oocytes retrieved and the woman's advancing age.

Paradoxically, it remains to maintain equal percentage ratio of the atretic oocytes in each of the age groups, i.e. the proportion of the atretic oocytes in the total number of produced oocytes is constant and equal, regardless of the age. This fact appears to be crucial with the decreasing of the total number of the oocytes retrieved with advancing age and this significantly reduces the substrate for embryos selection and transfer and, respectively, the chance of pregnancy.

The great advantage of age as a marker for the outcome of ART is that it has a predictive value in relation to the main aim of ART procedures – achieving a clinical pregnancy. There is a statistically significant decrease in the percentage of achieved clinical pregnancies, especially at the age over 35 years.

The results in regard to clinical pregnancies in the different age groups do not follow the smooth trend of inverse correlation, observed in regard to the number

of oocytes. In the group of young women up to 30 years and between 31 and 34 years, despite the significant difference in the retrieved oocytes, the percentage of clinical pregnancies is comparable. This suggests that even with a smaller number of oocytes, due to their good quality and that of the endometrium and, respectively, conditions for implantation in young women, the pregnancy percentage is equally good. With the advance of a woman age these conditions change – the number of oocytes becomes crucial to increase the chance of pregnancy. In women over 40 years of age, the percentage of clinical pregnancies decreases sharply, more pronounced compared to the degree of reduction of mean number of oocytes retrieved. The probable reason for these results is the high frequency of chromosomal and genetic defects in the oocytes, high frequency of premature oocyte luteinisation, reduction of their energy potential and suboptimal conditions for implantation. These data confirm the literature data that ovarian ageing is associated not only with a decrease in the number of oocytes, but also with a significant increase in the number of chromosomal defects, especially aneuploidies [10–15].

Conclusion. The modern socio-economic and cultural conditions and the related postponement of reproduction increase the age of the woman for the realization of her reproductive plans, including those for the birth of a subsequent child. As a consequence of these trends, the reduced ovarian reserve in quantitative but also in qualitative aspect and the poor response to COH are increasingly observed in today's treatment of infertility and at the same time the biggest challenge for specialists in reproductive medicine.

Age stands out in a convincing way as a major determinant of ART outcome. Infertility in young women has a far better prognosis than the chance of pregnancy in elderly women after the age of 34, even with normal OR. It has been proven that ARTs are not a means of overcoming ovarian ageing.

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