Survey of the influence of key factors on the number and percentage of preimplantation embryos obtained from patients with low ovarian reserve during IVF/ICSI procedure

Abstract

The current study presents the results of retrospective and prospective research of key factors such as age, patient's hormonal status, applied protocol for controlled ovarian hyperstimulation (COH), oocyte quality and method of insemination on the number and percentage of preimplantation embryos obtained in patients with reduced ovarian reserve.

Materials and methods: The study is carried out on 147 MII oocytes originating from 72 patients with diminished ovarian reserve, who have participated in an infertility treatment program through *in vitro* fertilization from February 2011 until February 2014. 2483 MII oocytes derived from 347 patients with normal ovarian reserve were used as a control group.

Results: Our study found that the patients with low and normal ovarian reserve statistically significant differ with respect to the number of embryos obtained ($p<0.0001$)–1.56 ± 0.93 and 5.97 ± 3.44, respectively. Statistically significant difference between the two groups of patients was also found regarding the percentage of embryos obtained ($p<0.05$) at 76.19% and 83.49% for the studied and the control group, respectively.

Conclusion: The examined key factors related to the *in vitro* fertilization process did not show a statistically significant influence on the percentages of the embryos obtained in patients with low ovarian reserve. Such a correlation was only established between patient's age and anti-Mullerian hormone (AMH) values and the number of embryos obtained.

Keywords: low ovarian reserve, preimplantation embryos, number/percentage of embryos obtained, IVF, ICSI

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Introduction

Essential to the success of an *in vitro* procedure infertility treatment cycle are the number and quality of the oocytes obtained, and the preimplantation embryos, respectively. Larger number of embryos allows a selective embryo transfer of the most viable embryos showing the best quality to be done which has been proved to increase the rate of clinical pregnancies and birth rates.[1,2] Achieving a satisfactory number of embryos is a result of an appropriate controlled ovarian hyperstimulation consistent with the patient's age and hormonal status, a properly selected method of insemination of the oocytes based on the morphological features of the gametes.[3]

Proper evaluation of the ovarian reserve is of the utmost importance for the prognosis and treatment of infertile patients. The term "ovarian reserve" includes the women's primordial and growing follicles at a given time of life.[4,5] It is closely related to the woman's reproductive potential. The larger the number of remaining oocytes, the greater the chance of achieving a satisfactory number of embryos and pregnancies and vice versa. The reduced ovarian reserve is characterized by a low number of oocytes remaining in the ovary and decreased oogenesis due to age, congenital, iatrogenic or idiopathic factors. Ovarian aging or ovarian reserve depletion is an individual process for each woman, but usually begins after 30 and ends up to about 50-51 years of age.[6]

The treatment of infertility in patients with low ovarian reserve is a challenge for reproductive medicine specialists both from an obstetric-gynaecological and biological point of view. The results of *in vitro* fertilization treatment procedures in these patients are associated with a small number of follicles after COH, a small number of oocytes aspirated usually with poor morphological features, a lower fertility rate, fewer and low-quality embryos obtained and low percentage of clinical pregnancies, respectively.[7]

Our study investigates the number and rates of IVF/ICSI preimplantation embryo procedures in patients with low ovarian response, and the impact that major process-related factors exert on them.

Materials and methods

The current study presents the results of a retrospective and prospective analysis of 147 MII oocytes derived from 72 patients with low ovarian reserve who participated in an infertility treatment program through *in vitro* fertilization from February 2011 until February 2014 in "Vita" Multidisciplinary Hospital for Active Treatment - Sofia, First Specialized Obstetrics and Gynaecology Hospital for Active Treatment "St. Sofia" - Sofia and Medical Center for Assisted Reproduction "Varna" - Varna, Bulgaria. As a control group to compare the results 2483 MII oocytes derived from 347

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patients with normal ovarian reserve were used. As patients with diminished ovarian reserve were diagnosed those meeting at least two of the following criteria: FSH levels on the third day of menstrual cycle (MC) >10 mIU/ml, AMH levels <1.2 ng/ml, antral follicle number ≤7, age ≥40 years, previous poor response to COH.[7]

Results and discussion

Concerning the number of embryos obtained in the two groups of patients, our study found a statistically significant difference (p<0.0001) at an average number of 1.56±0.93 for patients with low and 5.97±3.44 for patients with normal ovarian reserve (Mann–Whitney test). When compared by age group, where applicable, a statistically significant difference was not found in the first age subgroup (p>0.05) but was found in the second and third subgroups (p<0.0001) (Table 1).

Regarding the number of embryos obtained in patients with reduced ovarian reserve, a statistically significant difference between the second and fourth age subgroups was found, at an average of 2.19±1.11 and 1.16±0.83, respectively, with a tendency to decrease the number of the embryos obtained with age increasing (Kruskal-Wallis test and Dunn's Multiple Comparison Test).

Regression analysis was also applied in order to establish a relationship and if there is such to find how strong it is between: 1) the number of zygotes obtained from oocytes with normal morphology and the number of embryos obtained, and 2) the number of zygotes obtained from abnormal oocytes and the number of embryos obtained. Data from the analysis are presented in the table below:

The number of embryos obtained in patients with low ovarian reserve is expected to be lower than that in the control group due to the fact that the number of oocytes available for fertilization in this group is lower -2,56±1,39 and 8,22±4,72 for low and normal responders, respectively. For this reason, the percentages of the embryos obtained in both groups were calculated, and the statistical processing of the data was continued precisely by the percentage of embryos obtained, not by their number.

Regarding the percentage of embryos obtained, Fisher's exact criterion showed a statistically significant difference between the two groups of patients (p<0.05) at 76.19% and 83.49% for the studied and the control group, respectively. However, when compared by age subgroup, where applicable, the above difference did not occur in any of the three age subgroups compared in the examined population sample (p>0.05). The presented in Table 2 data show a tendency to decrease the percentage of embryos obtained with age increasing in patients with reduced ovarian reserve, but when comparing the results among the individual age subgroups, Fisher's exact criterion showed no statistically significant dependence in the population sample (p>0.05). The non-parametric Spearman's correlation coefficient (-0,104) in this case is statistically insignificant and therefore cannot be interpreted (p=0,386).

There was not found a linear correlation between the hormonal status of patients with reduced ovarian reserve and the percentage of embryos obtained. Such a weak but statistically significant and convincing negative dependence was only found between the number of embryos obtained and the AMH levels (correlation coefficient -0.251).

	Age and number of embryos obtained
Level of significance of the F-criterion	**< 0,0001** This value is less than 0.05, indicating that the selected regression model is adequate.
Correlation coefficient	**0.474** This means that the relationship between the two changes is moderate and one-way.
Determination coefficient	**0.224** This means that 32% of changes in the number of embryos obtained are due to different ages.
Standardized beta coefficient	**-0,474** This means that when the age factor is increased by 1, the number of embryos obtained will decrease by 0.474.

	Number of zygotes obtained from oocytes with normal morphology and number of embryos obtained	Number of zygotes obtained from oocytes with defects and number of embryos obtained
Level of significance of the F-criterion	**< 0,0001** This value is less than 0.05, indicating that the selected regression model is adequate.	**< 0,001** This value is less than 0.05, indicating that the selected regression model is adequate.
Correlation coefficient	**0.717223** This means that the relationship between the two variables is strong and unidirectional - as the number of zygotes obtained from normal oocytes increases, the number of embryos increases.	**0.392089** This means that the relationship between the two variables is weak and unidirectional - with the increase in the number of zygotes produced by oocytes with defects, the number of embryos increases.
Determination coefficient	**0.514409** This means that 51.4% of changes in the number of embryos are due to the number of zygotes obtained from oocytes with a normal morphology.	**0.153733** This means that 15.4% of changes in the number of embryos are due to the number of zygotes obtained from oocytes with defects.
Standardized beta coefficient	**0.717223** This means that when the number of zygotes produced by normal oocytes is increased by 1, the number of embryos will increase by 0.717.	**0.392089** This means that when the number of zygotes fertilized by oocytes with defects is increased by 1, the number of embryos will increase by 0.392.

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The Kruskal-Wallis test showed no statistically significant difference between the percentage of embryos obtained depending on the stimulation protocol applied (p>0.05) in the examined group of patients -75.96% for Microdose "flare up" protocol, 81.99%-Short antagonist protocol, 81.82%-Mild stimulation and 71.43%-natural cycle IVF/ICSI procedure.

No statistically significant dependence (p>0.05) between the insemination method and the percentage of embryos obtained in patients with low ovarian reserve was found -79.31% for ICSI and 77.76% for IVF, respectively.

Based on the morphological features of the aspirated oocytes, the patients were divided into three subgroups: 1) only with normal oocytes, 2) with normal and defective oocytes and 3) only with abnormal oocytes. The results obtained are presented in Table 3. No statistically significant difference in the percentage of embryos obtained in the three subgroups of patients was found (p> 0.05).

Table 1 Summarized statistical data of the number of embryos obtained from patients with low and normal ovarian reserve

Group	Age subgroups	Number of patients	Mean number of embryos	Standard deviation	Median
0 Normal responders	1 (<30 years)	87	5,79	3,35	6,00
	2 (31–35 years)	200	6,46	3,54	7,00
	3 (36–40 years)	60	4,62	2,92	4,00
	Total	347	5,97	3,44	6,00
1 Low responders	1 (<30 years)	3	2,00	1,00	2,00
	2 (31–35 years)	16	2,19	1,11	2,00
	3 (36–40years)	31	1,52	0,72	2,00
	4 (41–45 years)	19	1,16	0,83	1,00
	5 (>45years)	3	0,67	0,58	1,00
	Total	72	1,56	0,93	1,00

Table 2 Summarized statistical data of embryos obtained in patients with low and normal ovarian reserve

Age subgroups	Group	Number of inseminated oocytes	Number of obtained embryos	Percentage of obtained embryos	p - value
1 (<30 years)	0 Normal responders	591	504	85,28	>0,05
	1 Low responders	7	6	85,71	
2 (31–35 years)	0 Normal responders	1540	1292	83,90	>0,05
	1 Low responders	45	35	77,78	
3 (36–40 years)	0 Normal responders	352	277	78,69	>0,05
	1 Low responders	61	47	77,05	
4 (41–45 years)	1 Low responders	31	22	70,97	
5 (>45 years)	1 Low responders	3	2	66,67	
Total	0 Normal responders	2483	2073	83,49	<0,005
	1 Low responders	147	112	76,19	

Table 3 Percentage of embryos obtained depending on the oocyte quality in patients with poor ovarian response

	Types of oocytes		
	1 Normal only n=10	2 Normal and defective oocytes n=26	3 Abnormal only n=36
Percentage of embryos obtained	86.67	71.95	80

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Conclusion

Our study of 72 cycles of *in vitro* fertilization in patients with diminished ovarian reserve found and confirmed the literature data[8,9] of a tendency to reduce both the number and the percentage of embryos with increasing patients age. The regression analysis performed showed that the number of embryos was significantly affected by the quality of the oocytes from which they derived in favor of better results when a higher number of oocytes with normal morphology are available. Regarding the influence of patients hormonal status, a weak but statistically significant and persuasive relationship was found only between the number of embryos received and the AMH levels, confirming its reliable role as a predictive marker for the outcome of an assisted reproductive procedure.[10] The selection of stimulation protocol could slightly affect the number of oocytes and embryos obtained in this contingent of patients, but our study did not detect a statistically significant correlation between the percentage of embryos obtained and the COH protocol. Despite the practice of mass application of ICSI in patients with diminished ovarian reserve, our results did not establish a statistically significant correlation between the percentage of embryos obtained and the oocyte insemination method, and therefore reject the need of doing ICSI out of the cases with male factor of infertility.

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Conflict of interest

The author declares no conflict of interest.

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