DECLINE IN FEMALE FERTILITY AFTER 40 YEARS

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OPADANJE FERTILNOSTI KOD ŽENA STARIJIH OD 40 GODINA

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ABSTRACT

Important factor related to the conception possibility is women age. The decline in fertility with aging is proven and evident in literature. Infertility is increasing and many couples seek help in advanced techniques such as IVF (in vitro fertilization) in order to overcome the problem caused by aging, but the quality of the oocytes is a significant limiting factor. With the aging the quantity and quality of oocytes decreases, such as the quality of the embryo after fertilization. The accelerated rhythm of life, liberty and women inclusion in all kinds of professions brought many benefits to women, but also increasingly postponing births. Each person is unique individual, and can be more or less fertile compared to the average at same age. Unfortunately, some women has a rapid decline in fertility - accelerate aging, very early, already in the early twenties and when testing them with different methods and exams, the result is very low number of oocytes, low value of anti-Müllerian hormone and also very poor quality of these oocytes, or low ovarian reserve. The problem is that when you have accelerate aging, even IVF techniques can not be of great help in achieving pregnancy. The pregnancy rate (17,65%) and the childbirth rate (5,88%) with the patients older than 40 is very low, although comparable to the data from the scientific literature and speaks in favour of the fact that the success of assisted reproductive techniques is very modest with women older than 44.

Keywords: *Infertility, anti-Müllerian hormone; antral follicle count; ovarian reserve*

SAŽETAK

Starost žene je važan faktor kada je u pitanju mogućnost začeća. Opadanje fertilnosti sa starenjem je dokazano i očigledno u našem društvu. Mnogi parovi traže pomoć u naprednim tehnikama kao što su IVF (in vitro fertilizacija), da bi prevazišli problem nastao zbog starenja. Ipak kvalitet jajne ćelije je značajan limitirajući faktor.

Pravilnije je reći da sa starenjem žene opada kvantitet i kvalitet jajnih ćelija, što dalje vodi do lošeg kvaliteta embriona nakon fertilizacije. Ubrzani tempo života, sloboda žene i uključivanje u sve vrste profesija doneli su mnoge prednosti ženama, ali i sve češće odlaganje rađanja. Svaka individua je jedinstvena, i može biti manje ili više fertilna u poređenju sa prosekom i njenim godinama starosti. Mada je retko, moguće je da neka osoba ima brzo opadanje kvaliteta jajnih ćelija (ubrzano starenje) veoma rano, već u ranim dvadesetim godinama života i prilikom izvođenja različitih testova i pregleda možemo utvrditi veoma mali broj oocita u jajnicima, nisku vrednost anti-Müllerian hormona a takođe i loš kvalitet tih jajnih ćelija tj, lošu ovarijalnu rezervu. Naime, kada postoji ubrzano reproduktivno starenje, čak ni primena IVF tehnika ne može biti od velike pomoći u postizanju trudnoće. Stopa trudnoća i stopa porođaja kod pacijentkinja starijih od 40 godina je veoma niska (stopa trudnoća 17,65%, stopa porođaja 5,88%), mada komparabilna sa podacima iz literature i govori u prilog tome da je uspeh asistiranih reproduktivnih tehnika veoma skroman kod žena starijih od 44 godine.

Ključne reči: *Infertilitet, anti-Müllerian hormon; broj antralnih folikula; ovarijalna rezerva*

ABBREVIATIONS

FSH- follicle stimulating hormone,
E2-estradiol,
AMH- anti-Müllerian hormone,
AFC-antral follicles count,
IVF- in vitro fertilization,
ART-assisted reproductive technology.

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INTRODUCTION

During the intrauterine development of the female fetus with the help of three simultaneous processes: mitosis, meiosis and oogonial atresia number of germ cells in the gonads in the 20th week of gestation reaches 6-7 million, out of which about two thirds are intrameiotic primary oocytes. From that moment and on, processes of irreversible atresia are performing that reduce the number of germ cells in the gonads predominantly through follicular (oogonial) atresia, which occurs around the 6th lunar month, and continues during the reproductive age (1, 2). In human fetuses atresia generally occurs via apoptosis in oocytes until in adult age mainly through apoptosis of granulosa cells. It takes place during each stage of folliculogenesis, but it is more common in follicles that have reached a size that could be selected for ovulation (3). Thus, some of 300000 to 400000 of follicles are in the rest period at menarche, only about 500 follicles will ovulate, and the rest are subjected to atresion. Promptness of ovarian atresia is not constant and can be said to have a biexponential shape, with the changes that primarily determine the number of remaining oocytes in the ovaries, and less – women`s age. It is normal to register a significant decline in the number of ovarian follicles after 37-38 years, when the total number of remaining follicles is approximately 25 000 (4).

Based on numerous studies conducted by reproductive biologists, optimal fertility period is between 18 and 31 years (5). Several theories was formulated to explain the decline in the oocyte quality with aging. Hypothesis "production line" indicates that the quality of oocytes is established during fetal life and oocytes that are less susceptible to errors, for example - non-disjunction during meiotic division, or, as we can say, better quality oocytes ovulate first, leaving those of lower quality to ovulate later in life (6). For this reason, frequent "reproductive errors" might be more likely consequence of the reduced number of remaining ovarian follicles than women`s age.

With ageing the risk of non-separation during metaphase II of the second meiotic division increases, and this phenomenon can be explained by the accumulation of errors in the genome as a result of compromised function of granulosa cells: defective microcirculation around the biggest follicle which leads to the reduction of oxygen levels in follicular fluid, or a gradual increase in intracellular oxidative stress (7).

In older women meiotic spindles during metaphase II (the second meiotic division) are diffuse, show a lack of bipolarity and the chromosomes are not clearly arranged along the equator of the spindle but are loosely holding for the spindle and are in different locations along the spindle (8). This less regulated localization of chromosomes in oocytes of older women can be a cause of problems encountered during the separation and segregation of chromosomes. So we get embryos with a high percentage of chromosomal abnormalities that contain all possible combinations of monosomies or trisomies (9). Monosomies in conceptus are present in the same percentage as the trisomies, but these embryos are lost in a much earlier stage of pregnancy and almost never come to terms. Thus, the incidence of genetic abnormalities is much greater than we can imagine, and certainly grows with the age of a woman as much as a number of miscarriages in advanced age (10).

Women who are approaching 40 years of age, may be diagnosed in most cases an increase in basal serum FSH (FSH measured by the 2nd or 3rd day of the menstrual cycle) and this value directly indicate a decrease in the number of antral follicles capable of ovulating (11). This increase FSH levels usually starts about a decade or more before the onset of menopause. Jump FSH values occurs as a result of the negative feedback FSH-modulating ovarian proteins, mainly inhibin A and inhibin B. Since the Inhibin B (dimeric polypeptide secreted by ovarian granulosa cells) is predominantly secreted in the early antral follicles, decrease in the value of inhibin B in serum directly indicates a decrease in antral follicles "pull" in the ovaries (12).

The increase in serum FSH may be also independent of the age (13). In patients with this problem it can certainly be established poorer ovarian response after controlled ovarian hyperstimulation with fewer obtained oocytes similarly as in older women. Also a large number of studies and expert opinions are still divided as to whether ovaries will respond better to stimulation in younger women with elevated FSH and low values of inhibin B or ovaries of older women with normal FSH and inhibin B. However, in most cases, we can conclude that the ovarian reserve is a better predictor of oocyte production capacity or the number of oocytes obtained after ovarian hyperstimulation, and patient's age is a better predictor of the quality of obtained oocytes (14, 15). We should not forget that there are patients with mutated FSH receptor in which the amino acid asparagine in the receptors protein is replaced with serine at position 680, and this change leads to a conformational change in FSH receptor which is now much less active and require much higher levels of FSH hormone for normal functioning. In these cases, serum FSH levels certainly are not a reflection of accelerated reproductive aging (16, 17, 18).

Implantation of the embryo-blastocyst is a complex process with a lot of interaction between the blastocyst and endometrium, or the embryo and the endometrium, but to be successful requires two conditions: a vital embryo and receptive endometrium(window of implantation) (19, 20, 21). Another important factor affecting fertility in the advanced age is abnormal endometrial receptivity, and it is predominantly a result of the reduced number of progesterone receptors, as a result of reduced levels of estrogen receptors(22, 23).

With aging the possibility of the occurrence of many pathological changes in the endometrium that affect its receptivity increases and these changes include endometrial polyps, fibroids, adhesions, endometriosis and hydrosalpinges (24, 25, 26, 27, 28).

Expression of estrogen receptors (ER) at the implantation time has important role in endometrial receptivity and

in healthy women estrogen receptors are down regulated at that moment, but in women with endometriosis, there is up regulation of ER, which leads to low pregnancy rate (29, 30). Endometrial thickness measured by ultrasound, more than 9-10mm, is associated with significantly higher pregnancy rates relative to thickness below 6mm, usually seen in older patients with specific hormonal status (31).

Treatment of impaired endometrial receptivity is still in focus of many studies and some of them propose endometrial gene therapy, some endometrial stimulation using local injury, or endometrium priming with instillation of granulocyte colony-stimulating factor, piroxicam and human chorionic gonadotropin (hCG) (32, 33).

Quantity of remaining oocytes in the ovaries or ovarian reserve testing, probably has not great influence on natural fertility (if a woman trying to get pregnant the natural way). However, if a woman undergoes in vitro fertilization techniques, this parameter has a lot of influence in terms of ovarian response to drugs used to stimulate ovulation (34).

The more the number of remaining oocytes in the ovaries, the more oocytes may be obtained from the stimulated cycle, and it gives a greater chance for the realization of successful pregnancy.

Thus the reproductive age is best defined by ovarian reserve, the functional ovary potential at one moment, or the current number of follicles in the ovary that are able to ovulate. Before starting ART procedures, ovarian reserve of each patient can be determined:

1. ultrasonography-measuring the number of antral follicles (AFC-antral follicle count), ovarian volume and blood flow through the ovaries,
2. determining basal hormone levels 2nd or 3rd day of the menstrual cycle: FSH, LH, estradiol-E2, AMH (antiMullerian hormone), inhibin B, inhibin A, P-progesterone, FSH: LH ratio, P: E2 ratio, testosteon -T, VEGF (vascular endothelial growth factor), IGF 1 (insulin-like growth factor), IGF-BP I (insulin-like growth factor-binding protein) (35)
3. by implementation of the ovarian stimulatory tests, such as: CCCT (clomiphene citrate challenge test), EFORT-test (exogenous FSH ovarian reserve test) and GAST (gonadotropin agonist stimulation test) (35, 36).

The aim of these tests is to precisely predict the potential success for the patient, but to warn patients with poor ovarian reserve parameters on the likely poor outcome and all of that before starting IVF treatment (37, 38).

AMH is a dimeric glycoprotein hormone which belongs to the TGF family (transforming growth factor) - and is produced by the granulosa cells of ovarian follicles. In the literature it is also called MIS or Mullerian inhibiting substance and it was first investigated in sexual differentiation of male during embryogenesis (39).

It is first produced in the primary follicles which are formed from primordial follicles. At this stage, the follicles are microscopic and can not be visualized by ultrasound. The maximum production of AMH is in preantral and small antral follicles (smaller than 4 mm in diameter). AMH production decreases and then stops as the follicle grows, so that there is almost no AMH production in follicle diameter greater than 8mm (40). Thus, AMH levels are constant, can be determined any day of the menstrual cycle and are used to estimate the pull of growing follicles in women. Size of the pull, or the quantity of growing follicles is closely dependent on the number of remaining primordial follicles, and thus the value of AMH reflects the amount of remaining oocytes, or the size of the ovarian reserve (41).

According to many researchers, AMH can be considered to be a marker for the ovarian ageing process (42). AMH values are a good predictor of the IVF outcome, so the higher concentrations of AMH are associated with a greater number of mature oocytes obtained after stimulation, consequently, a large number of embryos, and finally a higher percentage of clinical pregnancies (43, 44, 45, 46).

Interesting is fact that AMH levels are reduced in smokers or women who had consumed long term hormonal contraception (almost 30% lower values than in controls) (47, 48, 49).

Measuring the ovary volume and antral follicle count (AFC) with ultrasound is currently one of the best methods for assessing ovarian reserve. Antral follicles are small follicles with a diameter of 2-8mm, which can be seen, measured and counted by ultrasound. Using a vaginal probe is certainly the method of choice, and measurement of the antral follicle count give us indirect information of the relative number of microscopic primordial follicles remaining in the ovary, which can potentially develop in the future. Number of antral follicles is a good predictor of the number of mature oocytes that can be obtained in stimulated cycle and the number of oocytes obtained directly correlates with the success of IVF (50). By measuring the number of antral follicles at the beginning of the stimulated cycle we can predict the response after controlled ovarian stimulation.

In patient selection prior to IVF it is necessary to determine both complementary factors: AMH provides information about number of very small, non-atretic follicles, and AFC provide information about follicle sizes and discrepancies in follicle size (51).

Measuring the volume of the ovary by ultrasound is also a reliable indicator of ovarian reserve. It is already well known fact that decreased volume of the ovaries is associated with advanced age. For women with ovarian volume less than 3 ml, we can say that there is a high risk of poor response to controlled ovarian stimulation during IVF, and high rate cancellation of cycle (52, 53).

Many studies suggest that about 13 years before menopause, ovary begin to show signs of accelerated aging, even if there are regular menstrual cycles (54, 49).

Therefore, it is useful to determine whether this physiological signals has started. But there is no gold standard for this testing, so assessment of ovarian reserve is possible using many laboratory hormone levels measurements and

stimulatory tests (55), by measuring mean LH, amplitude of LH and LH response to GnRH (56, 57).

Clomiphene test (or Clomiphene challenge test) is a dynamic type of testing that can detect some cases of decreased ovarian reserve, which still show normal levels of FSH day 3 of the menstrual cycle.

EFORT-test (exogenous FSH ovarian reserve test) and GAST test (Gonadotrophin analogue stimulating test- or ovarian response to the use of GnRH agonists) is type of testing usually used for prediction of patients response during ovarian stimulation prior to IVF. It is effective and simple method for determing good and poor responders in IVF (58, 59, 60, 61).

The quality and quantity of oocytes decreases rapidly from 38 years of age.

From 44 years onwards, with the help of IVF techniques and using the woman own oocytes, the chance for pregnancy almost does not exist. In fact, if using oocytes of women older than 44 years, the chances of success are below 2% per attempt. Oocyte quality is passed on to the quality of the embryo, which is by far the most important factor that determines the IVF procedures success (62).

THE AIM OF WORK

The aim of this work is to determine the different methods of ART in the treatment of infertility with women at the age from 40 to 45 and the contribution to the development of the strategy for the successful outcome of IVF with the patients of this age.

PATIENTS AND METHODS

This study is conducted on KGA KCS, and the data are collected during the traetment of infertile female patients from the department for *In vitro fertiliztion* in the period from 2008 to 2011.Patients were included in the study according to certain criteria: the age of the patient, BMI, the cause of fertility, if there were previous ART procedures and which ones, the lenght of stimulation, the type of stimulating process, the number of aspirated follicle, the number of obtained oocytes, the number of embryos as well as the procedure of formation of embryos (ICSI or IVF), embryotransfer and the chracteristics of the returned embryos. The obtained data were statistically processesed by using the SPSS program package and represented both graphically and tabularly

a) The patients were divided into six groups according to their age (40, 41, 42, 43, 44, i 45). Apart from the basic hormonal status, the level of FSH hormone on the 2nd or 3rd day of the menstrual period was tested seperately.
b) Based on the used protocol of the controlled stimulation of the patient‘s ovary (agonists or anti-agonists GnRH and gonadotropins by short or long protocol) the results after the stimulation were followed and compared in terms of determining: the number of obtained oocytes and their quality (M II, M I, germinative vesicles -GV or the degenerated oocyte), the rate of fertilization, the success of embryotransfers and the number of returned embryos (with the review of the quality of embryos), the rate of pregnancies and the rate of childbirths.

Table 1: The characteristics of the cycle with embryotransfer with women older than 40.

parameter	value
Age	42,04±0,1
How many ampoules of gonadotropine were used	38,61±1
The number of obtained oocytes	4,87±0,1
% MII oocytes	83,5
% 2PN	56,22
The number of embryos in ET totally	118
% returned embryos	2,23

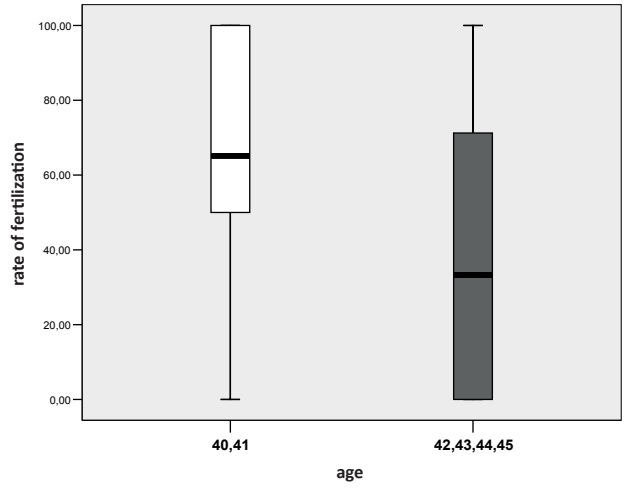
THE RESULTS

The outcome of ART technique with 68 patients of the age from 40 to 45 who were treated on KGA KCS during the period from 2008 to 2011 was analysed. Out of 68 analysed cycles of stimulation, the embryotransfer was done with 51 patients ie. in 75% of cases, and the cycle was cancelled – terminated with 17 patients (25%) for the following reasons: in 8 cycles there were no grown oocytes, in 6 there was no fertilization, in 2 there was a bad growth of embryos after the fertilization and one was cancelled due to extremely difficult embryotransfer so the embryos were frozen. (**Table 1.)**

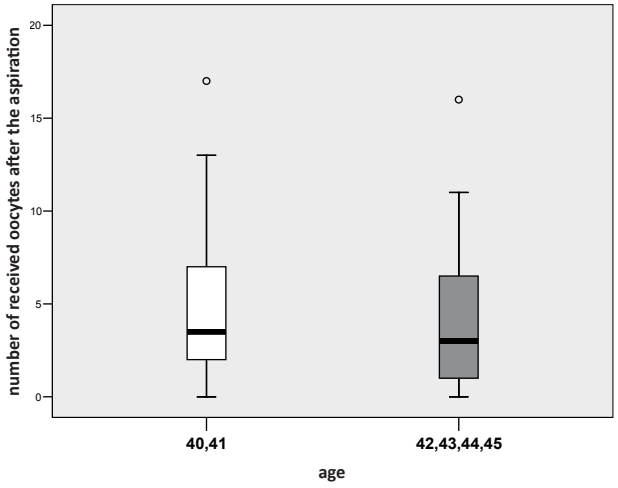
Out of 68 patients included in the research, for only 5 the clinical pregnancy was determined thereof one had ectopic pregnancy, with one patient the prenatal diagnostics that is the biopsy of chorionic cups (CVS) showed SY Down so the pregnancy was terminated and with one patient the pregnancy was cancelled before the term due to premature delivery in 8th lunar month. 51 embryotransfers were done, 5 embryos were returned with 2 patients, 4 embryos were returned with 7 patients, and 1 to 3 embryos were returned with all the others. Based on these data we have the rate of pregnancies per transfer in this research to be 17,65%, and the rate of childbirths per transfer 5,88%.

Considering the fact that the number of patients who belong to a certain age group (eg. 40, 41, 42 ...) is different, and in order to get a better interpretation of the statistical values, the patients were divided in two age groups: 1st group 40 and 41years old and 2nd group 42 and more. If we now consider the rate of fertilization of the obtained egg cells with the patients belonging to groups 1 and 2, we get a statistically significant difference. So there is a higher rate of fertilization with women aged 40 and 41 (median 65,16%) than with women older than 42 (median 33,33%) (Mann-Whitney U test (Z= -2,82, p=0,005<0,05) (**graph 1)**.

Graph 1



Graph 2



The statistical tests were done due to determining the normal distribution, and after that the non-parameter analises were done (Shapiro-Wilk).

Statistically there is a significant difference in the rate of fertilization with women aged 40 and 41 (median 65,16%) and with women aged 42-45 (median 33,33%).

If we analyse the success of the process of stimulating the ovulation ie analyse the number of received oocytes after the aspiration with the patients of these two age groups we come to the result that there is no significant difference: the group 40-41 (median 3,5) and the group 42-45 (median is 3). The analyses were done by Mann-Whitney U test (Z= -0,98, p=0,328>0,05) which showed that there is no statistically significant difference in the number of aspirated oocytes (**graph 2**)

The average values of the number of aspirated oocytes after the stimulation with the patients aged 40-41 (median is 3,5) and patients aged 42-45 (median is 3) are very similar.

If we want to prove more precisely the success of the applied ART techniques with the patients divided according to their age, it is necessary to analyse the percent of the obtained mature oocytes (MII) after the stimulation ie.one of the parameters that shows the quality of the egg cell.

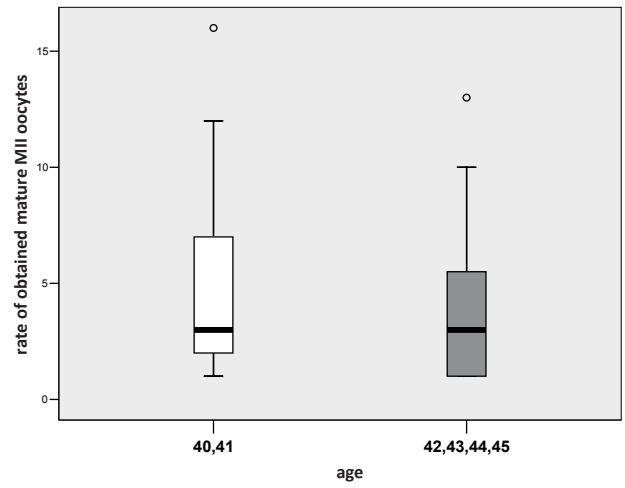
Mann-Whitney U test (Z= -0,776, p=0,438>0,05) was implemented which showed that there is no significan difference in the number of obtained mature MII oocytes with the patients aged 40-41 (median is 3) and patients aged 42-45 (median is 3) **(graph 3)**.

The implemented Mann-Whitney U test (Z= -0,776, p=0,438>0,05) did not show the significant differences in the number of obtained MII oocytes with the patients aged 40-41 and patients aged 42-45. Medians are the same and are 3.

By analysing the very procedure of stimulation, that is how many days of stimulation by gonadotropines are necessary to have a satisfyng result as well as the number of used ampoules of gonadotropinesthat were necessary for a successful stimulation , we get the results that show that there is no significant difference between these two age groups.

T test (t= -0,864, d=64,61, p=0,391>0,05) was implemented which showed that there is no statistically significant difference in the number of days of stimulation with the patients aged 40-41 (10,25±1,74) and patients aged 42-45 (10,61±1,70) **(Table 2), (Graph 4)**.

Graph 3



Graph 4

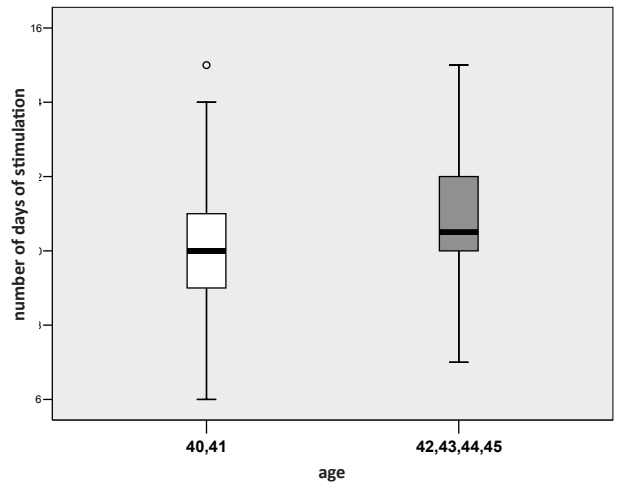


Table 2: The results of the t test which analysed the number of days of stimulation by gonadotropines related to the age of the patients in research – patients divided in two aged groups: 40-41 and 42-45.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
number of days of stimulation	Equal variances assumed	,096	,757	-,866	66	,390	-,361	,417	-1,194	,472
	Equal variances not assumed			-,864	64,613	,391	-,361	,418	-1,196	,473

The results of the t test which analysed the number of days of stimulation by gonadotropines related to the age of the patients in research – patients divided in two aged groups: 40-41 and 42-45.

T test (t= -0,864, d=64,61, p=0,391>0,05) was implemented which showed that there is no statistically significant difference in the number of days of stimulation with patients aged 40-41 (10,25±1,74) and patients aged 42-45 (10,61±1,70).

By analysing the data obtained after the stimulation of ovulation with the mentioned age groups of the patients, we get the results which show that there is no statistically significant difference in the number of used ampoules of gonadotropines for stimulation (p>0,05). The results were tested by Mann-Whitney U test (Z= -0,025, p=0,980>0,05) **(Table 3)** which showed that there is no significant difference in the number of used ampoules of gonadotropines with patients aged 40-41 (median is 36) and patients aged 42-45 (median is 35,5) **(Graph 5)**.

There is no statistically significant difference in the number of used ampoules of gonadotropines with patients aged 40-41 (median is 36) and patients aged 42-45 (median is 35.5)

By analysing the number of obtained embrios per each cycle with the patients older than 40 (divided into the same categories ie 40-41 and 42-45), we get the statistically significant difference, ie there is a better result of IVF tehniques with the younger patients than with the older ones, which corresponds to almost all literary data.

The statistical significance was determined by applying the Mann-Whitney U test. The test results are (Z= -2,140, p=0,032<0,05) **(Table 4)** and they point out the significant differences in the number of obtained embrios with the pa-

Test Statistics[a]

	number of ampoules
Mann-Whitney U	574,000
Wilcoxon W	1102,000
Z	-,025
Asymp. Sig. (2-tailed)	,980

Grouping Variable: age22

Table 3: The results of the statistical analyses of Mann-Whitney U test (p=0,980>0,05)

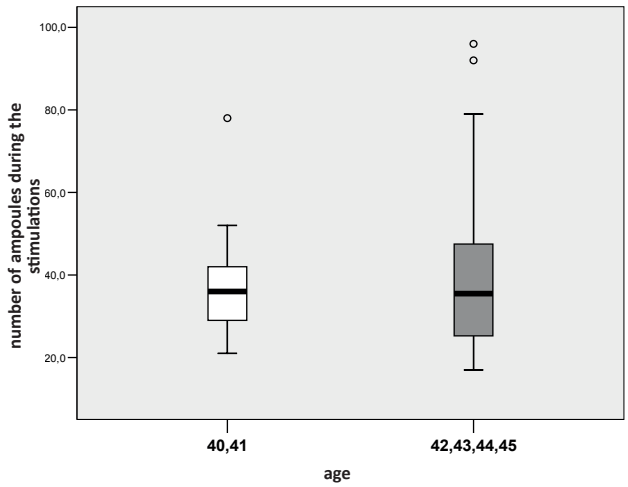
Test Statistics[a]

	number of embryos
Mann-Whitney U	320,000
Wilcoxon W	816,000
Z	-2,140
Asymp. Sig. (2-tailed)	,032

Grouping Variable: age22

Table 4: The statistical significance p=0,032<0,05 in the number of obtained embrios with patients of two age groups.

Graph 5



Graph 6

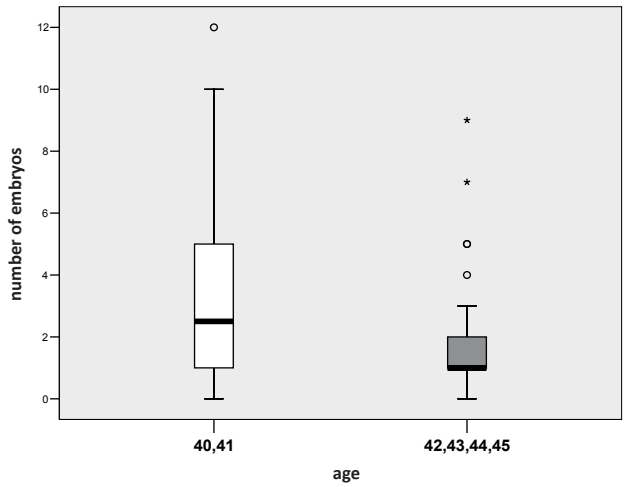


Table 5: The corellation tests which show the statistically significant negative corellation between the patients'age and the number of embryos.

Correlations

			starost	stopa_ fertilizacije	broj_ embriona
Spearman's rho	age	Correlation Coefficient	1,000	-,232	-,258*
		Sig. (2-tailed)	.	,057	,045
		N	68	68	61
	rate of fertilization	Correlation Coefficient	-,232	1,000	,467**
		Sig. (2-tailed)	,057	.	,000
		N	68	68	61
	number of embryos	Correlation Coefficient	-,258*	,467**	1,000
		Sig. (2-tailed)	,045	,000	.
		N	61	61	61

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

tients aged 40-41 (median is 2,5) and patients aged 42-45 (median is 1) **(Graph 6)**.

The median of the number of obtained embrios with patients aged 40-41 is 2.5, and with the patients aged 42-45 the median is 1 – statistically significant difference.

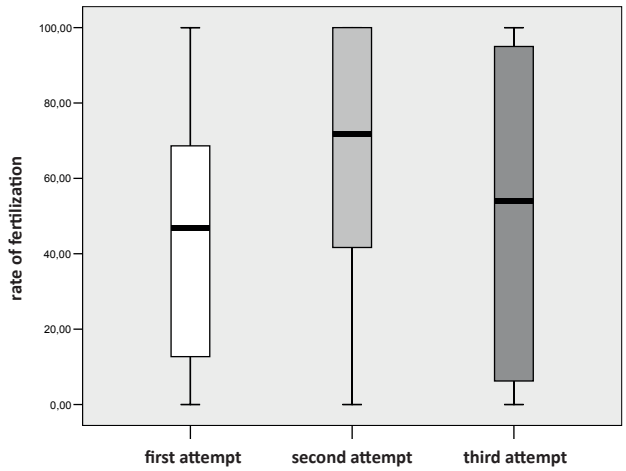
If we examine the obtained result by corellation tests, we also get the statistically significant negative corellation between the number of obtained embrios after the applied ART techniques and the age of the patients, ie the older group of patients the less number of embrios (Spearmans'rho = -0,258, p=0,045<0,05).

If we apply the corellation tests on the fertilization rate and the patients'age, we get the values which are near the significance (Spearmans'rho = -0,232, p=0,057>0,05). The results of the tests are shown in **Table 5.**

If we use the statistical analyses to test the fertilization rate related to the fact that this is the first attempt of in vitro fertilization or second, third or more, we obtain the result which points out that there is no significant difference (χ^2=2,505, d=2, p=0,286>0,05). **(Graph 7).**

Kruskal-Wallis test was implemented (χ^2=2,505, d=2, p=0,286>0,05) which showed that there is no significant difference in the fertilization rate with the patients with the first attempt (median is 46.88%) , second attempt (median is 71.82%) or more than two fertilization attempts (median is 53.98%).

Graph 7



DISCUSSION

The results of this clinical study point out that the characteristics and outcomes with the women aged 42 and older are different and mostly much worse comparing to the women aged 40 and 41 and they mostly match the majority of data available in the literature. The thing that makes the interpretation of the obtained results difficult is the relatively small number of patients involved in the research comparing to the literary data and uneven number of patients in each age group (40, 41, 42, 43, 44 and 45) so in order to obtain the decisive results during the statistical testing we divided the patients into two age groups 40 and 41 years old and 42-45 years old.

The rate of pregnancies per transfer in this study is 17.65%, as is shown in majority of studies which examined the success of ART techniques with women older than 40 where results vary from 11.6 % to 18.2 % (63, 64, 65). The rate of childbirths per transfer in this study is 5.88% whereas in the scientific literature it is 7-8.5% (66, 67). There were no multiple pregnancies and clinical pregnacies with the patients older than 43. The data from this study confirm the so far conclusions from the literature that the fertilization rate with the patients of younger age is significantly higher comparing to the older patients (in this case the statistically significant difference in the fertilization rate between age groups of 40-41 and 42-45).

However if we analyse the very process of stimulation of ovulation ie whether the short or long protocole is used, the number of days of stimulation as well as the number of ampoules of gonadotropines used for adequate stimulation of ovulation with these patients, there is a difference, but not statistically significant difference. We also do not find the significance by analysing the aspirated oocytes in these two age categories or

the number of mature MII oocytes in the total number of aspirated oocytes related to the age. All these data speak in favour taht it is possible to have a satisfying result with older patients after the stimulation in terms of good reaction of ovary on the applied stimulation protocole but the further outcome of the procedure, ie whether the clinical pregnancy and the childbirth will happen or not depend much more from the quality of the egg cell which is weakened in the older age, and which is confirmed by this study.

The statistically significan difference is also confirmed in the number of obtained embrios with women aged 40-41 and 42-45which is also proved in majority of cases in similar studies that involved much bigger number of examined patients (63).

When we analyse the success of ART techniques with women of older age, in many countries the number of embryos taht are returned by transfer is not limited (68). In this study, only one patient had 5 embryos returned, and 7 patients had 4 embryos each, and the rest 42 patients 1-3 embryos. There is no significance in the pregnancy rate or childbirths per transfer related to the number of returned embryos. Similar results were obtained in the majoruty of other recent studies (63, 64, 68, 69).

CONCLUSION

With the patients aged 40-41 we can expect relatively good response of the ovary after the stimulation by gonadotropine in terms of the number of obtained oocytes and the number of mature MII oocytes depending on the level of FSH and AMH hormones. However the successful outcome of ART techniques mainly depends on not only the number but the quality of obtained egg cells which definitely recedes with ageing.

The pregnancy rate and the childbirth rate with the patients older than 40 is very low, although comparable to the data from the scientific literature and speaks in favour of the fact that the success of ART techniques is very modest with women older than 44.

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