PRACE ORYGINALNE

niepłodność

Reproductive outcomes of intracytoplasmic sperm injection (ICSI) in good-prognosis patients who electively decided to limit the number of oocytes used for microinjection: a two-center comparative study

Ograniczenie liczby komórek jajowych wykorzystanych w docytoplazmatycznej iniekcji plemnika (ICSI) nie pogarsza wyników leczenia metodą zapłodnienia pozaustrojowego: dwuośrodkowe badanie porównawcze

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Abstract

Objectives: *The aim of the study was to compare the outcomes of intracytoplasmic sperm injection/embryo transfer (ICSI/ET) between two IVF centers with similar pregnancy rates and embryo transfer policy but with two different approaches to good-prognosis patients who intentionally chose to limit the number of oocytes used for ICSI.*

Material and methods: *It was a retrospective two-center comparative study. A total of 218 patients after successful retrieval of >10 mature oocytes following ovarian hyperstimulation were included in the study. The number of fertilized oocytes used during ICSI/ET was limited to 6 and 10 in 108 and 110 patients of the Centre for Reproductive Medicine KRIOBANK and VitroLive Fertility Clinic, respectively.*

Results: *No significant differences in the implantation rate (29.93% vs. 29.54%; p=0.94) and ongoing pregnancy rate (39.81% vs. 45.45%, p=0.40) were observed between patients who electively fertilized 6 as compared to 10 oocytes, respectively. However, in patients who deliberately limited the number of fertilized oocytes to 6 the following were observed: i) significantly fewer embryos available for ET (2.89±1.23 vs. 3.77±1.48, p<0.01); ii) considerably lower number of frozen embryos per cycle (1.05±1.30 vs. 2.00±1.67, p<0.01), and iii) lower rates of cycles with embryo cryopreservation (47.22% vs. 72.72%, p<0.01) as compared to patients with 10 fertilized oocytes.*

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Conclusions: *Elective fertilization of 6 vs. 10 oocytes does not adversely affect fresh ICSI/ET outcome in normal -responding patients. Restricted number of oocytes used for ICSI/ET may be a favorable alternative for couples who do not wish to cryopreserve surplus human embryos.*

Key words: **avoiding surplus embryos** / **cryopreservation** / **ICSI** / / **limited fertilization of oocytes** / **oocyte quality** / **oocyte numbers** /

Streszczenie

Cel: *Celem pracy było porównanie wyników leczenia metodą zapłodnienia pozaustrojowego z zastosowaniem docytoplazmatycznej iniekcji plemnika (ICSI) w dwóch ośrodkach leczenia niepłodności, które charakteryzują się zbliżonym odsetkiem uzyskiwanych ciąż oraz strategią transferu zarodków, ale różnią się w zakresie ograniczenia liczby komórek jajowych u par, które nie chcą wykorzystać wszystkich oocytów do mikroiniekcji.*

Metodyka: *Do badania zakwalifikowano 218 pacjentek, u których w trakcie punkcji jajników uzyskano ponad 10 komórek jajowych. U 108 kobiet leczonych w Centrum Leczenia Niepłodności Małżeńskiej Kriobank liczbę oocytów użytych do ICSI ograniczono do 6, u 110 kobiet leczonych w Centrum Ginekologii i Leczenia Niepłodności VitroLive liczbę oocytów ograniczono do 10.*

Wyniki: *W grupie pacjentek, u których do ICSI wykorzystano 6 oocytów, w porównaniu do tych u których użyto 10, stwierdzono niższą liczbę zarodków dostępnych do transferu (2,89±1,23 vs. 3,77±1.48, p<0,01), mniejszą liczbę zarodków poddanych mrożeniu na cykl (1,05±1,30 vs. 2,00±1,67, p<0,01), a także istotnie niższy odsetek cykli z krioprezerwacją zarodków (47,22% vs. 72,72%, p<0.01). Nie stwierdzono różnic pomiędzy dwiema badanymi grupami (6 vs. 10 oocytów) w zakresie wskaźnika implantacji (odpowiednio 29,93% vs. 29,54%; p=0,94) oraz odsetka ciąż klinicznych (odpowiednio 39,81% vs. 45,45%, p=0,40).*

Wnioski: *Uzyskane wyniki pokazują, że w grupie pacjentek dobrze odpowiadających na stymulację ograniczenie liczby wykorzystanych komórek jajowych nie pogarsza wyników w świeżym cyklu ICSI. Zredukowanie liczby oocytów użytych do ICSI może stanowić rozwiązanie dla par, które chcą uniknąć mrożenia dodatkowych zarodków uzyskanych w wyniku zapłodnienia pozaustrojowego.*

Słowa kluczowe: **nadliczbowe zarodki** / **krioprezerwacja zarodków** / **ICSI** / / **zapłodnienie ograniczonej liczby oocytów** / **jakość oocytu** / **liczba oocytów** /

Introduction

Several studies aimed to estimate the optimal number of oocytes in *in vitro* fertilization (IVF) [1-3]. The results indicate that retrieving fewer than 6-8 oocytes per cycle significantly reduces both, pregnancy rate (PR) and live birth rate (LBR) [4-5]. On the other hand, a high number of oocytes (over 18-20) is associated with an elevated risk of ovarian hyperstimulation syndrome (OHSS) [6-10]. Introduction of mild ovarian stimulation, whose aim was to obtain <8 oocytes, was associated with reduced risk of OHSS [5, 7, 11]. Additional benefits resulting from mild stimulation included higher competence of oocytes and embryo potential, and better endometrial receptivity [5, 12-14]. Recently, the results of a large retrospective study involving 400 135 IVF cycles published by Sunkara et al., showed a strong correlation between the number of oocytes and LBR rate, which rose with an increasing number of oocytes (up to 15), plateaued at the similar level with the number of 15-20, and progressively declined above 20 oocytes [15]. Also, Ji et al., in another large retrospective cohort study of 2455 patients, correlated the number of retrieved oocytes and LBR following the first IVF treatment cycle [16]. According to these authors, LBR after fresh transfer was the highest in the groups with 6–10 or 11–15 oocytes and reduced in the groups with 0–5 or >15 oocytes. Despite the fact that Ji et al., suggest the optimal number of oocytes for achieving a live birth to range between 6 and 15, the cumulative LBR after including frozen embryo transfer cycles increased along with the ovarian response.

The question whether the optimal number of oocytes is between 6 and 15 remains open. Also, the choice of an appropriate strategy to optimize the results of IVF in couples with high number of eggs (10-15 oocytes) who wanted to avoid creating surplus human embryos remains the topic of much heated debate. The aim of our study was to compare the results of intracytoplasmic sperm injection/embryo transfer (ICSI/ET) cycle outcome between two infertility centers with similar pregnancy rates and embryo transfer policy but with two different approaches to good-prognosis patients who decided to limit the number of oocytes used for ICSI. To the best of our knowledge, our study has been the first such comparison in the literature.

Material and methods

The trial was designed as a retrospective comparative two-center study. The recruited couples were qualified for ICSI/ET in two private Polish IVF centers: the Centre for Reproductive Medicine KRIOBANK in Białystok, and the VitroLive Fertility Clinic in Szczecin. A total of 218 couples who underwent their first controlled ovarian hyperstimulation (COH) retrieved a high number of oocytes (10-15) and decided to limit the number of eggs used for ICSI, were deemed eligible. The exclusion criteria were as follows: 1) maternal age >40; 2) at least one previous IVF-ICSI cycle with a fresh embryo transfer; 3) body mass index (BMI) of ≥ 28 kg/m^2; 4) ≥2 miscarriages; and 5) a high risk of OHSS (defined as estradiol concentration in blood serum ≥ 4500 pg/ml prior to administration of human chorionic gonadotropin

– hCG). In 108 consecutive patients treated at KRIOBANK the number of microinjected oocytes was limited to 6 and in 110 patients from VitroLive the number of oocytes used for ICSI was limited to 10.

The participants were scheduled for standard protocols of COH with the use of gonadotropin releasing hormone (GnRH) agonist triptorelin (Diphereline 3.75; Boufor Ibsen Pharma, France) or GnRH antagonist cetrorelix (Cetrotide; Merck Serono, Switzerland) and subcutaneous daily injections of human menopausal gonadotropin (hMG) (Menopur; Ferring Pharmaceuticals, Switzerland) or recombinant human follicle stimulating hormone (r-hFSH) (Gonal F; Merck Serono, Switzerland). The initial daily dose of gonadotropin was 150 IU, individually modified depending on the response of the ovaries. In order to achieve oocyte maturation, 6500 IU of hCG (Ovitrelle; Merck Serono, Switzerland) were administered subcutaneously and 24 hours after ovum retrieval the patients received luteal phase supplementation (depending on the clinic, either oral dydrogesterone (Duphaston; Solvay Pharma, Belgium) at a dose of 30 mg/day with 300 mg/day intravaginal progesterone (Luteina; Adamed, Poland), or intramuscular injections of microcrystalline progesterone suspension (Agolutin-Depot**;** BB Pharma, Czech Republic) at a dose of 100 mg/every 3 days with 300 mg/day intravaginal progesterone (Lutinus; Ferring Pharmaceuticals, Switzerland).

The standard embryological assessment, including characterization of the pronuclear morphology, was carried out 16-18 hours after ICSI on the basis of the classification presented by L. Scott et al. [17], followed by cell division evaluation, at 24-hour intervals, regarding number, symmetry and degree of blastomere fragmentation [18]. Good quality embryo on day 3 of culture was defined as an embryo with ≥ 8 blastomeres of equal size and regular shape (type A), or ≥8 blastomeres of different sizes and regular shape (type B), while high-quality embryos on day 3 were described as ≥ 8 equal-sized, non-fragmented blastomeres (type A). Blastocyst assessment on day 5 was done on the basis of the grading system proposed by Gardner and Schoolcraft, where three parameters are taken into consideration: appearance of the trophectoderm epithelium (TE), development of inner cell mass (ICM), and expansion of blastocyst cavity (numbers 1-4), where 1 is an early blastocyst with blastocoel being less than half the volume of the embryo and 4 is an expanded blastocyst with blastocoel volume larger than that of an early embryo and *zona pellucida* is thinning [19].

Good-quality blastocyst on day 5 of culture was defined when appearance of TE was described as tightly packed and many cells (type A) or loosely grouped with several cells (type B), and development of ICM was labeled as many cells forming cohesive epithelium (type A) or many cells forming loose epithelium (type B). Top-quality blastocysts were identified when many cells were tightly packed in TE and formed solid epithelium in ICM, while blastocoel at least completely filled an embryo (3AA and 4AA). The decision about the day and number of embryos to be transferred was made in accordance with the guidelines of the American Society of Reproductive Medicine (ASRM) [20].

Pregnancy was initially diagnosed by measuring serum β-hCG concentration 14 days after embryo transfer and confirmed 2 weeks later by transvaginal ultrasound. An ultrasound scan was repeated at about 8 weeks of gestation to confirm fetal heart rate and number of fetuses. Biochemical pregnancy was not taken into consideration in our study.

Statistical analysis

The main study endpoints were: number of embryos available for transfer; implantation rate defined as the number of gestational sacs, as assessed by ultrasound at 8 weeks of gestation, divided

Table I. Comparison of the ovarian response and embryological outcome in both groups.

	6 oocytes ICSI (n=108)	10 oocytes ICSI (n=110)	p
Number of retrieved oocytes	12.31 ± 2.42	16.11 ± 4.23	**<0.01***
Number of mature M2 oocytes	10.92 ± 1.82	13.27 ± 2.88	**<0.01***
Number of microinjected oocytes	6	10	
Mean number of fertilized oocytes 16-18h after ICSI	5.27	8.46	**<0.01***
Fertilization rate [%]	87.96 ± 15.75	84.64 ± 14.70	NS†
Mean number of developing embryos on day 2	5.16	8.46	**<0.01***
Embryonic development rate on day 2 [%]	86.11 ± 16.28	84.64 ± 14.70	NS†
Mean number of developing embryos on day 3	4.92	7.93	**<0.01***
Embryonic development rate on day 3 [%]	82.09 ± 22.97	79.33 ± 19.23	NS†
Number of embryos available for transfer	2.88 ± 1.23	3.77 ± 1.48	**<0.01***
Number of good-quality embryos available for transfer ¤	1.80 ± 1.42	2.87 ± 1.62	**<0.01***
Number of high-quality embryos available for transfer ‡	0.59 ± 0.87	1.39 ± 1.05	**<0.01***
Number of transferred embryos	1.83 ± 0.42	1.77 ± 0.50	NS*
Number of high-quality embryos per transfer‡	0.49 ± 0.71	1.00 ± 0.69	**<0.01***
Number of frozen embryos per cycle	1.05 ± 1.30	2.00 ± 1.67	**<0.01***

Unless otherwise stated, data are presented as mean value ± standard deviation. ICSI – intracytoplasmic sperm injection; * t-student; † χ^2; ¤: ⩾8A/8B for 3DET and AA/AB/BA/BB for 5DET; ‡: ⩾8A for 3DET and 3AA/4AA for 5DET; detailed description of embryo grading in the Material and methods section.

by the number of embryos transferred for each patient; ongoing pregnancy rate and cycles with cryopreservation rate.

Statistical analysis was performed using STATISTICA 10.0 (StatSoft Inc, Tulsa, USA). Continuous variables were presented as the arithmetic mean and standard deviation of a variable. Shapiro-Wolf test was used for continuous variables. Student's t-test (arithmetic) or Chi^2 (χ2) test were used for categorical variables when appropriate. The p-value of <0.05 was considered as statistically significant.

Results

A total of 218 women undergoing their first ICSI/ET cycle fulfilled the study criteria. Mean age of the patients with the number of oocytes limited to 6 was not significantly different than that with 10 limited oocytes (30.59±3.34 vs. 31.17±4.52 years, respectively). The comparison of ovarian response and embryological outcome is shown in Table I.

Mean number of the retrieved oocytes and mature oocytes was significantly lower in the group with 6 microinjected oocytes in comparison to 10 oocytes. Although fertilization rate and embryonic development rate on days 2 and 3 of culture were comparable in both studied groups, the number of embryos available for ET and the number of high quality embryos available for ET were significantly lower when 6 oocytes were fertilized in comparison to 10 oocytes (2.88±1.23 vs. 3.77±1.48, p<0.01 and 0.59±0.87 vs. 1.39±1.05, p<0.01, respectively). In the group with the number of fertilized oocytes intentionally restricted to 6 significantly fewer embryos were available for cryopreservation (1.05±1.30 vs. 2.00±1.67, p<0.01) and lower percentage of cycles with cryopreservation were observed (47.22% vs. 72.72%, p<0.01).

There were no significant differences in the implantation rate for the 6 oocytes group (29.93% vs. 29.54%, respectively), ongoing clinical pregnancy rate (39.81% vs. 45.45%, respectively), and ongoing twin-pregnancy (6.14% vs. 4.54%, respectively) as compared to the 10 oocytes group.

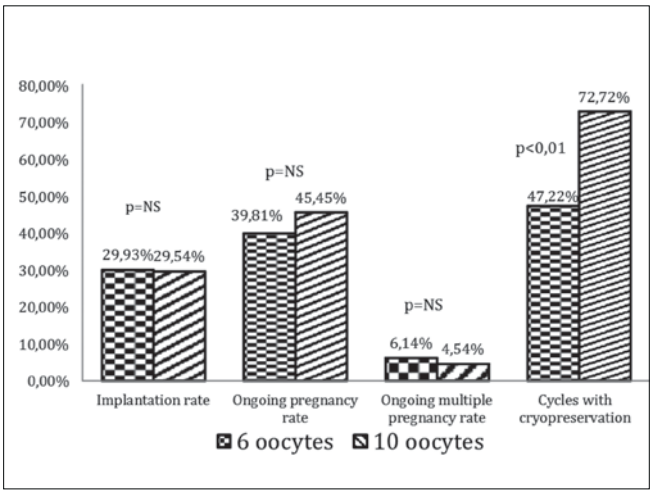

Figure 1. Comparison of the ICSI/ET outcomes in both groups.

Discussion

We chose to investigate a group of normal-responder patients undergoing IVF. The recruited couples, for various reasons, decided to restrict the number of oocytes available for fertilization. To the best of our knowledge, our study has been the first to demonstrate that with the retrieval of 10-15 mature oocytes following ovarian hyperstimulation, limitation of the number of oocytes for ICSI to 10, or even further to 6, did not affect the probability of conception in a fresh cycle. The implantation rate and ongoing pregnancy rate were comparable in both groups. Furthermore, when the number of the fertilized oocytes was intentionally restricted to 6, significantly fewer embryos were available for cryopreservation and the rate of cycles with cryopreservation was also considerably lower. The results of our study reveal several other implications regarding the optimal number of oocytes needed in IVF, approach to stimulation protocols, and patient counseling.

Studies investigating the relationship between the number of oocytes and pregnancy rates previously showed that pregnancy rates increased with a higher number of oocytes and the highest pregnancy rates were obtained with the number of oocytes ranging between 7–16 or 10–15 [15]. However, a high number of the retrieved oocytes can be associated with impaired receptivity of endometrium [21]. Several studies demonstrated a clear link between high preovulatory progesterone concentration and the number of ovarian follicles, and that progesterone concentration of >1.5 ng/ml was associated with reduced chances of pregnancy [22-24]. A recent study by Sunkara et al., indicated that earlier studies reported a small number of patients from single centers and, as such, had limited generalizability [15]. Furthermore, according to Sunkara et al., in order to maximize treatment success, the optimal number of oocytes to aim for in a fresh IVF cycle is between 11 and 15. Our study compared data of two IVF centers on patients with the optimal number of oocytes and revealed similar ongoing pregnancy rates (~40%) as those reported by Sunkara et al. (~31-41%). These findings may suggest that it is possible to reduce the number of the oocytes used for ICSI without unfavorably affecting the overall outcome on the basis of the number of the retrieved oocytes. However, it is important to emphasize that patients in both studied groups were normal responders who yielded a good number of oocytes and who should be distinguished from poor responders, who produce only a few oocytes, often resulting in embryos with poor implantation potential [25]. Therefore, our results do not show that there is no difference in the overall outcome between 6 and 10 retrieved oocytes.

Nowadays, a trend towards mild ovarian stimulation in IVF, with the emphasis on recovering fewer eggs than was previously considered optimal, is clearly visible [5]. Reduction of the amount of gonadotropins required for COH and, therefore, smaller costs would be another added value. However, although several retrospective studies have suggested that mild ovarian ovulation i.e. using a clomiphene citrate and low-dose gonadotropin protocol resulted in a pregnancy rate comparable to that of a group of patients who underwent normal COH protocol, it has been argued that mild stimulation may result in the reduction of the number of good-quality embryos available for transfer, and a decline in the pregnancy rate [1, 2].

Our findings seem to support the use of moderate stimulation protocols over mild COH protocols in IVF treatment. We also suggest that patients who satisfactorily respond to stimulation,

but have by choice fewer oocytes fertilized, produce a cohort of good-quality oocytes that become good-quality embryos capable of implanting.

Cryopreservation of surplus human embryos is an integral part of IVF procedures and the number of embryos in storage around the world is steadily increasing [26-27]. However, there is a group of patients who object to freezing supernumerary embryos that have been created in the course of an IVF treatment. Regardless of the reasons, counseling these patients appropriately presents a dilemma due to the relative lack of evidence about the outcomes of the various options [28]. According to Biggers et al., those patients should be given the option to decline IVF and consider adoption, or less efficient treatments, namely mild ovarian stimulation, limited insemination of the available ova or natural cycle IVF, where no surplus embryos are generated [29]. Our results demonstrate that a restricted number of oocytes used for ICSI can be a moderately safe solution for normal-responder (good prognosis) patients. This should be considered as a viable option for couples who, for ethical or moral reasons, object to cryopreservation of superfluous embryos that have been created for the ICSI treatment.

In conclusion, the literature offers limited data about the outcome of ICSI/ET treatment in normal-responder women who decided to limit the number of the microinjected oocytes. Our results suggest that, in a group of patients who choose to avoid creating surplus embryos for cryopreservation, limiting the number of oocytes to 6 did not affect the outcome of their treatment cycle. This information may be also useful in counseling patients on the available options of avoiding creating supernumerary embryos before undergoing ICSI/ET treatment. Further studies are needed to assess the relationship between limiting the number of the retrieved oocytes concerning the cumulative live birth rate including fresh and replacement of all frozen embryos generated from a single ICSI treatment cycle.

Oświadczenie autorów

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