

# Does the Number of Retrieved Oocytes Influence Pregnancy Rate After Day 3 and Day 5 Embryo Transfer?

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## 1. Introduction

Decisions associated to ovarian stimulation approach are an essential component of medically assisted reproduction (MAR). Considerable amount of research in this field has enhanced our understanding of certain biological phenomena taking place in the process and also brought along novel means of ovarian stimulation. Nevertheless, there is still not enough evidence to suggest the optimal number of oocytes collected at retrieval in order to predict the occurrence of successful pregnancy and most importantly, birth of a live baby. First, this chapter will describe certain historical developments in MAR. Next, current ovarian stimulation protocols and desired aims of modern era ovarian stimulation, embryo culture and embryo transfer outcome will be discussed. A single-centre MAR results analysis was performed in a ten year span in order to help resolve one of the ultimate questions: what is the optimal number of oocytes needed to achieve clinical pregnancy after embryo transfer?

## 2. Milestones in evolution of ovarian stimulation and embryo transfer

Ever since the beginnings of MAR, increasing the chance of a live birth has been the most important aim of researchers' efforts. The first successful embryo transfers (ETs) resulting in pregnancy by Edwards and Steptoe in the early 1970s were carried out in natural cycles. This means that only one oocyte was harvested at follicle aspiration (which was at the time performed laparoscopically). Hundred-and-one ETs were attempted before the first successful delivery of the world's first IVF (in vitro fertilization) baby Louise Brown in 1978 (Edwards & Steptoe, 1980). In these first attempts that paved the way for future extensive worldwide MAR success, Edwards and Steptoe described 65 natural cycles that yielded 45 oocytes from 44 patients and resulted in 3 successful deliveries. Thus, pregnancy rate was 9.1% and delivery rate 6.8% per oocyte retrieval.

Since then, research has brought along many improvements to MAR. In the early 1980s, one of the most important steps was the introduction of human menopausal gonadotrophin (HMG), which allowed for controlled ovarian hyperstimulation (COH) and multiple follicular growth. This enabled the retrieval of multiple oocytes at pick-up and thus substantially increased the likelihood of successful embryo replacement and subsequent

pregnancy. This finding has ignited further research and development that has improved gonadotrophin formulation and efficiency, but detailed illustration of this process is beyond the scope of this chapter.

### **3. Current challenges in ovarian stimulation**

#### **3.1 Ovarian hyperstimulation syndrome**

The development and implementation of gonadotrophins that allowed for controlled ovarian hyperstimulation (COH) in MAR has brought along a possibility of potentially life-threatening complication, namely ovarian hyperstimulation syndrome (OHSS) (Brinsden et al., 1995). It has been established that the risk of OHSS increases proportionally with an increase in ovarian response to stimulation as measured by the number of ovarian follicles, number of retrieved oocytes and serum estradiol concentration on the day of human chorionic gonadotrophin (hCG) administration (Asch et al., 1991). In our previous research, it has been demonstrated that the risk of OHSS increases with rising number of harvested oocytes and the risk is significantly higher in patients with more than ten collected oocytes (Reljić et al., 1999). Severe OHSS is a serious and potentially life-threatening complication of MAR treatment and has a mean incidence of 1-3% in MAR programmes involving standard ovarian stimulation protocols (Fauser et al., 1999). However, to date, there are no reliable predictors of its occurrence. Owing to these facts, many investigations in recent decade have been aimed towards less aggressive, milder forms of ovarian stimulation and to procedures that could eliminate the risk of OHSS (Revelli et al., 2011).

#### **3.2 Ovarian stimulation strategies**

Ovarian stimulation is the crucial component in medically assisted reproduction. In current practice, long acting gonadotrophin-releasing hormone (GnRH) agonist pituitary suppression combined with recombinant or purified urinary exogenous follicle stimulating hormone (FSH) is the most frequently used stimulation protocol (Macklon et al., 2006). However, in light of making MAR more “patient friendly”, there is a recent trend toward milder stimulation protocols in order to reduce the chances of complications and not lastly, to lower the costs of MAR treatment (Fauser et al., 1999). With the availability of GnRH antagonists in ovarian stimulation protocols, administration of FSH can be delayed to mid-follicular phase, thus reducing the amount of gonadotrophins used for stimulation and minimizing exogenous hormonal interferences that are present in conventional hormonal stimulation (Fauser & van Heusden, 1997). On the other hand, regarding reduction of OHSS, conventional stimulation protocols employing GnRH antagonists allow for substitution of human chorionic gonadotrophin (hCG) with GnRH agonist for ovulation triggering. Using this procedure, OHSS can be eliminated almost completely even in high responding patients with high numbers of retrieved oocytes (Humaidan et al., 2011).

Despite the novel approaches to ovarian stimulation, the number of retrieved oocytes is still considered to be an important prognostic variable in everyday MAR practice. However, the relation between the number of oocytes and MAR outcome is poorly understood as studies performed on this subject present with conflicting results (Hamoda et al., 2010; Letterie et al., 2005; Meniru et al., 1997; Sunkara et al., 2011; Yoldemir et al., 2010). Moreover, as IVF procedures are performed in increasing extent globally, new, restrictive legislation policies in certain countries limit the amount of oocytes per cycle that can be used in MAR

procedures. Due to the lack of reliable data, experts' opinions of such legislation effects on treatment success are too often contradictory. Thus, further research is needed to establish the role of number of harvested oocytes in MAR outcome prediction.

### **3.3 Embryo cultivation and transfer strategies**

In the scenario of transfer of more than one embryo, multiple gestations are the most common complication of pregnancies achieved through MAR. Thus, in recent times, scientific societies have propagated the idea that the goal of medically assisted procreation must be the achievement of a singleton pregnancy (European Society of Human Reproduction and Embryology [ESHRE] Task Force on Ethics and Law, 2003). The simplest way of reducing multiple gestation incidences is strict implementation of single embryo transfer (SET) strategy. However, this is related to significant declines of pregnancy rates. The development of advanced embryo culture media in the past two decades have allowed for extended, blastocyst cultivation of the embryos. Following the evolution of these media, many have advocated blastocyst transfer mainly due to better morphologic embryo assessment possibilities at blastocyst stage compared to cleavage stage embryos. Although the opinions on this subject are still not uniform, many recently performed studies have demonstrated significantly better outcome after blastocyst transfer (Papanikolau et al., 2008). Thus, a recent Cochrane review has shown a significant improvement in pregnancy and live birth rates for blastocyst transfer compared to transfer of cleavage stage embryos (Blake et al., 2007).

Currently, increasing number of legislation acts on MAR in developed countries impose mandatory single embryo transfer under certain circumstances (mostly in younger patients), in order to decrease the incidence of multiple gestations. Thus, the question remains; how to select the best embryos in order to achieve the highest success and at the same time lower the incidence of multiple gestations? With regards to these facts, additional studies should settle the issue in what scenario can the number of oocytes be a factor in decision for SET or multiple embryo transfer and whether embryos should be transferred at cleavage or blastocyst stage.

## **4. Influence of number of retrieved oocytes on embryo transfer success**

A retrospective study of retrieved oocyte number on MAR outcome was performed at Department of Reproductive Medicine and Gynaecologic Endocrinology, University Medical Centre in Maribor. Totally, 6989 consecutive in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) cycles resulting in follicle aspiration and oocyte pick-up were included. Ovarian stimulation protocols with combination of GnRH agonist/GnRH antagonist and recombinant FSH (Gonal-f®, Serono International SA, Geneva, Switzerland)/HMG (Menopur®, Ferring Pharmaceuticals Inc., Saint-Prex, Switzerland) were used and were previously described in detail (Vlaisavljević et al., 2000). Embryo quality was assessed by an experienced embryologist at day two and blastocysts were graded according to our established grading system at day five after oocyte pick-up (Kovačič et al., 2004). Embryo transfer was carried out three or five days after oocyte pick-up. Day five blastocyst transfer was performed if more than four fertilised oocytes were obtained and if more than three optimal embryos were available on day three according to our standard policies (Kovačič et al., 2002). After consultation with the patients, time of embryo transfer could be adjusted to

day 3 or day 5 according to doctor-patient agreement. Clinical pregnancy was defined as the presence of fetal heartbeat on ultrasound examination at six weeks of gestation. Frozen-thawed embryo transfer cycles and cycles with preimplantation genetic diagnostics (PGD) or in vitro matured (IVM) oocytes were excluded from the study.

Patients were stratified according to the number of oocytes collected at retrieval. Categorical variables were tested using Chi-Square test. Numerical values were tested bivariate using Student's *t*-test or Mann-Whitney *U* test. The analysis of variance test (ANOVA) with Tukey HSD or Games-Howell post-hoc test was used for analysing differences in continuous variables between groups stratified by the number of retrieved oocytes. To further explore the association between the number of oocytes and clinical pregnancy rate, a logistic regression model was constructed. The model was adjusted for confounding variables that affected pregnancy rate in univariate analysis (the history of previous MAR attempts, age and dose of gonadotrophins). Data were analysed using SPSS 17.0 (SPSS Inc., Chicago IL) statistical software package.

#### 4.1 Distribution of retrieved oocytes per cycle

In 6989 studied cycles, 61793 oocytes were collected at retrieval. The median number of retrieved oocytes was 8 [interquartile range (IQR) 4-12] and the median number of embryos created was 4 [IQR 2-7]. Figure 1 represents the distribution of collected oocytes in cycles resulting in oocyte pick-up.

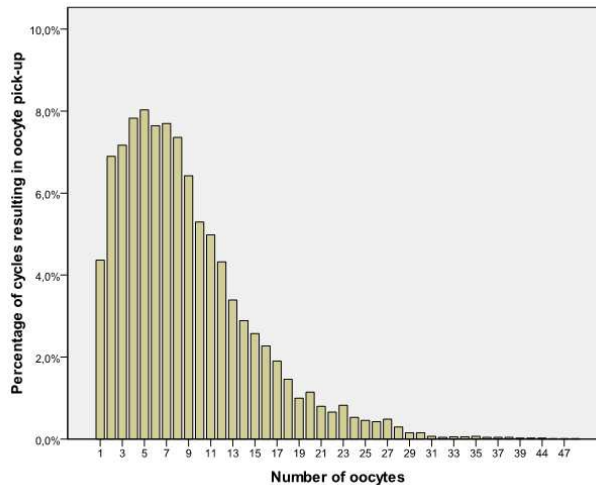


Fig. 1. Distribution of retrieved oocytes.

#### 4.2 Outcome of MAR cycles

Cycles were stratified according to the number of retrieved oocytes. Overall clinical pregnancy rate per cycle was 37.1%, calculated pregnancy rate per embryo transfer was 41.0%. Delivery rates per cycle and per embryo transfer were calculated to be 29.8% and 32.9%, respectively. Clinical outcomes are summarized in Table 1.

No. of oocytes	1-5	6-10	11-15	16-20	>20	TOTAL
No. of cycles	2396	2405	1269	544	375	6989
Age (SD)	36.1 (4.6) <sup>a</sup>	33.8 (4.6) <sup>b</sup>	32.6 (4.2) <sup>c</sup>	32.2 (4.4) <sup>c</sup>	32.0 (4.2) <sup>c</sup>	34.2 (4.7)
No of previous MAR attempts (SD)	2.0 (2.4) <sup>a</sup>	1.6 (2.1) <sup>b</sup>	1.5 (1.9) <sup>bc</sup>	1.4 (1.9) <sup>c</sup>	1.3 (1.6) <sup>c</sup>	1.7 (2.2)
No. of oocytes collected	7765	18823	16061	9571	9573	61793
Fertilised oocytes (2PN)	4910	11552	9668	5686	5510	37326
Day 2 embryos	4837	11352	9454	5565	5381	36589
ET(s)	2027	2241	1213	513	332	6326
Percentage of day 5 ETs	14.0% <sup>a</sup>	69.4% <sup>b</sup>	86.7% <sup>c</sup>	92.6% <sup>d</sup>	94.3% <sup>d</sup>	58.2%
Cancelled ET	15.4% <sup>a</sup>	6.8% <sup>b</sup>	4.4% <sup>c</sup>	5.7% <sup>bc</sup>	11.5% <sup>ab</sup>	9.5%
Transferred embryos	3502	4040	2123	828	489	10982
Cryopreserved embryos	295	2212	2544	1982	2150	9183
Clinical Pregnancies	532	954	638	286	184	2594
Avg. Fertilisation rate	75.6% <sup>a</sup>	77.2% <sup>ab</sup>	77.6% <sup>ab</sup>	79.3% <sup>ab</sup>	83.8% <sup>b</sup>	77.3%
Pregnancy rate/cycle	22.2% <sup>a</sup>	39.7% <sup>b</sup>	50.3% <sup>c</sup>	52.6% <sup>c</sup>	49.1% <sup>c</sup>	37.1%
Pregnancy rate/ET	26.3% <sup>a</sup>	42.6% <sup>b</sup>	52.6% <sup>c</sup>	55.8% <sup>c</sup>	55.4% <sup>c</sup>	41.0%
Cycles with blastocyst cryopreservation	8.3% <sup>a</sup>	38.9% <sup>b</sup>	58.3% <sup>c</sup>	73.0% <sup>d</sup>	83.5% <sup>e</sup>	37.0%

<sup>abcde</sup> Within each category, numbers with different letter superscripts are significantly different from each other, numbers with the same letter superscript are not significantly different ( $p < 0.05$ ).

Table 1. Outcome of MAR treatment according to the number of retrieved oocytes

### 4.3 Clinical pregnancy & delivery rates in relation to the number of retrieved oocytes

Clinical pregnancy and delivery rates were calculated per each number of collected oocytes. Clinical pregnancy rate rises constantly to peak at 11-15 oocytes and from then on remains constant until it declines slightly at high responders with more than 20 oocytes (Table 1 & Figure 2). Nonetheless, there are no statistically significant changes in pregnancy rates in groups of patients with more than 11-15 harvested oocytes. However, the risks related to ovarian stimulation increase with rising oocyte count, which is partially reflected in higher cycle cancellation rates, especially in patients with more than 20 harvested oocytes (Table 1). Published data about the number of oocytes influencing embryo transfer outcome are not consistent. Whilst some scientists claim that oocyte number plays no role in achievement of pregnancy after embryo transfer (Letterie et al., 2005; Yoldemir et al., 2010), others report of increasing pregnancy rates with increasing number of oocytes. The optimal numbers reported in these studies are usually in the range of 5 to 15 oocytes (van Gast et al., 2006; Meniru & Craft, 1997; Timeva et al., 2006). Most of these studies are however single-centre analyses performed on a fairly low number of patients. Recently though, Sunkara et al performed a large scale review of UK national IVF data and concluded that 15 is the optimal number of oocytes to be collected at retrieval (Sunkara et al., 2011). Results of our study that

included nearly 7000 cycles suggest that the optimal number of oocytes to aim for at ovarian stimulation in order to achieve clinical pregnancy should be between 11 and 15.

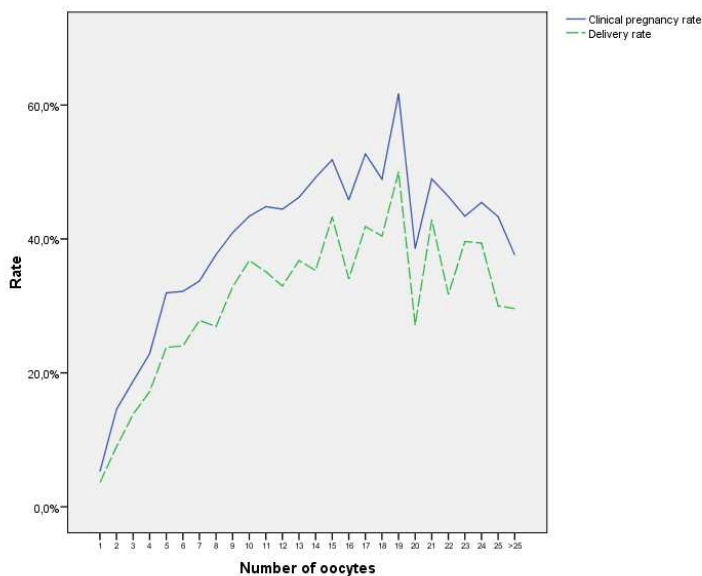


Fig. 2. Clinical pregnancy and delivery rates in relation to the number of retrieved oocytes

#### 4.3.1 Low number of oocytes

As noted above, pregnancy and delivery rates are significantly lower in cycles where less than 5 oocytes are retrieved at pickup. Accordingly, these cycles were analysed more thoroughly. Significant changes can be observed in pregnancy rates among each of the groups (Table 2). These differences remain significant even after controlling for the age factor and history of previous MAR attempts in multivariate analysis.

It has been demonstrated that low oocyte numbers after ovarian stimulation are related to ovarian ageing and the depletion of primordial follicle pool (Tarlantzis et al., 2003). Ovarian stimulation for women with low ovarian reserve has remained one of the most frustrating aspects of IVF (Revelli et al., 2011). However, direct correlation with pregnancy rate has not been thoroughly investigated.

Low oocyte number coupled with high gonadotrophin dose in conventional stimulation methods can imply lower oocyte quality. Furthermore, endometrial quality can also be hampered in high-dose stimulating protocols (Gougeon, 1996; Pal et al., 2008). On the other hand, higher number of oocytes simply allows for better selection of quality embryos from a larger cohort of available embryos (Devreker, 1999; Pal et al., 2008).

On the opposite, it has been theorised that milder forms of ovarian stimulation allow for higher quality oocytes and embryos with lower incidence of chromosome aneuploidies that can result in comparable pregnancy rates in spite of the lower number of harvested oocytes (Verberg et al., 2009). However, studies on this subject are relatively sparse and a recently

performed meta-analysis included only three studies featuring low number of participants (Verberg et al., 2009). All of the studies included report of relatively low pregnancy rates, 15-21%. What is more, the studies did not account for possible added benefits of embryo cryopreservation, which could shift the scale to the side of classic ovarian stimulation even further if adding freeze-thaw cycles to the analysis. In theory, mild stimulation seems a feasible choice that could be especially indicated in poor responding patients. Because of lower gonadotrophin dose, higher quality of oocytes and endometrium could be expected. But since there is very limited data available, current reports do not provide enough supporting evidence and in the end, additional studies are needed in order to define the role of mild stimulation protocols in routine MAR treatment.

Number of oocytes	1	2-3	4-5	TOTAL	
No. of cycles	305	983	1108	2396	
Age (SD)	37.9 (4.2) <sup>a</sup>	36.7 (4.4) <sup>b</sup>	35.2 (4.6) <sup>c</sup>	36.1 (4.6)	<i>p</i> <0.001
No of previous MAR attempts (SD)	2.0 (2.4) <sup>a</sup>	2.1 (2.7) <sup>a</sup>	1.9 (2.2) <sup>a</sup>	2.0 (2.4)	<i>p</i> >0.05
Gonadotrophin used (IE)	2979 <sup>a</sup>	2789 <sup>b</sup>	2518 <sup>c</sup>	2692	<i>p</i> <0.001
No of oocytes collected	305	2467	4993	7765	
Fertilised oocytes (2PN)	212	1574	3124	4910	
Day 2 embryos	205	1532	3100	4837	
ET(s)	185	828	1014	2027	
Percentage of day 5 ETs	0.5%	3.0%	25.4%	14.0%	
Cancelled ET	39.34% <sup>a</sup>	15.77% <sup>b</sup>	8.48% <sup>c</sup>	15.40%	<i>p</i> <0.001
Transferred embryos	186	1368	1948	3502	
Cryopreserved embryos	0	41	254	295	
Clinical Pregnancies	16	184	332	532	
Avg. Fertilisation rate	78.2% <sup>a</sup>	75.0% <sup>a</sup>	75.8% <sup>a</sup>	75.6%	<i>p</i> >0.05
Pregnancy rate/cycle	5.30% <sup>a</sup>	18.70% <sup>b</sup>	30.00% <sup>c</sup>	22.20%	<i>p</i> <0.001
Pregnancy rate/ET	8.70% <sup>a</sup>	22.20% <sup>b</sup>	32.70% <sup>c</sup>	26.30%	<i>p</i> <0.001
Cycles with blastocyst cryopreservation	0% <sup>a</sup>	3.30% <sup>b</sup>	15.10% <sup>c</sup>	8.30%	<i>p</i> <0.001

<sup>abc</sup> Within each category, numbers with different letter superscripts are significantly different from each other, numbers with the same letter superscript are not significantly different.

Table 2. Cycle characteristics in “poor” and “low” responders (<5 oocytes collected)

#### 4.4 Effect of age

Besides the number of oocytes, age was found to be one of the most important factors in predicting the success of the started cycle in our data. In further analysis, cycles were stratified according to the age of female patients. Advancing age significantly adversely affects the outcome of MAR cycles independently of oocyte count. Average clinical pregnancy rate was the highest in the youngest group of patients (20-34 years) at 45.6% and decreased to 16.8% in women over 40 years of age. On the other hand, mean oocyte count was also significantly lower in older women (20-34 years; 10.4 oocytes, 35-37 years; 8.5 oocytes, 38-39 years; 6.9 oocytes, 40-44 years; 5.7 oocytes, *p*<0.001). The results are illustrated in Figure 3.

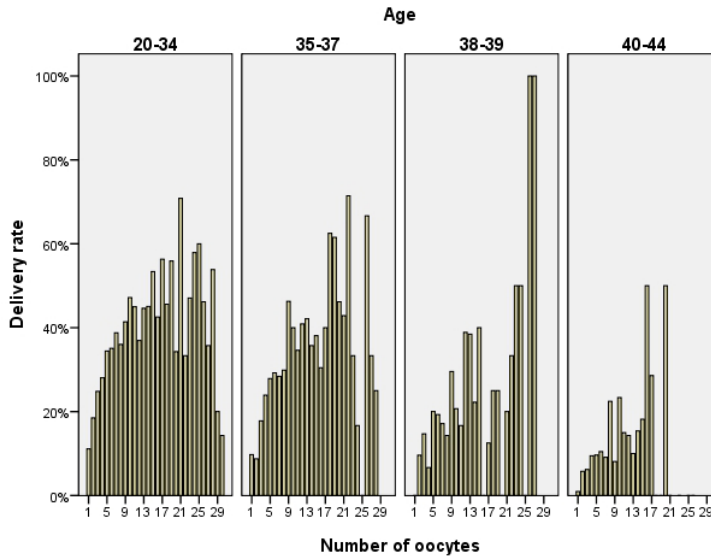


Fig. 3. Association between oocyte number and delivery rate stratified by the female patient age.

With ovarian ageing, the depletion of ovarian reserve usually involves adjusting stimulation protocols with higher doses of gonadotrophins that result in negative effects as discussed in previous topic. Although our study did not consider antral follicle count (AFC) and anti-Müllerian hormone (AMH) as a measure of ovarian reserve, it can be clearly seen from our results that age is independent predictor of lower oocyte count at retrieval. But even in the case of normal response to gonadotrophin treatment in older women, pregnancy rates are lower compared to younger patients. This could be related to the high proportion of embryo aneuploidies in these patients. This problem grows quickly after 40 years of age and after the age of 45 the birth of a healthy baby after MAR is very rare in spite of normal ovarian reserve tests (Forman et al., 2011).

#### 4.5 Embryo cryopreservation

Although the birth of a live baby preceded by the successful achievement of clinical pregnancy is the single most important outcome of MAR treatment, several other surrogate indicators were evaluated in order to assess the quality of MAR cycles.

Embryo transfer was performed in 91.5% of cycles after oocyte pick-up. In approximately 9.5% cycles transfer had to be cancelled. The rate of transfer cancellation is the highest in "poor" responding patients and approximately 40% of transfers were cancelled when only 1 oocyte was retrieved (Table 1 & Table 2). This can be contributed to the fact that there are fewer embryos available for transfer and also to the lower quality of oocytes available for treatment (Tarlantzis et al., 2003). On the other hand, cycle cancellation again increases significantly in high responding patients, with more than 20 oocytes collected at retrieval. Together with the higher rate of cycle cancellation, a drop in pregnancy and delivery rates is also observed beyond 20 harvested oocytes (Table 1 & Figure 2). In concordance with these



results, recently performed studies argue that the simple aim to harvest as many oocytes as possible is simply not justified (van der Gast et al., 2006; Sunkara et al., 2011). Our analysis has some limitations due to the fact that incidence of OHSS could not be analysed and cycles with cryopreservation of the whole embryo cohort due to high OHSS risk could not be excluded from the analysis. It could be reasoned that lower pregnancy rates and high embryo transfer cancellation can be attributed to ovarian hyperstimulation syndrome risk. But considering data in the literature, approximately 15% incidence of OHSS can be predicted at 20 collected oocytes and the risk increases with rising oocyte count (Reljić et al., 1999; Verwoerd et al., 2008). In addition, there is also evidence that high estradiol levels may negatively impact developmental potential of the embryos (Ertzeid & Storeng, 2001) and interfere with endometrial receptivity in the early luteal phase (Devroey et al., 2004; Horcajadas et al., 2008). Thus, pregnancy rates can be hampered in high responders even in the absence of ovarian hyperstimulation syndrome risk.

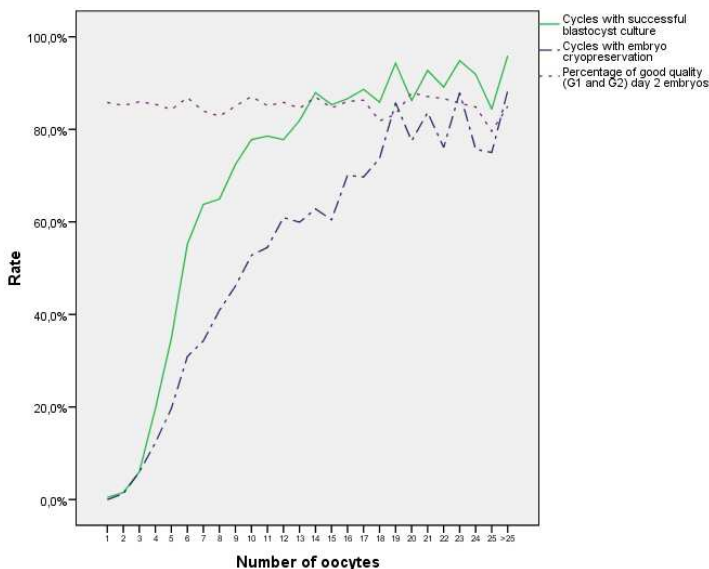


Fig. 4. Association of the number of retrieved oocytes to embryo cryopreservation, successful blastocyst culture and proportion of good quality embryos on day two.

Nonetheless, an increase in proportion of cycles with embryos available for freezing can be observed up until ~20 oocytes (Table 1 & Figure 4). The relationship was evident also in multivariate analysis after controlling for possible confounding variables (age, history of previous MAR attempts and dosage of gonadotrophins). Somewhat lower pregnancy rates in these patients could theoretically be recovered in subsequent cryo-thawed cycles. It should be noted though, that this group of patients included also couples with cancelled embryo transfer and cryopreservation of the whole embryo cohort due to high OHSS risk. Due to our study design, linkage between fresh and cryo-thawed cycles was not possible and the analysis of cumulative pregnancy rates could not be performed. Our results are comparable with findings of the research by Hamoda et al in which there was no increment in cycles with available oocytes for freezing beyond 18 harvested oocytes (Figure 4).

Furthermore, the storage of high numbers of cryopreserved embryos can also lead to logistic, administrative and ethical problems. Even so, in a unit with a successful cryopreservation programme, especially with the advances of embryo vitrification, cumulative pregnancy rates can be substantially improved (Kolibianakis et al., 2009).

Additionally, blastocyst transfer was performed significantly more frequently with rising number of harvested oocytes. Because day 5 blastocyst transfer allows for enhanced embryo selection and can optimize the chance of embryo transfer success (Blake et al., 2007; Kovačić et al, 2002; Papanikolaou et al., 2008), further analysis was aimed toward the comparison of different embryo cultivation and transfer policies.

#### 4.6 Embryo cultivation protocol

Couples were stratified according to the stage at which embryos were replaced in the uterus. At blastocyst stage, single embryo or two embryos were transferred, at cleavage stage one to three embryos were transferred depending on the age, history of the patients and embryo quality. Results of embryo transfer after different protocols are illustrated in Table 3.

In the beginnings of MAR, cleavage stage embryo transfer was traditionally performed. Physiology and energy metabolism of early embryos were not well understood and thus media could not be used to culture embryos beyond the four-cell stage. However, with scientific advances, new improved, sequential blastocyst media have enabled longer in vitro cultivation of embryos (Menezo et al., 1998). There are two central reasons why this should theoretically improve embryo transfer results. First of all, it is considered that blastocyst transfer mimics natural conception physiology as the embryo travels through Fallopian tubes and reaches the uterine cavity no sooner than the fourth day after conception. The uterus provides different nutritional conditions for the embryo and this may cause homeostatic stress and reduce embryo implantation rates after cleavage stage transfer (Blake et al., 2007). The second reason lies in the before mentioned chance of better morphologic selection of the embryos at blastocyst stage. However, in our previous studies, it was demonstrated that in the case of low number of embryos this advantages did not help to improve success rates of embryo transfer. Extended blastocyst culture did not prove to be of any value in improving pregnancy rates in the scenario when fewer than three embryos were available at day two after oocyte pickup. On the other hand, although transfer cancellation was significantly higher when waiting until day five for transfer, pregnancy and delivery rates per started cycle were comparable (Kovačić et al., 2002; Vlasisavljević et al., 2001). According to the present study, clinical and ongoing pregnancy rates were significantly higher in the case of blastocyst transfer compared to day three cleavage stage embryo transfer (52.7% vs. 25.3% and 43.1% vs. 18.6% respectively). Even in the multivariable logistic regression model, adjusting for the age of the patients and the number of harvested oocytes, these differences remained statistically significant. These results should however be interpreted in the light of the fact that these groups were composed of patients with uneven characteristics. Generally, patients with less than five harvested oocytes and fewer than three embryos on day two that have effectively worse prognosis underwent cleavage stage transfer on day three. The number of harvested oocytes was significantly lower in day 3 group transfer as compared to day 5. In spite of this, considering data in the literature (Blake et al., 2007; Papanikolaou et al., 2008), our data confirm that blastocyst transfer provides a higher chance of embryo implantation and subsequent clinical pregnancy.

Additionally, it can clearly be seen that double blastocyst transfer does not improve pregnancy rates compared to transfer of a single blastocyst. This can be observed even when analysing only cycles with at least one optimal blastocyst transferred. On the other hand, the incidence of twin deliveries rises dramatically with double embryo, especially double blastocyst transfer (38.1%).

	Day 5	SBT	DBT	Day 3	SET	DET	TET	TOTAL
No. of cycles	<b>3572</b>	1452	2120	<b>2647</b>	798	1377	472	<b>6219</b>
Age (SD)	<b>32.8</b> <b>(4.3)</b>	31.8 (4.2) <sup>a</sup>	33.4 (4.3) <sup>b</sup>	<b>35.8</b> <b>(4.6)</b>	35.7 (4.7) <sup>c</sup>	35.3 (4.5) <sup>c</sup>	37.5 (4.3) <sup>d</sup>	
Avg. no of oocytes (SD)	<b>11.8</b> <b>(5.6)</b>	12.5 (6.5) <sup>a</sup>	11.3 (5.1) <sup>b</sup>	<b>5.2</b> <b>(3.7)</b>	3.5 (2.8) <sup>c</sup>	5.6 (3.6) <sup>d</sup>	6.9 (4.0) <sup>e</sup>	<b>8.8</b> <b>(6.1)</b>
% transfers with optimal quality blastocyst	<b>53.2%</b>	60.6%	48.2%		.	.	.	.
Clinical pregnancy/ET	<b>52.7%</b>	52.2% <sup>a</sup>	53.1% <sup>a</sup>	<b>25.3%</b>	15.2% <sup>b</sup>	30.5% <sup>c</sup>	27.3% <sup>c</sup>	<b>41.1%</b>
Implantation rate	<b>43.9%</b>	53.1% <sup>a</sup>	37.6% <sup>b</sup>	<b>16.6%</b>	15.1% <sup>cd</sup>	19.2% <sup>c</sup>	11.3% <sup>d</sup>	<b>31.2%</b>
Ongoing pregnancy/ET	<b>43.1%</b>	(625) 43.1% <sup>a</sup>	(912) 43.2% <sup>a</sup>	<b>18.6%</b>	(92) 11.6% <sup>b</sup>	(313) 22.8% <sup>c</sup>	(84) 17.9% <sup>c</sup>	<b>(1663)</b> <b>32.9%</b>
Twins	<b>24.5%</b>	(8) 1.7%	(303) 38.1%	<b>17.6%</b>	(1) 1.4%	(49) 20.1%	(19) 25.3%	<b>(380)</b> <b>22.8%</b>
Triplets	<b>0.4%</b>	0	(5) 0.8%	<b>0.3%</b>	0	(1) 0.4%	0	<b>(6)</b> <b>0.4%</b>

<sup>abcde</sup> Within each row, numbers with different letter superscripts are significantly different from each other, numbers with the same letter superscript are not significantly different ( $p < 0.05$ )

Legend: »SBT«: single blastocyst transfer  
 »DBT«: double blastocyst transfer  
 »SET«: single cleavage stage embryo transfer  
 »DET«: double cleavage stage embryo transfer  
 »TET«: triple cleavage stage embryo transfer

Table 3. Embryo cultivation strategies and clinical outcome

The detailed investigation of the incidence of multiple gestations reveals that the incidence of higher order multiple pregnancies was low, as only 6 triplet pregnancies were observed during the ten-year study period. Conversely, birth of twins was recorded in 22.8% of deliveries and as mentioned before, this was especially high in the transfer of two blastocysts. In our publications, it was demonstrated that the age of the female patient and additional spare blastocysts available for cryopreservation are an important risk factor for multiple gestation after blastocyst transfer (Vlaisavljević et al., 2004). Our current data has shown, that even in the group of patients older than 40 years, after double blastocyst transfer, there were 27.7% twin gestations. This exemplifies the fact that the only option to minimize the risk of multiple gestations is strict implementation of single embryo transfer, even in the case of dealing with older patients.

## 5. Conclusion

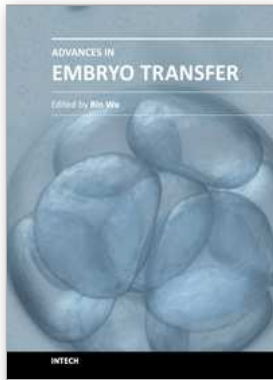
Introduction of exogenous gonadotrophins to ovarian stimulation represents one of the most important events in MAR that substantially improved the results of infertility treatment. The first successful pregnancies achieved with MAR were the result of a natural cycle IVF. Since then, ovarian stimulation protocols using GnRH agonists or antagonists in the combination with recombinant or highly purified gonadotrophins have become an everyday routine in MAR practice. Our results show that current protocols of ovarian stimulation should be used in a way to avoid excessive follicular development and to achieve moderate stimulation of the ovaries. This approach provides superior results compared to either aggressive or mild stimulation protocols. According to our study, oocyte pick-up resulting in eleven to fifteen harvested oocytes presents the optimal outcome of ovarian stimulation. Day five blastocyst transfer enables for higher pregnancy rates compared to transfer of cleavage stage embryos, especially when high number of developed embryos is available on day three after oocyte pick-up. Extended blastocyst culture also allows for transfer of reduced number of embryos without decreasing the overall pregnancy rate. However, only strict implementation of single embryo transfer can lead to a decrease in the incidence of multiple gestations. These steps, coupled with successful laboratory embryo cryopreservation programme, present as an optimal choice for MAR.

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## **Advances in Embryo Transfer**

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Embryo transfer has become one of the prominent high businesses worldwide. This book updates and reviews some new developed theories and technologies in the human embryo transfer and mainly focus on discussing some encountered problems during embryo transfer, which gives some examples how to improve pregnancy rate by innovated techniques so that readers, especially embryologists and physicians for human IVF programs, may acquire some new and usable information as well as some key practice techniques. Major contents include the optimal stimulation scheme for ovaries, advance in insemination technology, improved embryo transfer technology and endometrial receptivity and embryo implantation mechanism. Thus, this book will greatly add new information for readers to improve human embryo transfer pregnancy rate.

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