

# The comparison of two different embryo culture methods in the course of in vitro fertilization program.

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**Abstract:** The objective of the study was to compare two different embryo culture methods in the course of in vitro fertilization program by means of fertilization rate, embryo development, total time and cost. 98 patients undergoing assisted reproduction procedures due to infertility were analyzed. The inclusion criteria for the study: first IVF-ET program, at least 10 MII oocytes, no indications for ICSI. Oocytes were divided into two study groups: group A - open culture (oocytes placed in four-well dishes together, then inseminated and cultured in successive wells) and group B - a closed culture (oocytes placed in microdroplets, each embryo cultured separately). The fertilization rate was assessed around 18 hours from insemination. The embryos were classified into four classes. The best embryos were chosen for transfer. In the group A the fertilization rate obtained was lower than in group B (68% vs. 78%, respectively). The microdroplet culture required more time on the insemination day and on the second day of culture, while the four-well dish method required more time on the first day of culture and on the day of transfer. On analyzing the total cost of the above procedures (MI medium and oil costs) it occurred that the microdroplet culture was more expensive than the four-well dish method (due to the intake of paraffin oil). However, the difference was of no practical importance. In the conclusion, microdroplet culture gives a higher fertilization rate than four-well dish culture, probably due to a homogenous sperm distribution. Despite the differences in time outside the incubator and laboratory expenses (which are after all insignificant) microdroplet culture allows a better control over the embryo development. The embryos of best developmental potential can therefore be chosen for ET.

**Key words:** Human embryo culture - Microdroplet culture - Four-well dish culture

## Introduction

In vitro fertilization (IVF) has been present in clinical practice for as many as 29 years. It is a commonly used and accredited method in the process of infertility treatment. However, the knowledge of fertilization process in humans is rather confined due to the limitations of ethical nature. There are still many questions that clinicians are unable to answer.

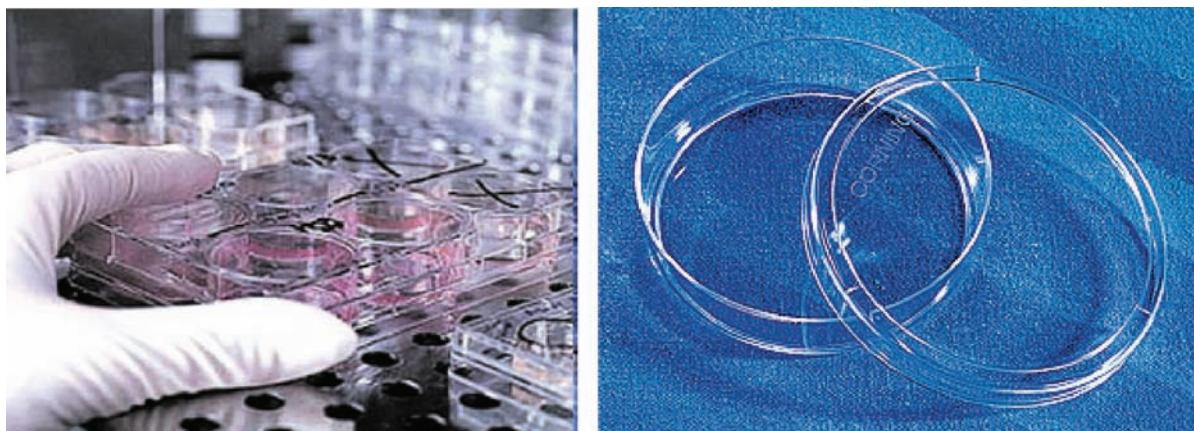
Beside a properly chosen stimulation protocol, embryo culture and all the procedures taking place in an embryology laboratory are the elements of great importance influencing the efficacy of IVF method. They aim at obtaining embryos of the largest development potential. The above mentioned laboratory factors include all the technical aspects (properly carried out procedures, the time in which the gametes and

embryos are outside of the incubator, experienced embryologist) and the means of embryo culture itself. Therefore, a search of an ideal culture method, which would allow an observation of particular stages of oocytes and embryos development, continues.

Nowadays many laboratories worldwide use two different methods of embryo culture - an open and a closed system. In the first above mentioned method both the insemination of cumuli and embryo culture take place in a four-well Nunc dish. In the closed system the insemination of a single cumulus and culture of one embryo is carried out in microdroplet (0.01ml) of medium on Petri dish under a layer of mineral oil [1-3] (Fig. 1).

The aim of the study was to compare two different embryo culture methods in the course of in vitro fertilization: microdroplet and four-well dish. Moreover, we tried to assess the efficacy of both systems by means of fertilization rate and embryo development, to evaluate the time the gametes and embryos were outside of the incubator and finally to estimate the intake of laboratory materials.

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**Fig. 1.** Multi-well dishes and plates used for culture in microdroplets.

## Material and methods

**Patients.** The study was carried out in the group of 98 patients who underwent assisted reproduction procedures due to infertility. A long stimulation protocol was used in each case. The inclusion criteria for the study were: first IVF-ET program in a patient's history, at least 10 mature MII oocytes retrieved during oocyte pick up, no indications for intracytoplasmic sperm injection (ICSI). At the time of oocyte pick up the oocytes were haphazardly divided into two study groups. Group A was an open culture. Mature oocytes were placed in four-well dishes (a maximum of 9 per well in 0.5ml of medium) and later inseminated. The culture was carried out in the same dish but in successive wells. However, only properly fertilized oocytes and embryos fulfilling morphological developmental criteria were moved into the following well. On the day of embryo transfer (ET) the selected ones were placed in the fourth well. The remaining, if any, were left in the third well to undergo embryo freezing.

The oocytes in group B were placed in microdroplets on Petri dishes (each drop of 0.01 culture medium under a 3 ml of paraffin oil). Each embryo was cultured in a different drop. On the day of transfer all chosen embryos were placed in one drop (Table 1).

The fertilization rate was assessed around 18 hours from insemination in both groups. After the gametes were cleaned of granulosa cells it was possible to see and count pronuclei. Afterwards the embryos were classified according to Cummins *et al.* scale, and divided into four classes.

**Statistical analysis.** T-Student test was used in statistical analysis and p value below 0.05 was considered significant.

## Results

In group A (four-well dishes) the fertilization rate obtained was lower than in group B (microdroplets) - 68% vs. 78%, respectively ( $p<0.05$ ). Moreover, the percentage of triploid zygotes was higher in four-well dish culture (6% vs. 3% in microdrops,  $p<0.05$ ) (Fig. 2). There were no significant differences in embryo stages on consecutive culture days, including the day of ET. The microdroplet culture required more time on the insemination day and on the second day of culture (embryo classification). However, the four-well dish method required more time on the first day of culture (pronuclei assessment) and on the day of transfer.

Despite the differences, the overall time the zygotes/embryos were outside the incubator was longer in group A by an average of 16 seconds.

The four-well dish culture used up a total of 2ml of MI medium and 2ml of paraffin oil per cycle. In group B, however, the intake of MI medium was ten times smaller (0.2-0.35ml of MI), while it required as much as 12ml of paraffin oil. In group A one dish was used per cycle, while in group B - three Petri dishes per cycle.

On analyzing the total cost of the above procedures it occurred that the microdroplet culture was more expensive than the four-well dish method. However the difference in expenses equaled 6 PLN (2.5 USD or 1.8 Euro), which is of no practical importance.

## Discussion

Proper embryological laboratory is one of the major factors of a successful assisted reproductive technology (ART) program, as well as an adequate stimulation protocol and embryo transfer technique. The choice of culture system used is a matter of individual preferences and previous experience; there are two methods widely and successfully used: microdroplets under paraffin oil and four-well plates. Each of these techniques has its own advantages and disadvantages [1].

Culturing in an open system in multi-well dishes exposes the oocytes and sperms to changes of the environment - pH and temperature fluctuations. That raises the question, what happens when the oocyte-corona cumulus complex, as well as sperms, are all co-incubated in small volumes of media - would not the oocytes be affected by the competition for nutrients between the thousands of cumulus cells and sperms or would some metabolites have any effect on the oocyte? Those theoretical questions raise, however, both systems work equally well, as evidenced by pregnancies obtained by laboratories using different kinds of culture conditions [1,4,5].

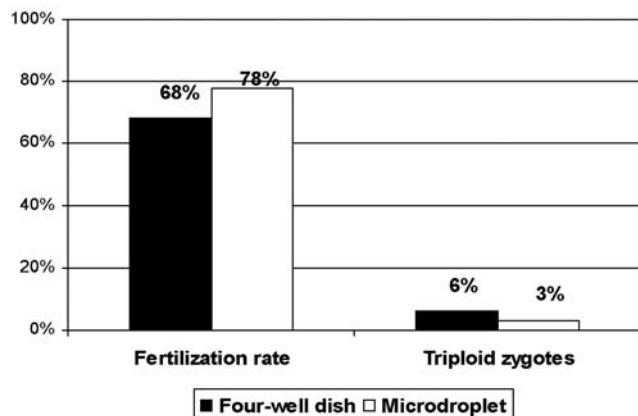
**Table 1.** The characteristics of studied women

Parameter	Group A	Group B
Average age of the patients	31.2	30.8
The average number of stimulation days	12.1	11.3
The average number of oocytes per cycle	13.4	12.7

Culture of embryos in groups in drops of culture medium under an oil overlay is the preferred and most effective method of culturing embryos to date. It has been reported in mouse, sheep and cow that cleavage rate and blastocyst formation increase when embryos are cultured in groups. There are publications claiming a much better embryological outcome when human embryos are cultured in groups than a single embryo per drop. In our study we did not find any differences among two groups according to the embryo development [6-8].

Unfortunately, there is a lack of publications in the field of type of embryo culture. Although the two "main" systems are well known, there are lots of small diversifications of the method used according to experience, habits and knowledge of embryologist in charge. It seems the embryo culture systems require unification worldwide [3].

The microdroplet culture is characterized by a higher fertilization rate, probably due to a homogeneous sperm distribution in the culture medium. This method allows a better control over the embryo development (the ability of frequent monitoring without exposing the embryos to harmful conditions outside the incubator). The embryos of best developmental potential can therefore be chosen for ET. It seems that the only advantage of an open culture is a paracrine influence of several cumuli cultured together. The differences in time outside the incubator are of no practical importance, nor are the differences in laboratory expenses.

**Fig. 2.** The rate of fertilization in two different culture methods

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