GYNEMEDIA

In vitro culture of human embryos up to day five in simplex optimized medium GM 501

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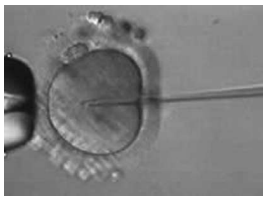
Summary

This study retrospective compared the clinical pregnancy rates after in vitro culture of human embryos in a simplex optimized medium (Gynemed GM 501) or various other commercially available media. During this study, the usual protocol and laboratory routine of 14 participating centres was maintained for all media used including GM 501.

Only the two inclusion criteria, age of patients and number of failed treatment cycles had to be observed for the selection of patients.

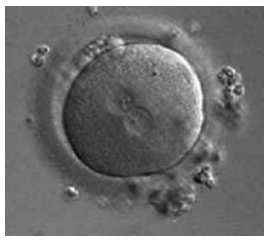


A total of 838 treatment cycles (486 in GM 501 and 352 in other media) of 14 working groups were recorded. The results show that the in vitro culture in simplex optimized ready-to-use medium GM 501 from the zygote to the blastocyst yields pregnancy rates at least as high as other media routinely use in the individual centres.

Introduction

In reproductive medicine, the development of media for in vitro culture was influenced by two philosophies. The development of sequential media is based on the „Back to the nature“ principle. Here, media were developed which correspond to the different environments in the fallopian tubes and the uterus. However the fluid exchange between fallopian tube and uterus, and thus a blending, is not taken into consideration. Thus the embryo is in a micro-environment, whose composition cannot be clearly defined and most probably varies dynamically. Although analysis of the composition of collected oviductal and uterine secretions may not represent the particular microenvironment due to various technical factors e.g. stabilisation of the components until analysis. In addition, the change of the medium during in vitro development can cause stress to the embryo, as it needs to adapt its metabolism due to the sudden change in the medium's composition. Furthermore substances with a beneficial effect, e.g. growth factors produced by the embryo, are diluted.

The other philosophy, namely the simplex optimized media, is based on the „Let the embryo choose“ principle. The basis for the development of such a „computer-optimized-medium“ is a mathematical model,



which made it possible to optimize simultaneously the combination and concentration of various components. Such a medium contains all the components an embryo needs during in vitro development from day 1 to day 5 (Summers and Biggers, 2003, Biggers et al. 2005). KSOM is such a medium developed by Biggers et al. in Boston at the beginning of the nineties. SOM in KSOM stands for „Simplex Optimization Medium". Within the scope of further optimization, KCL was added and let to the name of KSOM. The advancement into KSOM^{AA} is based on the addition of essential and non-essential amino acids (Biggers, 2005).

Gynemed GM 501 is a modified KSOM^{AA}. The composition and concentrations of some compounds were adjusted according to the latest knowledge and publications regarding media intended for culturing human embryos.

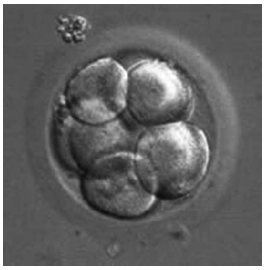
Materials and Methods

To maintain similarity between both groups, only those patients were included which were younger than 37 years and had at least no more than two unsuccessful treatment cycles. The assignment of the patients into either study group GM 501 or control group was left to the centres. In total, 12 centres in Germany and two centres in Austria used the medium Gynemed GM 501 between 2005 and April 2006.

A total of 838 treatment cycles (486 in GM 501 and 352 in other media) were recorded and analysed. The data were pooled although IVF and ICSI was used as ART. Clinical pregnancy was defined as distinct intrauterine gestational sac seen on transvaginal ultrasound. There were no given specifications about the use of the medium so that all centres could employ the medium within their specific routine laboratory conditions. The embryo transfer took place between day 2 and day 5. Next to the in vitro culture in Gynemed GM 501, gametes and embryos of the control group were cultured in media obtained from Medicult, Vitrolife or Sage.

Statistical analysis was performed by the SPSS statistical package. The patient parameters such as age, treatment cycle, number of collected oocytes, fertilisation rate, transfer rate and number of transferred embryos from both groups were compared with the nonparametric Mann-Whitney test. A P value of $< 0,05$ was considered as significant.

Results



In total, 838 cycles were reported (486 in GM 501 and 352 in routinely use media). The transfer rate, the age of the patients, the number of collected oocytes, the number of injected or inseminated oocytes and the number of embryos transferred were not significant different in both groups. Only the number of treatment cycle was significantly different between both group, although all patients met the inclusion criteria of no more than two unsuccessful previous cycles (Tab. 1). Additionally the number of fertilized oocytes was significant different.

	GM 501	routine media	p value
No. of cycles	486	352	
No of transfers	465	338	
Transfer rate in %	96%	96%	NS
No. of clinical pregnancies	187	129	
age (years) (mean+/-SD)	31,3 +/-3,4	32,0+/-3,0	NS
No. of treatment cycle (mean+/-SD)	1,54+/- 0,7	1,76+/-0,8	< 0,05
No. of oocytes (mean+/-SD)	10,1+/- 5,7	9,5+/- 5,8	NS
No. of injected/inseminated Oc (mean+/-SD)	8,8+/- 5,1	8,6 +/- 5,7	NS
No. of fertilized oocytes (mean+/- D)	5,5+/- 3,8	4,9+/- 3,7	< 0.05
Fertilisation rate (%)	62%	56%	
No. embryos/transfer (mean+/-SD)	1,9+/- 0,6	1,9+/- 0,6	NS
Pregnancy rate/puncture (%)	38%	37%	NS
Pregnancy rate/transfer (%)	40%	38%	NS

Tab. 1: *Parameter, fertilisation rates and pregnancy rates in ART using GM 501 medium*

In this retrospective analysis, there was no statistical difference in the clinical pregnancy between both groups according to the follicle puncture. The pregnancy rate was 38% after development in Gynemed GM 501 and 37% after in vitro culture in other media employed (Fig. 1). In reference to the embryo transfer the clinical pregnancy rate is also not statistical different with 40% in GM 501 IVF and 38% in other media (Fig. 1).

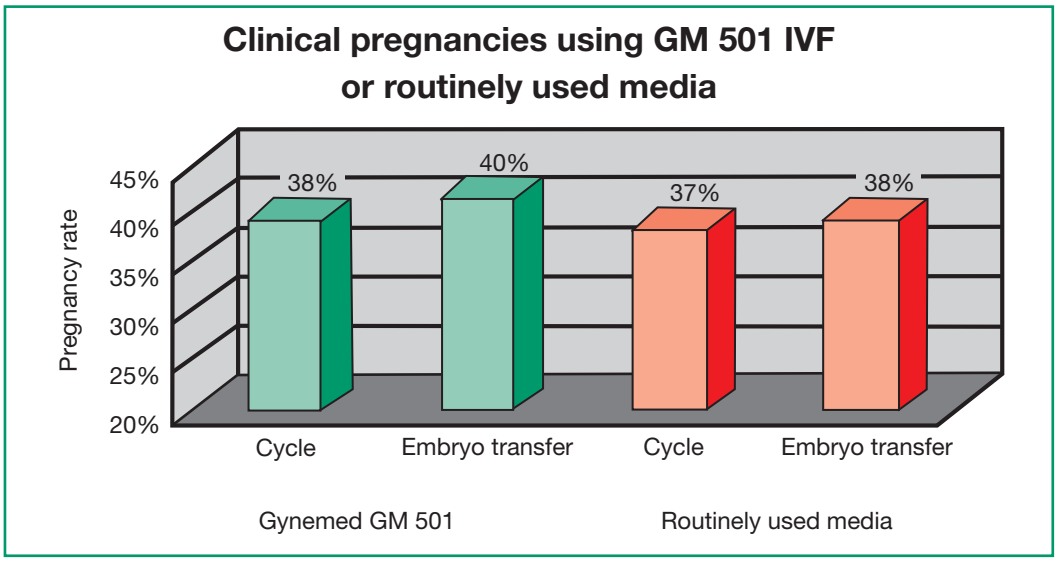
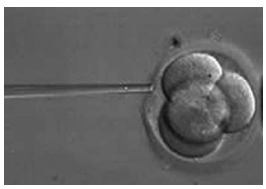


Fig. 1: *Pregnancy rate after in vitro culture in GM 501 or in routinely used media*

Discussion

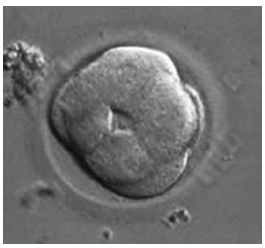
The results show that good pregnancy rates are possible when Gynemed GM 501 medium is used in comparison with other commercial media. A limiting factor was that 12 clinics work under the German Embryo protection law. This law allow only embryo selection at the pronuclear stage and the transfer of no more than three embryos. Despite the number of treatment cycles the patient characteristics in both groups are not significantly different. All patients met the inclusion criteria no more than two failed previous treatments. The reason for this difference is not clear. But we can assume, that the influence on the pregnancy rate between the first, second and third cycle is not so strong, that the groups are not comparable. Interestingly the fertilisation rate is significant higher in the GM 501. One explanation could be, that the medium contains Glucose, a important energy supply for the spermatozoa.



Biggers and Racowsky (2002) showed that the embryo development of human zygotes to blastocysts in KSOM^{AA} does not provide any significant differences when compared with sequential media. Stecher et al. (2005) also showed that the use of a simplex optimized medium (Global One) does not have an adverse effect on the development of human zygotes. During a total of 135 treatment cycles they could achieve a pregnancy rate of 49% with embryo culture in Global One. Using a sequential medium by Vitrolife, a pregnancy rate of 41% was achieved. Aoki's study (2005) compared the development of human embryos and the pregnancy rate when the embryos were cultured in four different media. Once again it was shown that the embryo development was

identical or even better during culture in Global One compared to culture in sequential media. The clinical pregnancy rate did not significantly differ in all four culture media. Greenblatt et al. (2005), when comparing the Media Global One by Live Global and G1/G2 by Vitrolife, also came to the conclusion that the embryo development in Global One was better than in G1/G2 cultures by Vitrolife. However the pregnancy rates were comparable.

An essential contribution to the adequate development of human embryos can be seen in the addition of amino acids to the medium. Ho et al. (1995) were able to show in murine embryos that the development of embryos in KSOM to which amino acids had been added did not differ from the in vivo development. Biggers et al. (2000) arrived at the same conclusion. Adding amino acids to the medium had a positive effect on the development to blastocysts. Also Rinaudo et al., (2004) observed a higher cleavage rate, an earlier cavitation and hatching of murine embryos cultured in KSOM^{AA} in comparison to in Whitten's medium without amino acids.



Gardner et al. (2003) compared the development of human embryos during culture in G1/G2 and KSOM^{AA}. It was shown that in G1/G2 more viable blastocysts developed than in KSOM^{AA}. He suspected the reason to be the addition of ammonium compounds, caused by the breakdown of the amino acid glutamine. The toxic effect of glutamine suspected by Gardner et al. (2003) could however not be confirmed by other working groups. Summers et al. (2005) compared the development of murine embryos, which were cultured either in a medium with twice the regular amount of glutamine or in a medium with the more stable glycyl-L-glutamine compound, to blastocysts and foetuses. They were not able to ascertain any abnormal foetal development in the mice, even though there were, dependent on the time frame, measurable amounts of ammonium compounds in the medium. The development to blastocysts was slightly better in the medium with the more stable glycyl-L-glutamine compound. Another option to prevent the formation of toxic ammonium compounds is the replacement of the amino acid glutamine with the much more stable peptide alanyl-glutamine (Summers et al. 2005).

For this reason the amino acid glutamine in Gynemed GM 501 was replaced with the more heat stable na-alanyl-glutamine compound to prevent possible toxic effects.

Research by Rinaudo et al. (2004) on the gene expression in murine blastocysts gave another indication that a simplex optimized medium supports the adequate development of embryos.

They compared the gene expression of murine blastocysts that were cultured either in Whitten's medium or in KSOM^{AA} with the gene expression of blastocysts developed in vivo. This was done by using the oligonucleotide micro-array technique. It was shown that the embryos grown in Whitten's medium displayed a defective expression pattern in 114 genes (1,28% of the genome), whereas only 29 genes were misexpressed after culture in KSOM^{AA}.

The use of sequential media offers the advantage of being able to consider the embryo's changing needs and to dilute any potential negatively effective substances by renewing the medium (Gardner und Lane, 2004). It was shown, however, that adequate concentrations of neither glucose nor EDTA hinder the early development of the embryos (Summers et al, 2003, Biggers et al, 2005). Neither did the renewal of a medium in order to dilute the possibly harmful substances lead to a better development of the embryos. Biggers et al. (2005) concluded from these results that sequential media do not offer any advantages for the in vitro development of murine embryos. Biggers and Racowsky (2002) also showed that a renewal of the medium is not necessary for adequate development of human embryos.

For a better assessment only cycles (n=486) which met the inclusion criteria such as female age < 37 years and no more than two previous failed treatment cycles were analyzed in this study. However the Gynemed GM 501 media was also used for older patients and patients with more than three treatment cycles in at least 120 cycles. The pregnancy rate dropped down to 26% according to the follicle puncture and 28% according to embryo transfer. The mean age in this group was 38,2 +/- 2,3 and the mean of the treatment cycle was 2,6 +/-2,0. This data show that the Gynemed GM 501 also support the development of embryos of older patients or patients with a not ideal prognosis.

Next to a good pregnancy rate, a cost benefit analysis is of utmost importance. Greenblatt et al. (2005) compared the costs of culturing embryos in Global One by Live Global and G1/G2 by Vitrolife. The costs for the sequential medium were four times higher than the costs for the simplex optimized medium.

In conclusion, in vitro development of embryos in Gynemed GM 501 let to the same pregnancy rate as in other commercially available media. Apart from the comparable pregnancy rate, Gynemed GM 501 is user-friendly, easy to implement due to its ready-to-use formulation and has a favourable cost benefit. It supports the development of embryos from

the zygote to a blastocyst stage in one single medium. Another important and relevant advantage of Gynemed GM 501 is the extended shelf life of 6 months which keeps logistics and ensuing costs simple and manageable for the laboratory.

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