LARGE EVALUATION STUDY CONFIRMS: NEW FORMULATIONS OF SOLUTIONS FOR FREEZING AND THAWING OF CLEAVAGE STAGE EMBRYOS RESULT IN OUTCOMES SIMILAR TO AFTER VITRIFICATION IN MORE THAN 1000 PATIENTS

Vitrification has become the most common cryopreservation method for oocytes and blastocysts due to superior results compared to slowfreezing. Many clinics worldwide however still employ slow-freezing for cleavage stage embryos despite lower survival rates compared to vitrification. The reasons may be that the slowfreezing method is comparatively easy to learn and is time efficient when there are many oocytes or embryos available for cryopreservation. The freezing machine is also often already in place. With the aim to investigate if survival rates could be improved, the study[1] abbreviated below evaluated a new formulation of solutions for slowfreezing of cleavage stage embryos.

Methods

The evaluation was divided into two parts. In the first retrospective part, results with the new formulations of freeze and thaw solutions (108 patients) were compared to the results of the older products which had been used in the clinic for a long time (400 patients).

The second part of the study was prospective. Group A included 1273 embryos from 542 patients and group B included 2274 embryos from 897 patients.

Embryos were frozen on day 3 of development. The primary endpoint was survival rate.

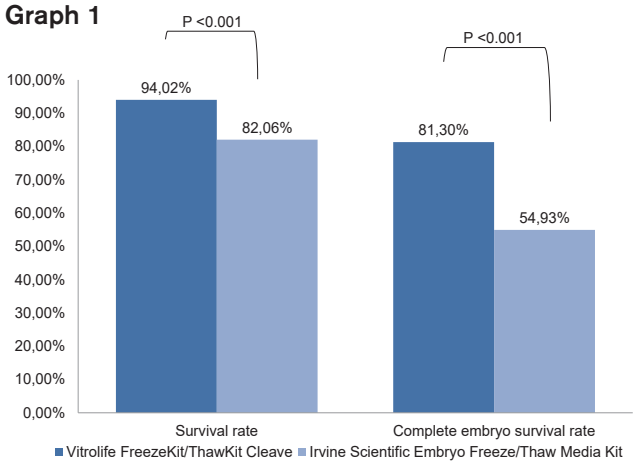
Embryos were cultured in the G-Series™ media (Vitrolife Sweden AB). The products used for cryopreservation were FreezeKit Cleave™ and ThawKit Cleave™ (new formulations, Vitrolife Sweden AB, group A) and Embryo Freeze media and Embryo Thaw media (old formulations, Irvine Scientific, USA, group B). The new formulations contain cryoprotectants according to Edgar et al[2] and the base medium contains amino acids for support of embryo viability with the addition of MOPS buffer for physiological pH maintenance plus hyaluronan which has been shown to embryo survival after cryopreservation[3,4].

Results

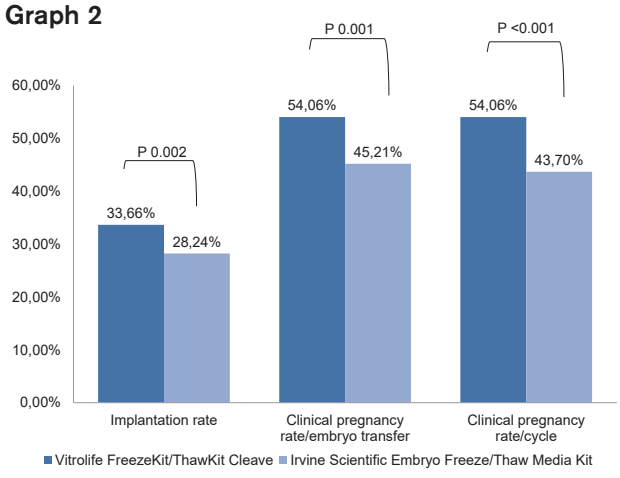
There were no differences between the groups regarding age of female patients, number of retrieved oocytes, fertilisation rates, number of good quality embryos or number of embryos per transfer. During the first evaluation period significantly improved survival rates were obtained in group A, and the pregnancy rate per cycle was also significantly higher. In group B, 18 embryo transfers had to be cancelled due to no surviving embryos while no transfers at all had to be cancelled in group A.

During the second evaluation period the difference in cancelation rate remained. Significantly improved survival rates as well as the clinical pregnancy rate were also shown in this extended part of the evaluation. When compiling the results from the complete evaluation period, it was clear that survival rates, implantation rate and clinical pregnancy rate both per embryo transfer and per cycle was significantly higher in group A while cancellation rate was higher in group B. Graph 1, 2, and table 1.

Graph 1



Graph 2



Vitrolife

1.161121.1

Table 1

	Vitrolife FreezeKit/ThawKit Cleave	Irvine Scientific Embryo Freeze/Thaw Media Kit	P value
Age of female patients	30,82 ± 4,26	30,58 ± 4,31	0.464
Number of freezing cycles	1065 (108 + 957)	1590 (400 + 1190)	
Number of thawing cycles	542 (108 + 434)	897	
Number of transfers	542 (108 + 434)	867 (485 + 382)	
Cycle cancellation rate	0% (0/42)	3,34% (30/897)	<0.001**
Survival rate	94,02% 1197 (240 + 957)/1273	82,06% 1866 (822 + 1044)/2274	<0.001**
Complete embryo survival rate	81,3% 1035 (203 + 832)/1273	54,93% 1249 (528 + 721)/2274	<0.001**
Implantation rate	33,66% 379(69+310)/1126 (217 + 909)	28,24% 492 (195 + 297)/1742 (750 + 992)	0.002**
Clinical pregnancy rate/embryo transfer	54,06% 293(60 + 233)/542 (108 + 434)	45,21% 392 (174 + 218)/867(382 + 485)	0.001**
Clinical pregnancy rate/cycle	54,06% 293(60 + 233)/542 (108 + 434)	43,7% 392 (174 + 218)/897 (400 + 497)	<0.001**

**Significant (P < 0.01) difference between group Vitrolife and Irvine

Conclusion

With the new formulation of solutions for freezing and thawing of cleavage stage embryos significantly improved survival rates, clinical pregnancy rates and implantation rate were achieved. In fact, a survival rate of above 90% using the new formulations of solutions is very similar to that obtained after vitrification.

1. Fang L, Jin L, Li E, Cui L. and Ye Y. (2016). Clinical evaluation of two formulations of slow-freezing solutions for cleavage stage embryos. J Assist Reprod Genet 33(10): 1389-1393. 2. Edgar DH., Karani J. and Gook D. (2009). Increasing dehydration of human cleavage-stage embryos prior to slow cooling significantly increases cryosurvival. Repr Biomed Online 19(4): 521-525. 3. Gardner DK., Maybach J. and Lane M. (2001). Hyaluronan and RHSA increase blastocyst cryosurvival. Proc 17th World Congress on Fertility and Sterility, Melbourne. P 226. 4. Lane M., Maybach J., Hooper K., Hasler JF. And Gardner DK. (2003). Cryosurvival and development of bovine blastocysts are enhanced by culture with recombinant human albumin and hyaluronan. Mol Reprod Dev. 64: 70-78.

Vitrolife