

## EFFECTIVE CULTURE METHOD FOR GAMETES AND EMBRYOS OF POOR PROGNOSIS PATIENTS

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Objective: calcitonin (Ct) and Plastoquinone derivatives (PQ) potentially can affect upon several specific for aged oocytes features: mitochondrial function, ATP-level, intracellular calcium level, adenylyl cyclase activity and antioxidant insufficiency. Our task was to identify in vitro beneficial effects of these compounds on embryo developmental dynamics in poor prognosis patients.

Design: prospective case-control study.

Materials and methods: to identify the ability of Ct (20 ng/ml) and PQ ( $10^{-13}$  mol/l) to improve embryo development we formed two groups of patients with poor prognosis: group 1 (n - 11) - women under 35 years with repeated IVF attempt with "grievous" embryo development in previous treatment cycle (maximum 1 blastocyst, or no blastocysts from at least of 6 zygotes); group 2 (n - 11) - women 40-44 years old, with at least 4 oocytes. In each group, study and control cohorts were evenly formed from sibling oocytes. Ct and PQ were added to fertilization medium, where gametes were inseminated/placed after ICSI. After pronuclei checking, zygotes were placed to nonmodified single step medium. Embryos were cultured in drops, in groups, in low O<sub>2</sub> atmosphere, in benchtop incubators.

Embryos with at least 6 even blastomeres and fragmentation level less than 20% on 72 hours recognized as good; good blastocysts are BB3 and better. For statistical analysis we used paired t-test.

Results: In patients of advanced age we found statistically significant improvements: in embryo quality on 72 hours and blastocyst rate. In young women with unfavorable embryo development history we obtained significant increase in blastocyst rate. Besides this, Ct+PQ embryos has absolutely another dynamic they often show day 3 early compaction and blastulation on early day 4.

Group 1 (women under 35 years)			
	Control	Study	P
Oocytes	84	81	
Fertilization	60	68	>0,05
Good quality embryos on 72 h	45	58	>0,05
Blastocysts	9	25	<0,05
Group 2 (women 40-44 years)			
	Control	Study	P
Oocytes	51	51	
Fertilization	40	43	>0,05
Good embryos on 72 h	29	40	<0,05
Blastocysts	8	20	<0,05

Conclusion: gamete culture in Ct+PQ medium during early hours after oocyte pick up has a strong positive effect on embryo development in women of advanced reproductive age, and in young women where we had none or very small amount of blastocysts in previous treatment cycles. Finally, we believe that it can be the way to improve treatment results in cohort of "difficult" patients. Changes in embryo developmental dynamics, probably, can be more important for elder women and can be beneficial for embryo-endometrial synchronicity.

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