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human reproduction ORIGINAL ARTICLE *Infertility*

Treatment with Ca^{2+} ionophore improves embryo development and outcome in cases with previous developmental problems: a prospective multicenter study

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STUDY QUESTION: Does calcium ionophore treatment (A23187, calcimycin) improve embryo development and outcome in patients with a history of developmental problems/arrest?

SUMMARY ANSWER: Application of A23187 leads to increased rates of cleavage to 2-cell stage, blastocyst formation and clinical pregnancy/live birth.

WHAT IS KNOWN ALREADY: Studies on lower animals indicate that changes in intracellular free calcium trigger and regulate the events of cell division. In humans, calcium fluctuations were detected with a peak shortly before cell division. Interestingly, these calcium oscillations disappeared in arrested embryos. Mitotic division blocked with a Ca^{2+} chelator could be restored by means of ionophores in an animal model.

STUDY DESIGN, SIZE, DURATION: This prospective, multicenter (five Austrian centers), uncontrolled intervention study (duration 1 year) includes 57 patients who provided informed consent.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Inclusion criteria were complete embryo developmental arrest in a previous cycle (no transfer), complete developmental delay (no morula/blastocyst on Day 5), or reduced blastocyst formation on Day 5 ($\leq$15%). Severe male factor patients and patients with $<$30% fertilization rate after ICSI were excluded because these would be routine indications for ionophore usage. The total of the 57 immediately preceding cycles in the same patients constituted the control cycles/control group. In the treatment cycles, all metaphase II-oocytes were exposed to a commercially available ready-to-use ionophore for 15 min immediately after ICSI. After a three-step washing procedure, *in vitro* culture was performed as in the control cycles, up to blastocyst stage when achievable.

MAIN RESULTS AND THE ROLE OF CHANCE: Fertilization rate did not differ (75.4 versus 73.2%); however, further cleavage to 2-cell stage was significantly higher ($P < 0.001$) in the ionophore group (98.5%) when compared with the control cycles (91.9%). In addition, significantly more ($P < 0.05$) blastocysts formed on Day 5 in the study compared with the control group (47.6 versus 5.5%, respectively) and this was associated with a significant increase ($P < 0.01$) in the rates of implantation (44.4 versus 12.5%), clinical pregnancy (45.1 versus 12.8%) and live birth (45.1 versus 12.8%). All babies born at the time of writing (22/28) were healthy.

LIMITATIONS, REASONS FOR CAUTION: The frequency of patients showing embryo developmental problems was expected to be low; therefore, a multicenter approach was chosen in order to increase sample size. In one-third of the cycles, the clinician or patient requested a change of stimulation protocol; however, this did not influence the developmental rate of embryos.

WIDER IMPLICATIONS OF THE FINDINGS: This is the first evidence that developmental incompetence of embryos is an additional indication for ionophore treatment. The present approach is exclusively for overcoming cleavage arrest.