

Growth differentiation factor 9 (GDF-9) levels in spent culture media and pregnancy outcome

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Objective: GDF-9 is a growth factor that is secreted by the oocyte during folliculogenesis and also from the embryo. The objective of this study was to measure GDF-9 levels in spent culture media from embryos and compare them to pregnancy outcome.

Design: Prospective study.

Materials and methods: A total of 113 patients with frozen embryo transfers were included in the study. Following ICSI, oocytes were cultured in an EmbryoScope™ time lapse incubator for up to 6 days. The EmbryoScope™ was set at 37C, 5.5% CO₂ and 5.0% O₂, with image acquisition every 10 minutes. Embryos were biopsied for preimplantation genetic analysis and vitrified on Day 5 or 6, at which time 22µL of spent culture media were removed and placed in protein-free PCR tubes, and frozen at -80°C until time of analysis. Videos of embryo culture were annotated for various time points according to cell divisions. Euploid embryos were warmed for transfer and the corresponding spent culture media was thawed and analyzed for the presence of GDF-9 using the AL-176 GDF-9 ELISA kit. Pregnancy outcome was quantitatively determined 14 days post embryo transfer (biochemical) and by ultrasound 6-7 weeks after transfer to assess viability (clinical).

Results: Day 5 embryos yielded significantly higher biochemical and clinical pregnancy rates as well as GDF-9 values compared to Day 6 embryos (Table 1). For day 5 embryos, there were no significant differences in time lapse morphokinetic (TLM) parameters between the non-pregnant, clinically pregnant and miscarriage groups. However, for Day 6 embryos, the non-pregnant group showed significant differences in TLM than the clinically pregnant group in most of the time points up to the 8-cell stage and start of blastulation. GDF-9 did not show any significant differences between non-pregnant and pregnant groups of Day 5 or Day 6 embryo transfers. No GDF-9 was detected in the control spent media (without embryos), showing that the GDF-9 was produced by the embryo.

Table 1: Pregnancy outcome and GDF-9 levels in spent media of Day 5 and Day 6 embryos

	Biochemical pregnancy (%)	Clinical pregnancy (%)	GDF-9 (pg/mL)
Day 5 (n=57)	46/57 (80.7 ^a)	39/57 (68.4 ^b)	6,456.6 ± 3,851.6 ^c
Day 6 (n=56)	35/56 (62.50 ^a)	27/56 (48.21 ^b)	3,933.6 ± 2,705.9 ^c

^aP=0.0326; ^bP=0.0302, ^cP=0.0002. Values expressed as mean±SD

Conclusions: GDF-9 can be detected in spent culture media as a by-product from the embryo and decreases significantly from Day 5 to Day 6, with corresponding decreases in biochemical and clinical pregnancy rate. Even though no significant differences were noted in GDF-9 in the pregnancy groups on Day 5 or Day 6, ongoing studies are underway to evaluate the feasibility of GDF-9 as a marker for embryo quality.

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