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Embryo production by intracytoplasmic injection of haploid germ cells derived in vitro from bovine neonatal gonocytes.

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An effective procedure for recapitulating spermatogenesis in vitro would greatly facilitate mechanistic studies of the in vivo process while providing a biological basis for treating male infertility and genetically modifying the male germ line. We developed a novel long-term culture system which spermatocytogenesis and meiosis can be achieved even when starting with an infantile testis. The objective of this study was to investigate whether presumptive round spermatids produced by in vitro culture of neonatal testis cells are developmentally competent. The testis of a 3 day-old bull was decapsulated mechanically and seminiferous tubules were dissociated to recover Sertoli cell and germ cells by enzyme treatment. Some of dissociated cells were transfected by lipofection with the pIRES2-EGFP vector containing the enhanced green fluorescent protein (EGFP) coding region. Transfected or non-transfected cells were washed, re-aggregated and encapsulated into tubule-like structures, which were then cultured for 14 weeks in modified MEM/F12 media at 32°C, 5% CO2 in humidified air. At 14 weeks after culture, presumptive round spermatids (7-10μ m) are obtained from cultured cell aggregate, aspirated into an injection pipet, and injected into denuded, in vitro matured, activated oocytes. During culture for 10 days after round spermatid injection (ROSI), embryonic development was monitored and developing embryos were evaluated for presence of the Y-chromosome based on PCR with Y-chromosome specific primers or by karyotyping , and for transfection based on GFP expression.Embryonic cleavage rate was not different between ROSI and activation control groups, but the rate of blastocyst formation in the ROSI group (24/56, 42.9%) was higher than activated controls (12/50, 24.0%). 36.8% (14/38) of blastocysts and 50% (14/28) of embryos obtained from ROSI were Y-chromosome positive and diploid. Also, transfection efficiency was high (~70%) and GFP expression remained high in all cell types throughout the 14-week culture period. And, 56.7% (21/37) of blastocysts obtained from ROSI exhibited GFP expression. None of the blastocysts obtained from activation alone contained a Y-chromosome or expressed GFP. We conclude that presumptive round spermatids obtained by a novel culture procedure were competent to produce diploid bovine embryos.