Gonadal development following blastocyst complementation in mice

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Blastocyst complementation is a technique in which embryonic stem cells (ESCs) are injected into genetically deficient blastocyst that lack certain cell types in an organ. Without the competition from the host, the injected ESCs are able to populate the niche and rescue the phenotype. Such technique has been used in organs such as pancreas and brain. We hypothesize that injected mouse ESCs (mESCs) into a mouse blastocyst that has been genetically modified to not develop gonads, will populate the gonadal niche and gonads will develop from the injected mESCs. These cells can be modified prior to injection and a screening tool can be established to streamline the study of genes that are important for gonadal development. To test this hypothesis, we injected wildtype or genetically modified mESCs into recipient blastocysts that are unable to form gonads. The gonad-depleted blastocysts were generated by crossing the *Nr5a1*-cre mice with the Rosa-DTA mice, resulting in apoptosis of all gonadal supporting cells and adrenal glands. The absence of gonads and adrenals in the recipient blastocyst provides a niche for donor ES cells to colonize and form gonad and adrenals of mESC origin. We injected either XY mESCs labelled with the red fluorescent protein (XY-RFP) or XX mESCs labelled with the green fluorescent protein (XX-GFP). The injected *NR5a1-cre;Rosa-DTA* blastocytes were then transferred to pseudo-pregnant females and collected at different stages of development. *Nr5a1-cre;Rosa-DTA* control embryos without ES cell injection did not develop gonads or adrenals as expected. On the other hand, blastocyst injected with XY-RFP mESCs developed testis with proper appearance of Sertoli cells, Leydig cells, and interstitial cells, regardless of the genetic sex of the recipient blastocyst. When XX-GFP mESC were injected, embryos developed ovaries with granulosa cells and oocytes. Injection of XY mESCs with null mutation of the testis-determining gene *Sry* resulted in the development of ovaries in the injected embryos. When XX-GFP mESCs that carry the *Sry* transgene were injected, the resulting gonads in the injected blastocyst developed as testes. The somatic cell compartment of all gonads that developed following mESC injections originated from the injected mESCs. These results provide evidence that blastocyst complementation can be used as a tool to screen the functional roles of candidate genes in sex determination and gonadal development.