90. FGF9: A MALE SEX-DETERMINING FACTOR IDENTIFIED IN THE OVARY

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The presence of sex-reversed cells (Sertoli and Leydig cells) in the estrogen-deprived, aromatase knock out mouse (ArKO) ovary (1), suggests that estrogen is essential for the maintenance of the 'female' ovarian phenotype. In the course of investigating the expression of 'male'genes (MIS, Sox9, fgf9) by the ArKO ovary we found that fgf9 was present in normal ovary. Fgf9 has been implicated in testicular cord formation (2) and XY fgf9-/- gonads are sex reversed showing predominantly female reproductive structures (oviducts and fused uteri) and histology (3). The apparent pro-male role of fgf9 probably accounts for the lack of data on fgf9 expression by the ovary. The preliminary studies described here, were undertaken to establish if fgf9 mRNA and protein were present in rat ovaries and thus whether fgf9 is regulated by estrogen. Ovaries were collected from Sprague-Dawley rats, 4, 8 and 12 days of age, and 21- and 24-day-old animals that had been treated with DES for 4 or 1 days, respectively. At least 3 groups of ovaries were collected for each age or treatment. Adult rat testis was used as a positive control. RNA was extracted, reverse-transcribed and real time PCR performed. Fgf9 primers amplified a 222 bp cDNA fragment. GAPDH was used for data normalisation. Fgf9 protein was immunolocalised on formalin-fixed, paraffin-embedded sections of ovary (age and treatments as above) using a specific antibody. Levels of FGF9 mRNA were highest in the rat ovary 4 days after birth, declining to approximately 20% of these levels on postnatal days 8 and 12, which was similar to levels expressed by adult testis. DES treatment increased FGF9 mRNA expression by the ovary within 24 hours, ultimately reaching 2-fold after 4 days of treatment. Fgf9 protein was localised to theca and interstitial cells of postnatal and DES-treated ovaries. Fgf9 protein was undetectable in granulosa cells and oocytes. Based on its localisation to the ovary (mRNA and protein) and its regulation by estrogen, the data support our hypothesis that fgf9 is a local regulator of ovarian function. Supported by the NH&MRC of Australia (Regkey 241000).

(1) Britt *et al.* (2002) *FASEB J.* **16**: 1389–97. (2)Cotinot *et al*. (2002) *Semin. Reprod. Med*. **20**: 157–68. (3) Colvin *et al*. (2001) *Cell* **104**: 875–89.