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Review

Role of androgen and gonadotrophins in the development and function of the Sertoli cells and Leydig cells: Data from mutant and genetically modified mice

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ABSTRACT

Development and maintenance of the male phenotype and establishment of fertility are all dependent upon the activity of the Sertoli cells and Leydig cells of the testis. This review examines the regulation and function of these cell during fetal and post-natal development. Fetal Leydig cells are sensitive to both luteinising hormone (LH) and adrenocorticotrophic hormone (ACTH) but Leydig cell function appears normal in fetal mice lacking both hormones or their receptors. Post-natally, the Sertoli cells and Leydig cells are reliant upon the pituitary gonadotrophins. Leydig cells are critically dependent on LH but follicle-stimulating hormone (FSH), presumably acting through the Sertoli cell, can also affect Leydig cell function. Testosterone secreted by the Leydig cells acts with FSH to stimulate Sertoli cell activity and spermatogenesis. Study of animals lacking FSH-receptors and androgen-receptors shows that both hormones can act to maintain the meiotic germ cell population but that androgens are critical for completion of meiosis.

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1. Introduction

The testes are regulated by the pituitary gonadotrophins follicle-stimulating hormone (FSH) and luteinising hormone (LH). In the post-natal animal LH stimulates Leydig cells to secrete testosterone and both testosterone and FSH act to promote spermatogenesis through direct stimulation of the Sertoli cells. Developmental changes in circulating hormone levels and some of the major landmarks in testis development are shown in Fig. 1. Most of this basic description of testicular regulation has been known since the 1920s and 1930s, through the pioneering work of Smith, Greep and others (Smith and Engle, 1927; Greep and Fevold, 1937; Walsh et al., 1934). However, through the introduction of highly purified and recombinant gonadotrophins and, more recently, the generation of genetically modified mice (Huhtaniemi, 2006) we have been able to define more clearly both the role of these hormones and their mechanism of action at the cellular level. This article both reviews and

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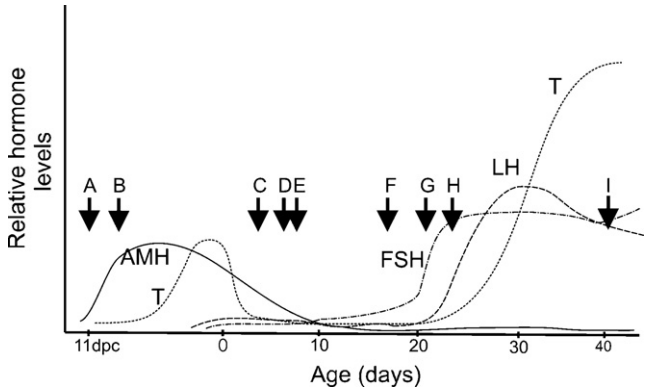

Fig. 1. Relative changes in circulating hormone levels during development in the male mouse. The approximate timing of landmark events in the development of the testis is shown by vertical arrows. (A) Testis differentiation; (B) fetal Leydig cell differentiation; (C) spermatogonial differentiation; (D) adult Leydig cell differentiation; (E) onset of meiosis; (F) end of Sertoli cell proliferation; (G) formation of Sertoli cell barrier; (H) testis descent; (I) mature sperm present in testis.

presents novel data on the development and hormonal regulation of Leydig cell and Sertoli cell function. Our data relates specifically to the mouse but at the end of this article we have also tried to show how relevant the mouse is as a model for understanding control of testis development and function in the human.

2. Leydig cells

2.1. Fetal Leydig cells

Fetal Leydig cells first appear in the mouse at about 12.5 days post-coitum (dpc), developing from mesenchymal-like stem cells within the interstitial space between the tubules (Byskov, 1986). Differentiation requires desert hedgehog (DHH), platelet-derived growth factor (PDGF) (possibly PDGFC) and expression of the X-linked Aristaless-related homeobox gene (*Arx*) (Pierucci-Alves et al., 2001; Yao et al., 2002; Brennan et al., 2003; Kitamura et al., 2002). DHH is secreted primarily by the Sertoli cells but *Pdgfc* is not of Sertoli cell origin and *Arx* expression is limited to peritubular myoid cells, endothelial cells and fibroblast-like cells in the fetal testis (Kitamura et al., 2002) showing that cells, other than the Sertoli cells, are involved in the differentiation process.

The fetal Leydig cells are essential regulators of masculinisation. Secretion of testosterone acts to rescue and stimulate growth of the male reproductive tract and external genitalia while a combination of testosterone and insulin-like growth factor 3 (INSL3) ensures appropriate testicular descent (Klonisch et al., 2004). In the mouse and rat it is not, however, clear how fetal Leydig cell function is regulated. Post-natal Leydig cells are critically dependent on LH and fetal Leydig cells respond to LH *in vitro* but Leydig cell function is normal in the fetus in the absence of LH or its receptor (O'Shaughnessy et al., 1998; Zhang et al., 2001; Ma et al., 2004). Interestingly, in mice lacking a pituitary gland through a null-mutation in the *T/ebp* gene, testosterone levels are markedly reduced in late gestation indicating that for fetal Leydig cell function to be maintained in the normal range pituitary hormone support is essential (Pakarinen et al., 2002). Levels of pituitary-derived adrenocorticotrophic hormone (ACTH) are high in late fetal life and we have shown that fetal Leydig cells express the ACTH receptor (Johnston et al., 2007) and respond to ACTH *in vitro* with an increase in testosterone production (O'Shaughnessy et al., 2003). Study of mice lacking ACTH showed, however, that fetal testosterone levels were normal in these animals and that ACTH alone was not, therefore, responsible for fetal Leydig cell function (O'Shaughnessy et al., 2003). Nevertheless, the sensitivity of fetal Leydig cells to both LH and ACTH raised the possibility that both hormones may act, in a redundant fashion, to regulate fetal testosterone production *in vivo* (i.e. loss of one hormone or its receptor may be compensated by the presence of the other hormone). To test this hypothesis we generated mice lacking both ACTH (Pomc-null) and the LH receptor (LuRKO) and measured testosterone levels in late fetal and early neonatal development (Fig. 2). Results showed that testosterone levels were normal in double knockout mice in late gestation and while there was reduced testosterone on the day of birth (day 1) this was not significantly different to LuRKO mice. This data indicates that LH and ACTH do not regulate fetal Leydig cell function and suggests either that another pituitary hormone is required or that the loss of fetal Leydig cell function in the *T/ebp*-null is not related to the loss of the pituitary and that other factors such as extra-pituitary hormones or paracrine factors may be involved. It is noteworthy that a number of putative paracrine factors have been shown to regulate the steroidogenesis of fetal, but not adult Leydig cells (El Gehani et al., 1998).

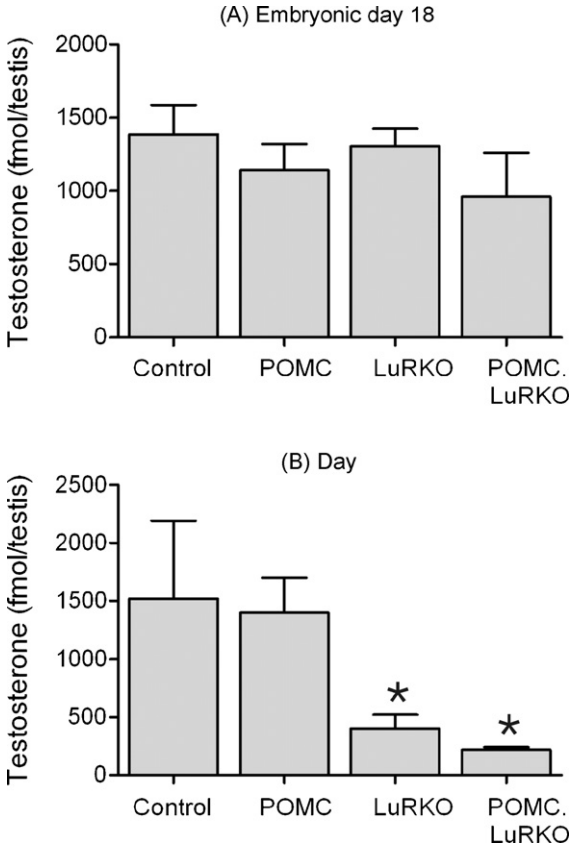

Fig. 2. Intratesticular testosterone levels in normal (control), LuRKO, POMC-null (POMC) and LuRKO/POMC-null (LuRKO·POMC) mice. Intratesticular testosterone levels were measured on day 18 post-coitum (A) and on post-natal day 1 (B). Results show the mean ±S.E.M. of 3–8 animals in each group. Two-factor analysis of variance was used to analyse the data at each age. At post-coitum day 18 there was no difference between groups. At day 1 there was a significant effect of the LuRKO modification (*$P < 0.05$) but no effect of POMC-null and no interaction.

2.2. Adult Leydig cells

After birth, at about day 7 in the mouse, a second population of Leydig cells starts to differentiate (Baker et al., 1999; Nef et al., 2000). This "adult" population of Leydig cells gives rise to the pubertal increase in testosterone levels which is required for adult fertility. Unlike the fetal Leydig cell population, the factors regu-