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INVITED RESEARCH HIGHLIGHT

Male Health

Reduced fetal androgen exposure compromises Leydig cell function in adulthood

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Disruption of normal fetal development can influence functioning of organs and cells in adulthood. Circumstantial evidence suggests that subtle reductions in fetal androgen production may be the cause of adult male reproductive disorders due to reduced testosterone production. The mechanisms through which these fetal events affect adult testosterone levels are largely unknown. A recent paper of Kilcoyne *et al.* provides evidence that fetal reduction in androgen production or signaling results in a reduced Leydig stems cell number after birth and concomitant Leydig cell failure in adulthood. This implies that fetal androgen deficiency can lead to negative programming of adult Leydig cell (ALC) function, which may have implications for general health, aging, and longevity.

Leydig cells are the main producer of androgens in the body and not only play an essential role in the paracrine regulation of spermatogenesis in the testis, but also have various systemic endocrine effects, androgenic and anabolic. Knowledge about the origin of the ALC population and the regulation of Leydig cell function is important for reproductive and metabolic performance. It will also help to understand the aging-related gradual decline in testosterone levels with consequences for not only sperm production and libido but also for general well-being as it affects for instance muscle mass and bone mineral density.

Androgen production in fetal life is initiated with the formation of the fetal Leydig cell (FLC) population, and plays a crucial role in masculinization of the male fetus. After birth, the FLC population regresses and androgen production is low until puberty, when a new population of Leydig cells starts to develop, the ALC population. The definitive origin of the ALC stem cell has not been conclusively defined. Although both ALC and FLC have a common function in androgen production, ALC are not derived from preexisting FLC[1] but have their origin in a stem cell population in the interstitium of the postnatal testes. In 2006 Ge *et al.*[2] were the first to identify and characterize the potential stem cell for the ALC, being a steroidogenically inactive spindle-shaped cell that expresses receptors for platelet-derived growth factor receptor-α and leukemia inhibiting factor, but does not express the luteinizing hormone (LH) receptor or steroidogenic enzymes. There are indications that the stem cells from which the ALCs develop find their origin in the fetal testis,[3,4] but no definitive proof for this hypothesis was available until the publication of Kilcoyne *et al.*[5] in the Proceedings of the National Academy of Sciences USA.

Chicken ovalbumin upstream promoter transcription factor II (*Coup-tfII*), an orphan nuclear receptor of the steroid/thyroid hormone receptor superfamily, has been implicated as an important factor in the development of the ALC population. Based on the studies by Qin *et al.*[3] Kilcoyne *et al.*[5] hypothesize that the *Coup-tfII* expressing cells in the fetal testis that are negative for Leydig cell markers such as LH receptor and 3β-hydroxysteroid dehydrogenase (*Hsd3b*), may be the stem cells for the ALC. The authors further suggest that these stem cells might be susceptible to programming by androgens produced by FLCs during fetal life.

As a first step to prove their hypothesis Kilcoyne *et al.* investigated whether Leydig stem cells are present in the adult testis, using the ethane dimethyl sulphonate (EDS) treated rat as a model. EDS is a compound that specifically destroys Leydig cells in the adult rat testis, followed by a complete regeneration of the ALC population several weeks after a single EDS administration.[6] After the ablation of the ALC population, numerous spindle-shaped *Coup-tfII* positive cells could be detected in the interstitium. By 2 weeks after the EDS injection the first regenerated Leydig cells were observed that expressed *Coup-tf-II* and *Hsd3b*. With progressive regeneration of the Leydig cell population the number of *Coup-tfII*^{pos}/*Hsd3b*^{neg} cells decreased, demonstrating that the new Leydig cells have developed from the *Coup-tfII* positive stem cells. The next step of the authors was to demonstrate that the *Coup-tfII*^{pos}/*Hsd3b*^{neg}, cells, which are also present in the fetal testis, develop into ALC after birth. Using transgenic Cre-recombinase mouse lines Kilcoyne *et al.*[5] are the first to show convincingly that *Coup-tfII*^{pos}/*Hsd3b*^{neg} cells in the fetal testis give rise to the ALC in the postnatal testis; these fetal cells also express the androgen receptor (AR).

Indirect evidence suggests that subtle reductions in fetal androgen production may be the cause of adult male reproductive disorders due to reduced testosterone production. The authors, therefore, investigated whether suppression of FLC androgen production could influence the functioning of the ALC population. To reduce fetal intratesticular testosterone levels pregnant female rats were treated with dibutyl phthalate. This treatment resulted in a 40% decrease in adult Leydig stem cell numbers at the time of birth. Although ALC numbers were normal in adulthood, Leydig cell functioning was severely affected as a consequence of a significant reduction in the expression of steroid acute regulatory protein (*StAR*), a protein that is responsible for the transport of cholesterol from the cytoplasm into the mitochondria of Leydig

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cells and therefore essential for testosterone synthesis.

In order to explain how altered fetal androgen action can reduce *StAR* transcription, and thus ALC function, in adulthood, epigenetic changes via histone methylation were investigated. Altered methylation of the proximal-1 promoter region of *StAR* is crucial for regulating the expression of this gene. The level of H3K27me3, a well-known transcriptional repressor, upstream of the coding region of *StAR* appeared to be significantly increased. H3K27me3 protein was present in a proportion of the ALC of rats in which fetal androgen production was repressed, whereas this epigenetic mark was virtually absent in ALC of control animals. H3K27me3 protein also appeared to be present in the *Coup-tfII*^{pos}/*Hsd3b*^{neg} stem Leydig cells in the adult testis, implicating a possible mechanism through which deficiency in fetal androgen action on stem cells can reprogram ALC function by influencing the transcription of *StAR*. These data fit with increasing evidence from studies in humans, in whom reduced fetal androgen production is shown to be associated with reduced adult sperm count.[7] In line with this assumption, men with reduced sperm count commonly exhibit compromised Leydig cell function. The publication by Kilcoyne *et al*. is the first to show that there may indeed be a relationship between deficits in fetal androgen exposure and ALC function, with consequences for fertility, but also a range of other disorders in men related to inadequate androgen production such as decreased bone mineral density, chronic fatigue, cancer and coronary heart disease.[8] Moreover, since fetal testosterone levels correlate with maternal testosterone levels,[9] this publication potentially adds to scientific literature on the importance of maternal physiology on later life health, in this case reproductive health, of the offspring.

COMPETING INTERESTS

All authors declare no competing interests.

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