Goldilocks famously rejected items that did not fit her criteria of “just right.” In the endocrinology of reproductive development, appropriate feedback along the hypothalamo-pituitary-gonad axis, and indeed the other axes (thyroid, adrenal, etc), is essential in humans and other species in which the fetal hypothalamo-pituitary-gonad axis is active during gestation (1). This complicates our understanding of appropriate levels of developmentally important hormones because feedback effects actively modulate the endocrine system, even in utero. Androgens, principally secreted from the developing fetal testes, are a classic example of critical endocrine signaling during development (2) and are the subject of an important new study in this volume by Connolly et al (25).

It is well known that in mammals the establishment of the male phenotype and development of a functionally healthy male adult, is androgen dependent. This is clearly seen when there is disruption of androgen signaling (eg, reviewed in Ref. 3) leading to a gradient of impaired masculinization that, at the extreme of complete androgen insensitivity syndrome, leaves the individual with an apparent normal female phenotype, although infertile (4). Less extreme versions, partial and moderate androgen insensitivity syndrome, are characterized by greater degrees of masculinization of the individual and are associated with less severe androgen receptor mutations. Therefore, in general the availability of inadequate androgen or inadequate androgen signaling is associated with a clear spectrum of disorders of male reproductive development.

From the other perspective, in the XX fetus developmental excess of androgen has serious consequences leading to masculinization of the external genitalia (virilization). In humans the most common problems arise from congenital adrenal hyperplasia (CAH), which leads to excess androgen production by the adrenal gland. In addition to virilization of the female fetus (although there is no evidence for retention of the Wolffian ducts, and the Mullerian ducts develop normally), individuals of both sexes with CAH are more likely to show increased blood pressure, obesity, and poor hormone control (5, 6). In addition, adult men with CAH have impaired fertility and lower testosterone to estradiol ratios (7), although it remains uncertain by exactly how much the total androgen tone was raised in these males in utero (Figure 1). Another example of abnormally raised gestational androgens is polycystic ovarian syndrome (PCOS). Maternal androgen levels are thought to be a prime driver of PCOS in female offspring, but in a recent study (8) female neonates had very high levels of testosterone (similar to unaffected male neonate levels) if their mother had PCOS. In contrast, in male neonates from women with PCOS, testosterone was at levels similar to unaffected males. This is likely to be due to the fact that normal endogenous fetal androgen levels are high and, in the human, approach adult male levels (9).

The developmental trajectory programmed by in utero androgen action is important for more than just male reproductive competence. In a number of studies, men with lower testosterone have health deficits such as metabolic syndrome, including increased incidence of obesity, diabetes, and cardiovascular disease (10–12). Therefore, from both a reproductive and a health and well-being point of view, it is very important that the developing male
fetus is set upon the appropriate developmental trajectory, with androgen levels in the “just right” zone.

The paper by Connolly et al (25) in this issue of Endocrinology provides a salutary reminder of just how complex this issue is. The authors have examined the effects of excess testosterone in utero on male development using the sheep as a model system. In the sheep many developmental processes are similar to the human including a relatively long gestational period of around 145 days and the need for active hypothalamo-pituitary regulation of the testes during gestation (1, 2). This study builds on the authors’ reports of excess androgen in the female fetus (13–15) and in both sexes by other researchers (16–19).

Male fetuses were exposed to additional testosterone during the male programming window — the critical period during which fetal masculinization is set in motion (20). The administration of exogenous androgen, via the mother, in the form of testosterone propionate (TP), was followed up by exhaustive analyses of the fetal testes and endocrinology. The points made about endocrine adaptability above were born out since the authors found that TP administration from day 62 of gestation did not affect circulating testosterone in male fetuses (unlike females), although the corollary of this was reduced LH production. This was accompanied by a reduction in testicular androgen synthesizing machinery and increased basal but not human chorionic gonadotropin (hCG)-induced testosterone section in vitro. The latter suggests fundamental changes in Leydig cells that may occur through increased testicular androgen receptor expression at day 90 potentially affecting intratesticular signaling. These findings are difficult to interpret for the human, however, who is exposed to very high levels of hCG during the presumptive male programming window (21). The authors also found disturbed Leydig cell development with an abnormal pattern of intratesticular cell distribution, effects that were lost after a cessation of androgen administration. Connolly et al (25) also report somewhat different, more severe effects of TP administration from day 30 of gestation. In this case Sertoli and germ cells were also affected along with the Leydig cells. Other studies using the sheep have shown that the adverse effects of androgen treatment from day 30 will persist into adulthood in males (22), highlighting an important area for further study.

One potential confounder in these studies might have been action of the fetal liver, or the placenta, during gestation. Both organs have a highly active aromatase system (23, 24), and it is likely that added TP would lead to an increase in circulating estrogen levels. Connolly et al (25) have addressed this confounder by direct fetal administration of either TP or diethylstilbestrol (DES) and have shown that the latter does not reproduce the effects of the

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**Figure 1. Overview of Multiple Factors Contributing to Androgen “Tone” in the Fetus.** The endocrine signaling underpinning fetal reproductive development and masculinization is tightly regulated by exchange, cross-metabolism, and differential signaling between organs. The overall degree of androgenicity at the tissue level in the male will be heavily dependent upon the maternal/placental signaling, such as hCG (excludes species such as rodents) in the first trimester in humans, the provision of substrates by mother, placenta and fetal adrenal and liver, and the production of androgens, especially testosterone, by the fetal testis. The latter is also in active signaling up and down the fetal hypothalamo-pituitary-testis axis (excluding species such as rodents). The production of sex hormone-binding globulin and α-fetoprotein by the fetal liver also plays a role in modulating steroid hormone signaling. AR, androgen receptor; DHEA, dehydroepiandrosterone; CG, chorionic gonadotropin.
TP. This is very strong evidence that it is not hepatic or placental conversion of androgen to estrogen that produces the phenotypic and functional effects of increased androgen exposure that they report.

In conclusion, our understanding of developmental androgen excess in the male has been significantly advanced by the publication by Connolly et al (25). In terms of the “goldilocks” concept of “too little” (well established), “too much” (some evidence), and “just right”, their findings, combined with those in the literature, suggest that there is an element at which the homeostatic mechanisms of interdependence in endocrine signaling and feedback during development can be pushed too far, resulting in disturbed Leydig cell development and impaired androgen production. Their study also adds the question of when during development excess androgen may be a problem for the developing fetus, as is the case with PCOS, and this aspect is an area for further research, especially with follow-through to adulthood.

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