The Brain Phenotype in Polycystic Ovary Syndrome (PCOS): Androgens, Anovulation, and Gender

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1.1 Introduction

Polycystic ovary syndrome (PCOS) is a common condition with reproductive and metabolic features. Recent studies confirmed that women with PCOS have multiple genetic allelic variants that are independently associated with hyperandrogenism, gonadotropin regulation, timing of menopause, depression, and metabolic disturbances, including insulin resistance [1]. Of note, the data cited above showed that not all women with PCOS possess the full complement of the 14 genetic variants identified. Genetic heterogeneity results in clinical heterogeneity. We have long recognized that there is a spectrum of clinical presentation, with some women having a more pronounced reproductive phenotype and others presenting primarily with metabolic features. Despite variation related to PCOS genotype and phenotype, however, two long-recognized pathogenic themes remain the same: excess androgen exposure and insulin resistance. Since androgens and insulin modulate of brain architecture and function, it is not surprising that PCOS is associated with a brain phenotype, but also one that presents variably. Building on the notion that the brain is a target of hormones of all classes, in this chapter we characterize the brain phenotype in PCOS and explore the evidence that the brain phenotype is the result of androgen exposure that not only predisposes to anovulation and obesity but also has the potential to skew gender identity and sexual orientation.

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1.2 Anovulation Reflects the PCOS Brain Phenotype

A key paradox in the presentation of PCOS is chronic anovulation despite an abundance of oocytes (polycystic ovaries). This paradox was one of the first clues to the unique brain phenotype in PCOS associated with reproductive dysfunction. Subsequent studies found that the primary cause of anovulation in PCOS was not resistance to FSH but an insufficient rise in FSH to initiate folliculogenesis. It is now widely appreciated that exogenous FSH administration readily initiates folliculogenesis in women with PCOS and that follicle development is often so exuberant that ovarian hyperstimulation results. Not only are FSH levels insufficient, paradoxically, LH levels are tonically high. The elevated LH/FSH ratio characteristic of women with PCOS catalyzed an investigative search for an explanation. As shown in Fig. 1.1, one likely contributor to increased LH and reduced FSH levels is increased GnRH-LH drive [2, 3]. As shown in Fig. 1.2, GnRH-LH pulse frequency in women with PCOS approaches that of men, namely, one LH pulse per hour, rather than one pulse every 90 minutes observed in eumenorrheic, ovulatory women [2]. Studies in men with idiopathic hypothalamic hypogonadism revealed that the more rapid the pulse frequency of exogenously administered GnRH, the higher the

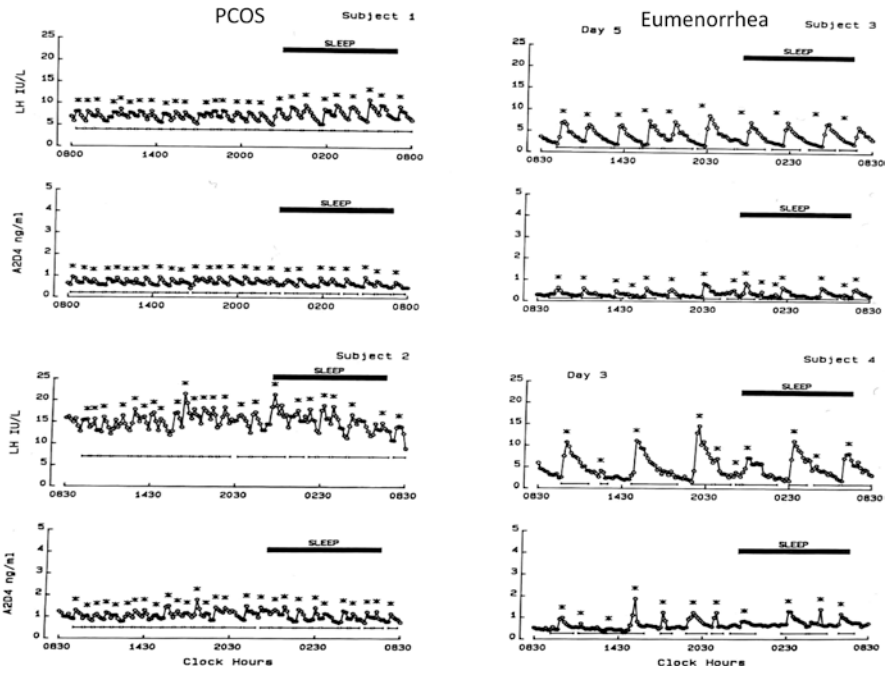

Fig. 1.1 GnRH-LH and alpha-subunit pulse patterns in 9 women with polycystic ovary syndrome (PCOS) (left) and 9 eumenorrheic, ovulatory women (right). Blood samples were obtained at 10-min intervals fro 24 h from an indwelling intravenous catheter and pulse patterns were analyzed using a computer-based algorithm. Berga et al. [2]

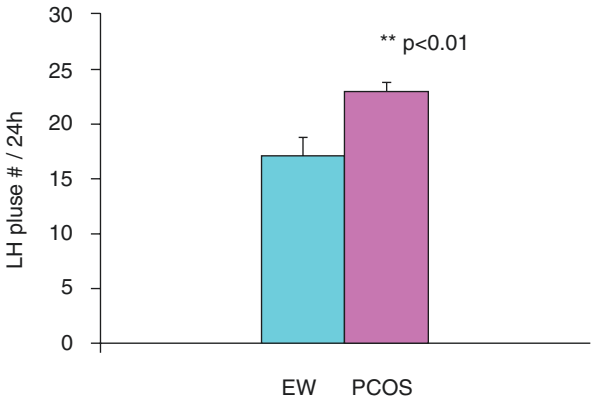

Fig. 1.2 24-h LH and alpha-subunit number in eumenorrheic, ovulatory women eumenorrhea and women with polycystic ovary syndrome (PCOS). EW mean 17.1 ± 1.7 (SEM) vs PCOS 23.0 ± 0.7, $p < 0.01$. The mean interpulse interval was 84 min for EW and 63 min for PCOS. Original graph. Berga et al. [2]

LH and the lower the FSH levels [4]. Subsequent studies showed that low dose testosterone increased GnRH-LH pulse frequency in eumenorrheic women and that high dose testosterone increased GnRH-LH pulse frequency in women with PCOS. Further, we and others also showed that the increased GnRH-LH drive in PCOS was resistant to suppression by sex steroids [5, 6] and that sensitivity to sex steroid suppression was restored by the androgen receptor blocker flutamide [7], but not by metformin [8]. The above evidence suggests that androgen exposure causes the rapid GnRH pulse frequency and explains the skewed LH/FSH ratio observed in women with PCOS.

1.3 Neuroregulation of GnRH and the Brain Phenotype in PCOS

An explosion in knowledge regarding the regulation of GnRH over the last 30 years has afforded us the opportunity to identify factors that mediate the development of the brain phenotype in women with PCOS. We now understand that neurodevelopment and neuroregulation is much more than sex steroid exposure, although clearly androgens, estrogens, and progesterone are major modifiers of both. However, other hormones, including peptides, growth, and immune factors also influence neurodevelopment and neuroactivity.

The discovery that the kisspeptin peptide system serves as a key proximate regulator of GnRH pulsatility revolutionized our understanding of the neuroregulation of reproductive function. Within the arcuate nucleus, kisspeptin/neurokinin B/dynorphin (KNDy) neurons release the prohormone kisspeptin, a 145 amino acid protein that is enzymatically cleaved to a 54 amino acid peptide known as kisspeption-54. The kisspeptin receptor, abbreviated GPR54 for G protein-coupled receptor 54, is expressed on GnRH neurons, allowing kisspeptin to activate GnRH neurons [9]. Exogenously administered kisspeptin exerts a profound stimulatory effect on gonadotropin secretion in animal and human models. Both testosterone and

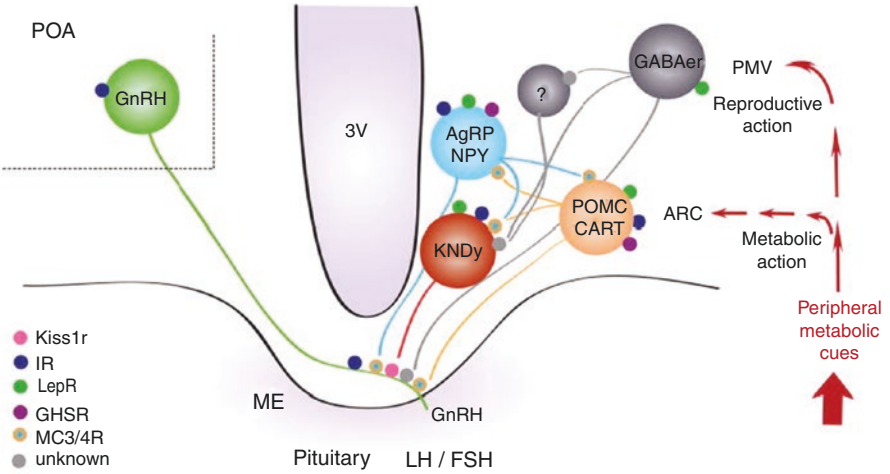

Fig. 1.3 Schematic representation of neural interactions between metabolic and reproductive functions depicting likely sites of action of leptin, insulin, and ghrelin to control GnRH release. 3 V, third ventricle; ARC, arcuate nucleus; ME, median eminence; PMV, ventral pre-mammillary nucleus; POA, preoptic area. Navarro and Kaiser [11]

estradiol regulate *Kiss1* gene expression. In addition to activating GnRH neurons, kisspeptin neurons also form synapses with GnRH neuron terminals in the median eminence, where GnRH release (exocytosis) is stimulated by kisspeptin [10]. Figure 1.3 shows the central cascade that regulates GnRH and highlights the role of KNDy neurons [11].

As shown in Fig. 1.3, GABA (gamma-aminobutyric acid) neuronal input modulates the entire cascade, including kisspeptin neurons, and directly and indirectly regulates GnRH drive. Importantly, the GABAergic network integrates external environmental and internal host signals to align reproductive function with individual circumstance. Thus, stress, sex steroids, and metabolic signals regulate GABAergic tone and the entire cascade by direct and indirect mechanisms. For example, in a monkey model, the administration of the CRH antagonist, astressin B, reversed the impact of the chronic social stress of subordination on GABA-A receptor binding in the prefrontal cortex, a site implicated in the regulation of the limbic-hypothalamic-pituitary-adrenal, −gonadal, and -thyroidal axes [12]. A recent study found that chronic administration of letrozole to female mice induced polycystic ovaries, anovulation, elevated testosterone, increased LH pulsatility, and elevated kisspeptin and neurokinin B gene expression in the arcuate nucleus [13]. In a murine model, leptin-responsive GABAergic neurons regulated fertility through pathways that reduced kisspeptinergic tone [14].

Androgens play a fundamental role in the organization and activation of the hypothalamic circuitry shown in Fig. 1.3. The mechanisms by which androgens act are many. Androgens increase GABAergic innervation of KNDy neurons and alter sex steroid feedback sensitivity [15]. Administration of dihydrotestosterone (DHT), a non-aromatizable androgen, to mice increased GnRH firing activity [16]. In a sheep

model of PCOS, prenatal testosterone exposure increased GABAergic synaptic inputs to and stimulation of GnRH and KNDy neurons [17]. Androgen exposure acting via an androgen receptor mechanism also impaired progesterone receptor transcription, impaired negative feedback, and resulted in GnRH neuronal hyperactivity [18]. Absence of progesterone signaling in kisspeptin neurons disrupted the LH surge and impaired fertility in female mice [19]. In a mouse model of DHT-induced PCOS, selective deletion of the androgen receptor (AR) in neurons, but not granulosa cells, reversed the impact of DHT, leading the investigators to conclude that neuroendocrine genomic AR signaling is an important extra-ovarian mediator of the PCOS phenocopy in mice [20]. The above preclinical studies likely explain why GnRH drive in women with PCOS was resistant to suppression by progestin and progesterone feedback [5–7]. Thus, as shown in Fig. 1.3, KNDy neurons and kisspeptin-GPR54 receptors form the final common pathway in the hypothalamic circuitry that regulates GnRH drive [9]; GABAergic tone modulates the function of the kisspeptinergic pathway and confers feedback sensitivity to sex steroids and metabolic signals.

The term hyperandrogenic anovulation parsimoniously conceptualizes PCOS and conveys the notion that androgens of ovarian origin initiate and maintain the brain phenotype responsible for anovulation, namely, increased GnRH-LH drive and chronic insufficiency of FSH. To investigate the role of androgens and GABA in human PCOS, we compared cerebrospinal fluid (CSF) levels of GABA, testosterone, and estradiol in eumenorrheic, ovulatory women and those with PCOS [21]. Figure 1.4 shows that women with PCOS not only have higher CSF levels of

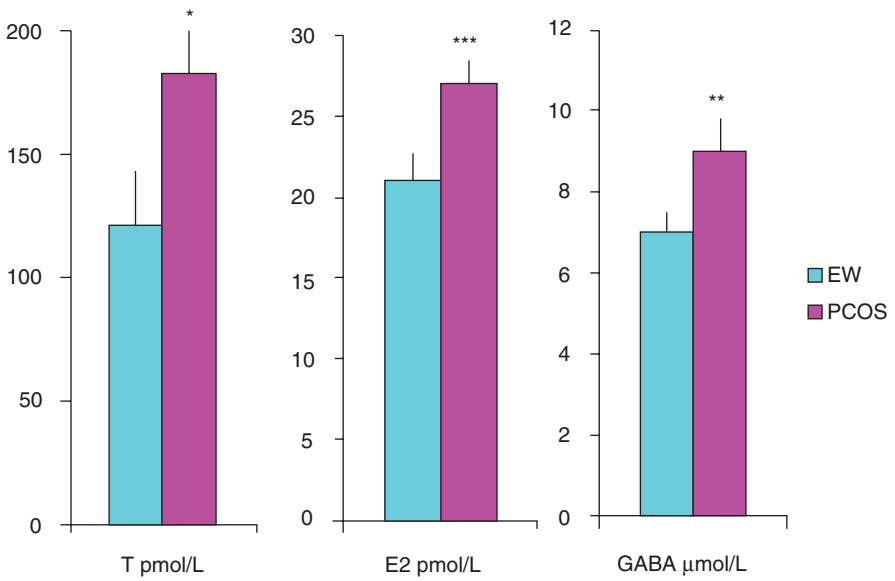

Fig. 1.4 Increased cerebrospinal fluid levels of GABA, testosterone (T), and estradiol (E2) in 12 women with polycystic ovary syndrome as compared to 15 eumenorrheic, ovulatory women (EW). Original graph. Kawwass et al. [21]

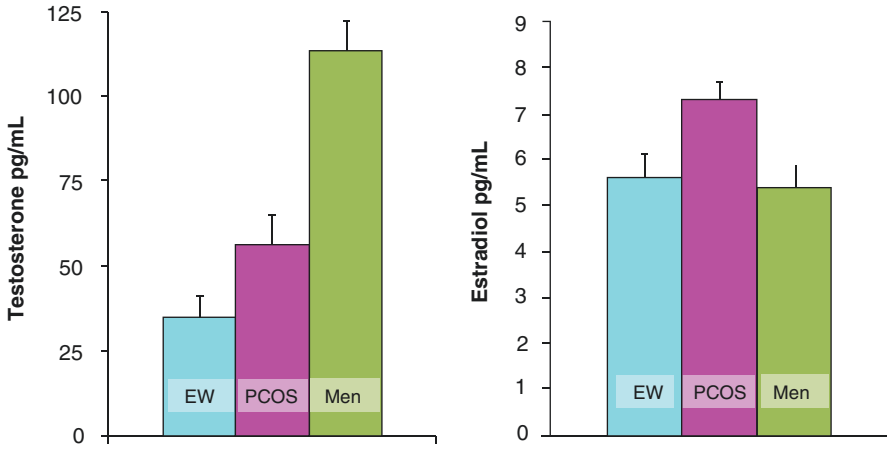

Fig. 1.5 Cerebrospinal fluid levels of testosterone and estradiol in 15 eumenorrheic, ovulatory women, 14 women with PCOS, and 6 men. Unpublished data from Berga lab

testosterone and GABA but also higher CSF levels of estradiol. While the CSF levels of testosterone in PCOS were not as high as the levels in men (Fig. 1.5), they were clearly higher than the levels in eumenorrheic, ovulatory women. Thus, the brain phenotype in PCOS that predisposes to chronic anovulation despite increased oocyte endowment most likely results from chronically increased androgen exposure, which, in turn, reflects an increased oocyte pool, as androgen levels and oocyte endowment correlate in PCOS [22]. As shown in Fig. 1.5, women with PCOS also displayed higher CSF levels of estradiol as compared to both eumenorrheic women and men. Higher CSF estradiol levels may differentially suppress FSH more than LH, contribute to the brain phenotype in PCOS, and explain the paradox of increased oocyte endowment and chronic anovulation. Ultimately, higher brain exposure to both androgens and estradiol imprints the brain in other ways that remain to be better elucidated, including gender identity and sexual orientation.

Another recently reported regulator of hypothalamic GnRH function is anti-Möllerian hormone (AMH). In both humans and mice, GnRH neurons expressed AMH receptors. In mice, AMH potently activated GnRH neuron firing rate and accentuated GnRH-dependent LH release from the pituitary [23]. Since AMH and testosterone are correlated with oocyte endowment [22], AMH also could play a fundamental role in the development and maintenance of the brain phenotype in PCOS that results in chronic anovulation. If so, this may explain why women with PCOS display more regular cycles as they age because AMH levels and oocyte endowment drop [24, 25]; a later age at menopause [1, 26, 27]; and better fertility than eumenorrheic women after age 40 [28, 29].

Androgen excess may also explain at least some of the metabolic features of PCOS including insulin resistance. In female mice, excess androgen receptor activation in neurons caused peripheral insulin resistance and pancreatic beta cell dysfunction [30]. In contrast, selectively knocking out the androgen receptor in neurons

of female mice decreased glucose and insulin levels in fasted and fed states as compared to wild-type female mice [31]. Ultimately, the most parsimonious explanation for PCOS, including the reproductive brain phenotype, is androgen excess in an XX genotype.

1.4 Gender Identity and Sexual Orientation

The brain orchestrates many functions in addition to reproductive function. What are the possible consequences of brain androgenization in women with PCOS other than increased GnRH pulsatility? Both insulin resistance and a tendency to weight gain likely reflect brain androgenization. Other behavioral variables that could be attributable at least in part to brain androgenization include stress sensitivity, mood, gender identity, and sexual orientation. While sex refers to genetic sex, which is readily determined because it is a biological attribute, gender refers to a set of behavioral expectations assigned according to genetic sex. However, gender is a cultural construct; the attributes considered male and female varies somewhat across cultures. Some cultures define gender as male, female, and other, while other cultures have a strictly binary view. Currently, cultures around the world are grappling with a more expanded perspective on gender.

At least two key important questions deserve increased clinical attention to better care for women with PCOS. First, do women with PCOS differ in terms of gender identity from eumenorrheic, ovulatory women? Second, do women with PCOS differ in terms of sexual orientation from eumenorrheic, ovulatory women? Given that our understanding of the role of prenatal and postnatal hormone exposures as contributors to brain organization and activation is limited, it should not be surprising that our understating of the impact of hormones on gender identity and sexual orientation is also constrained. However, current evidence based on neuroimaging and clinical studies suggests that women with PCOS differ from eumenorrheic women in terms of the proportion that report nonconforming gender identity and lesbianism.

To delve into the topic of gender identify and sexual orientation requires an appreciation of the notion that sex steroid exposures in utero organize the brain. At the time of puberty and during the ensuring reproductive years, gonadal hormones activate the already sexually dimorphic brain, which results in gender asymmetries and sex-specific attributes [32, 33]. There are many clinical studies showing that sex hormone exposures modulate attention, comprehension, reaction time, and memory. One of the critical behavioral consequences of gonadal hormonal exposures is altered information processing [34]. In a recent review, McCarthy and Arnold suggested that estradiol is a masculinizing hormone and exerts multiple region-specific effects via distinct cellular mechanisms [35]. During the perinatal sensitive period, estradiol promotes cell survival, cell death, and cell proliferation in separate brain regions and promotes the formation of new dendritic spine synapses in some brain regions while suppressing them in others. Essentially, hormonal exposures "sculpt" the brain. The enduring organizational effects of exposure to estradiol are mediated in part via epigenetic changes to the DNA and

chromatin in processes that are region-specific. Given the organizational complexity of the brain and the spectrum of hormonal exposures, the potential for neurocomplexity is enormous. Unfortunately, neither our lexicon nor our cultural and medical constructs adequately capture the neurocomplexity of gender identity and sexual orientation. Certainly, it is unlikely that gender is dichotomous. At this time, it might be best to assume that the actual range of neurodiversity is not "visible" due to the dissonance between biological complexity and cultural stereotypes that constrain individual expression.

Our study of CSF levels of estradiol and testosterone in women with PCOS revealed increased brain exposure to both estradiol and testosterone as compared to eumenorrheic women [21]. For women with PCOS, altered sex steroid exposure likely began in utero, resumed at puberty, and continued at least until menopause. The altered steroid milieu differentially organizes the brain architecture and then differentially activates brain function. As McCarthy and Arnold [35] suggest, altered sex steroid hormone exposures likely result in a spectrum, mosaic or hybrid of brain masculinization versus feminization that might be best termed gender neurodiversity.

Few investigations have directly determined the gender identity and sexual orientation of women with PCOS. Agrawal et al. [36] found that 80% of lesbian women versus 32% of heterosexual women had polycystic ovarian morphology (PCOM) on ultrasound. Nearly all transmen (female to male transgender) had PCOM [37]. Women with congenital adrenal hyperplasia showed increased rates of bisexual and homosexual orientation that correlated with prenatal androgenization. Bisexual and homosexual orientation also correlated with global measures of masculinization of non-sexual behavior and was predicted by childhood behavior [38, 39].

Neuroimaging of women with PCOS and congenital adrenal hyperplasia has revealed additional neurocomplexity that likely reflects the interaction of hyperandrogenism in an XX genotype, including sex-specific hormone action. The findings do not easily fit into the conventional mindset that gender identity and sexual orientation exist on a spectrum of maleness to femaleness. Rather the data suggest that gender nonconforming is a unique brain state. Lentini et al. [40] analyzed the contributions of genetic sex and androgen exposure and found that cerebellar and precentral gray matter volume was related to X-chromosome escapee genes in the amygdala, parahippocampus, and occipital cortex and that gray matter volume correlated with testosterone levels regardless of sex. They concluded that brain asymmetries are attributable to sex hormones and X-chromosome genes in a regionally differentiated manner [41]. Using PET scanning with $^{15}O\text{-}H_2O$, Savic et al. [42] showed that sex-specific pheromones elicited sex-differentiated hypothalamic activation in heterosexual women and men; however, homosexual men and women responded to pheromone exposure according to sexual orientation rather than biological sex [43, 44]. Two parameters previously shown to be sexually dimorphic are hemispheric asymmetry and functional connectivity. Extending earlier studies, Savic and Lindström [45] found that PET and MRI revealed differences in cerebral asymmetry and functional connectivity between homo- and heterosexual subjects.

Heterosexual men and homosexual women showed rightward cerebral asymmetry while all homosexuals showed sex-atypical amygdala connections. They concluded that these results were not due to learning. Other investigators found that white matter microstructure was altered and cognitive function was compromised in young adults with PCOS independent of education and BMI [46]. Further, women with PCOS who displayed insulin resistance had greater regional activation during an emotion task than controls, and this difference resolved with metformin therapy [47]. The best synopsis of the few neuroimaging findings currently available is that brain function in women with PCOS is neither strictly male nor female and reflects a hybrid or mosaic of features.

The brain is complex. There are more than 86 billion neurons in the mammalian brain, which exceeds the number of stars in the Milky Way. In the cortex, each neuron forms about 10,000 synapses with target cells. Astonishingly, a cubic millimeter of brain contains as many as 90,000 neurons. The human brain is also an energy hog; rodents require about 5% of daily energy intake to fuel their brains while monkeys require 10%, human adults 20%, and human infants 60%. In contrast the brain is only 2% of the human body by weight, requires only 16% of cardiac output, and 25% of oxygen consumption. No wonder our brains drive us to eat. Indeed, insulin resistance may well be an adaptive response to constrain energy utilization that until recently would have represented a survival advantage as it would have rendered insulin-resistant humans "fuel independent" relative to other humans. It is important to consider PCOS in the context of human evolution and recognize that genes that conferred "energy parsimony" may have provided reproductive and survival advantages in fuel-deficient environments, which until recently have been normative. Humans generally now have fuel abundance as even nutrient poor foods fuel the brain. One has to consider that hyperandrogenism in women may have increased rather than decreased reproductive opportunity by allowing survival and conferring prolonged fertility [29]. Thus, while women with PCOS display chronic anovulation and obesity in fuel replete settings, they also display neurodiversity with regard to cognition, behavior, gender identity, and sexual orientation. Clinically it is best to acknowledge and recognize that women with PCOS may not conform to cultural expectations with regard to gender identity and sexual orientation and that they may initiate care for a variety of reasons including gender-affirming hormone therapy.

1.5 Summary

PCOS is generally understood to be a reproductive condition characterized by chronic anovulation, hyperandrogenism, obesity, and metabolic dysfunction. In PCOS, chronic anovulation reflects increased GnRH drive resulting in chronically suppressed FSH. The most likely explanation for increased GnRH drive is prenatal and sustained postnatal brain androgenization. Recent studies suggest both genotypic and phenotypic variability. Given the complexities of brain development, brain androgenization not only manifests as altered reproductive function but also

gender neurodiversity. Thus, gender identity in women with PCOS may be culturally nonconforming. Gender diversity carries psychological consequences for individuals, families, and society and must be recognized and managed for better overall health. Clinical decision trees need to incorporate the variation in, and complexity of, the clinical presentation of PCOS. The medical profession must be able to offer more than ovarian suppression with oral contraceptives, ovulation induction for infertility, and metformin for metabolic dysfunction. Women with PCOS will undoubtedly benefit from holistic diagnostic and treatment algorithms that incorporate recognition of gender identity and sexual orientation and screening for mood disorders [48]. A deeper appreciation of the nuances of PCOS affords an opportunity for individualized and improved care.

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