Reviewer 1
1. l.80, 82. The terms “cystic follicles” or “cystic ovary” are inappropriate for PCOS. Polycystic ovaries grow too many non-dominant antral follicles that undergo atresia before becoming dominant. Animal models with cystic structures do not emulate PCOS. Those with excess numbers of antral follicles, on the other hand, do emulate PCOS.

   ➔ The authors deeply appreciate the in-depth comments of reviewer. The words, cystic follicles, polycystic ovary or cystic ovary has been removed or replaced as ‘too many number of antral follicles’ or ‘those with excess numbers of antral follicles’ accordingly.

2. l.88. Hyperandrogenism is a major clinical hallmark of PCOS, but since there is the normo-androgenic phenotype for PCOS, it is not “the” hallmark.

   ➔ The authors agreed and appreciate the in-depth comments of reviewer. The sentence is now revised as ‘one of a clinical hallmark of PCOS patients is hyperandrogenism’.

3. l.99. Point out that 3-week old female mice are pre-pubertal.

   ➔ The authors appreciate the constructive comments of referee. The paragraph of study using 3-week old female mice has been deleted.

   Long-term administration (90 days) of DHT to 3-week old female mice induced acyclic estrous, polycystic ovary morphology, excess dominant follicles and adipocyte hypertrophy (Caldwell et al., 2014). In this case, the serum DHT level was 8-fold higher than that in the control group.

4. l.109. LH pulse frequency is also elevated in prenatally androgen exposed mice, sheep and monkeys.
The authors appreciate the comments of referee. The sentence ‘In addition, the frequency of LH secretion was increased in this model’ has been corrected as ‘LH pulse frequency is also elevated in prenatally androgen exposed sheep and monkeys.’

5. l.112. Silva’s 2018 paper addresses a more contemporary understanding of neuroanatomical changes from androgen exposure.

The authors appreciate the comments. Commented reference has been cited and updated as follows.


6. l.113. Both prenatally androgen exposed sheep and monkeys show evidence of ovarian morphology suggestive of polycystic ovaries. This is not true of mouse and rat equivalents.

The authors appreciate the in-depth review and comments. The sentence has been modified as follows.

Animals such as sheep and monkeys exposed to androgens prenatally have also developed the hallmarks of PCOS phenotypes. Rats treated prenatally (embryonic day 16 to 19) with testosterone or DHT presented irregular estrous cycle and elevated serum testosterone, estradiol, and luteinizing hormone (LH) levels.

7. l.127-130. In fetal sheep and monkeys, androgen exposed female only experience fetal male levels of testosterone. There is no “excessive” exposure of “males and females”. In addition, newborn daughters of women with PCOS demonstrate a reliable biomarker of fetal androgen exposure, elongated anogenital distance. This also occurs in adult women with PCOS. Other biomarkers of fetal androgen exposure are also found in PCOS daughters or women with PCOS. To discount PCOS pregnancies in humans as devoid of increased androgen exposure reflects bias in selection of literature reviewed.

The authors agree with the reviewer. The sentence has been revised as follows.

‘Although the experiments demonstrated that androgen exposure can alter endocrine homeostasis and lead to the development of ovarian dysfunction as well as its accompanying metabolic symptoms, it is unclear where the circulating androgens arise.’

The sentence with exposure of “males and females” phrase has been removed.

In addition, considering both human males and females may not be exposed to such excessive hormonal disturbance in their lives.
8. Table 1. To be a model for PCOS, the animal studied has to exhibit at least two of the three diagnostic criteria. Elevated circulating AMH levels could provide a surrogate measure for increased numbers of antral follicles. Such considerations only apply to studies 1, 2, 4, 5, 10 and 17. For example, study 6 animals have regular cycles and are not hyperandrogenic. They have no relevance for PCOS. Obesity per se is not a precursor to PCOS or all obese women would have PCOS. The text at l.158-162 makes this very point. The authors cannot therefore include such animals as “models of PCOS”.

> Animal studies not fit two or more criteria for PCOS has been removed and Table 1 has been modified as recommended. Additionally, title of Table 1 has been revised as Table 1.

Transgenic and genetically modified animal models including PCOS-like symptom.

Table 1. Transgenic and genetically modified animal models including PCOS-like symptom

<table>
<thead>
<tr>
<th>Types</th>
<th>Author</th>
<th>Year</th>
<th>Species</th>
<th>Strain</th>
<th>Estrous cycle</th>
<th>Ovarian cyst</th>
<th>Androgen level</th>
<th>Metabolic features</th>
<th>LH level</th>
<th>Time point of examination (post-natal)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenic</td>
<td>[59]</td>
<td>2009</td>
<td>Mouse</td>
<td>17NF</td>
<td>Prolonged</td>
<td>Yes (hCG)</td>
<td>↑ (PMSG)</td>
<td>N/R</td>
<td>↓ 28 days</td>
<td></td>
<td>Enhanced sympathetic input affects trkA receptor and p75NTR signaling in the ovary</td>
</tr>
<tr>
<td>Transgenic</td>
<td>[60]</td>
<td>2014</td>
<td>Mouse</td>
<td>17NF</td>
<td>Prolonged</td>
<td>N/R</td>
<td>↑</td>
<td>N/R</td>
<td>↑ 19 weeks</td>
<td>10, 20 weeks</td>
<td>Increased ovarian sympathetic excitement</td>
</tr>
<tr>
<td>Transgenic</td>
<td>[39]</td>
<td>2010</td>
<td>Mouse</td>
<td>Ar KO</td>
<td>Acyclic</td>
<td>Yes (therapeutic)</td>
<td>↑</td>
<td>N/R</td>
<td>↑ 14 - 42 days</td>
<td>2 - 6 months</td>
<td>The HPG axis dysregulation, IR negative feedback</td>
</tr>
<tr>
<td>Transgenic</td>
<td>[51,52]</td>
<td>1995</td>
<td>Mouse</td>
<td>LH β-CTP</td>
<td>Prolonged</td>
<td>Yes</td>
<td>↑</td>
<td>N/R</td>
<td>↑ 6 weeks, 12 weeks</td>
<td>ERα KO in theca cells predispose the ovary to develop cysts</td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td>[33]</td>
<td>2009</td>
<td>Rat</td>
<td>JCR:LA-cp strain</td>
<td>Irregular</td>
<td>Yes (therapeutic, 5mg of PMSG or hCG)</td>
<td>↑</td>
<td>N/R</td>
<td>6 weeks, 12 weeks</td>
<td>Leptin receptor malfunction, IR insulin and leptin level</td>
<td></td>
</tr>
</tbody>
</table>

# = papers supporting similar study; N.S. = non-significance; NR = not reported; ↑ = significantly increased/up-regulated; ↓ = significantly decreased/down-regulated; Ar = aromatase; AT2R = angiotensin II type 2 receptor; ER = estrogen receptor; hCG = human chorionic gonadotropin; HPG axis = Hypothalamic-pituitary-gonadal axis; IGF = insulin-like growth factor; LH = luteinizing hormone; NGF = Nerve growth factor; PMSG = pregnant mare serum gonadotropin

9. l.168-194. These paragraphs are more informative as they dissect out potential causative and non-causative factors contributing to PCOS-like traits. To call all such models “models for PCOS”, however, is misleading. Contrasting different phenotypes related to different genetic manipulations is a useful exercise, but by definition it means that some of the models are not PCOS-like.

> The authors totally agree with reviewer. The headline and subheading have been revised as follows.

2.2. Animal models with PCOS or similar symptoms induced by indirect hormonal perturbations

2.2.1. Genetically engineered or genetic animal models including PCOS-like symptoms

2.2.1.3. Other transgenic rodent animal models

10. Table 2. Analogous issues to those found with Table 1.
The authors totally agreed the question raised by reviewer. Table 2 has been modified with removal of some references and the title has been modified as follow.

Table 2. Diet- or environmentally or chemically induced animal models including PCOS-like symptom

<table>
<thead>
<tr>
<th>Types</th>
<th>Author</th>
<th>Year</th>
<th>Species</th>
<th>Methods</th>
<th>Estrous cycle</th>
<th>Ovarian cyst</th>
<th>Androgen level</th>
<th>Metabolic features</th>
<th>LH level</th>
<th>Ages before the intervention (days)</th>
<th>Intervention/observation period</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical</td>
<td>[79]</td>
<td>2012</td>
<td>Mouse</td>
<td>D-galactose (S.C.)</td>
<td>Irregula r</td>
<td>Yes</td>
<td>↑</td>
<td>N/R</td>
<td>N/R</td>
<td>7-8 weeks</td>
<td>6 - 7 weeks</td>
<td>Increased AMH level; Formation of ROS and AGEs products</td>
</tr>
<tr>
<td>Environment</td>
<td>[68]</td>
<td>2014</td>
<td>Rat</td>
<td>Constant light</td>
<td>N/R</td>
<td>Yes</td>
<td>↑</td>
<td>N/R</td>
<td>N/R</td>
<td>6 weeks</td>
<td>16 weeks</td>
<td>Melatonin absence; SCN regulation</td>
</tr>
<tr>
<td>Environment</td>
<td>[70]</td>
<td>2004</td>
<td>Rat</td>
<td>Constant light</td>
<td>N/R</td>
<td>Yes</td>
<td>↑</td>
<td>N/R</td>
<td>N/R</td>
<td>3-4 months</td>
<td>8 month</td>
<td>Melatonin absence; Gonadotropin release dysregulation</td>
</tr>
<tr>
<td>Environment</td>
<td>[75]</td>
<td>2008</td>
<td>Rat</td>
<td>Cold stress</td>
<td>Irregula r</td>
<td>Yes</td>
<td>↑</td>
<td>N/R</td>
<td>N.S.</td>
<td>7-8 weeks</td>
<td>3 hr/day, 8 weeks</td>
<td>Increased noradrenergic activity response to cold stress</td>
</tr>
<tr>
<td>Chemical</td>
<td>[85]</td>
<td>2010</td>
<td>Rat</td>
<td>Bisphenol A (S.C.)</td>
<td>N/R</td>
<td>Yes</td>
<td>↑</td>
<td>N/R</td>
<td>N/R</td>
<td>-</td>
<td>P1 to 10 treated; 4 - 5 months (observation period) GaRH pulse disruption</td>
<td></td>
</tr>
<tr>
<td>Chemical</td>
<td>[91]</td>
<td>2003</td>
<td>Rat</td>
<td>Letrozole (P.O.)</td>
<td>Acyclic</td>
<td>Yes</td>
<td>↑</td>
<td>N/R</td>
<td>↑</td>
<td>6 weeks</td>
<td>3 weeks</td>
<td>Elevated testosterone and LH level</td>
</tr>
<tr>
<td>Chemical</td>
<td>[92]</td>
<td>2013</td>
<td>Rat</td>
<td>Letrozole (S.C./pellet)</td>
<td>Acyclic</td>
<td>Yes</td>
<td>↑</td>
<td>Insulin resistance, hyperinsulinemia</td>
<td>↑</td>
<td>3 weeks</td>
<td>5 weeks, 10 weeks</td>
<td>High androgen levels and low estrogen by inhibited aromatase activit</td>
</tr>
<tr>
<td>Chemical</td>
<td>[82]</td>
<td>2016</td>
<td>Rat</td>
<td>Monosodium-L-glutamate</td>
<td>Irregula r</td>
<td>Yes</td>
<td>N.S.</td>
<td>Obesity, fat accumulation, N.S.</td>
<td>-</td>
<td>P 2 to 10 treated; P 75 (observation period) Increased AMH level on the</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>[97]</td>
<td>2008</td>
<td>Cynomolgus monkey</td>
<td>Naturally occurred</td>
<td>Prolong ed</td>
<td>Yes</td>
<td>↑#</td>
<td>Obesity, increased glucose level, hyperinsulinemia</td>
<td>N/R</td>
<td>During &gt;56 months (observation period) Endometrial hyperplasia with hyperinsulinemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>[98]</td>
<td>2017</td>
<td>Rhesus monkey</td>
<td>Naturally occurred</td>
<td>N.S.</td>
<td>N/R</td>
<td>↑</td>
<td>N.S.</td>
<td>↑</td>
<td>&gt; 5 years (observation period)</td>
<td>Suggested environmental, epigenetic, prenatally programmed hyperandrogenism suggested</td>
<td></td>
</tr>
</tbody>
</table>

# = papers supporting similar study; * = a review paper; N.S. = non-significance; N/R = not reported; ↑ = significantly increased/dropped-regulated; ↑# = increased, compared to references,

11.356-360. It is unclear what the authors mean here.

The authors appreciate the constructive comment by the reviewer. The paragraph has been deleted.

Damage to the noradrenergic nucleus locus coeruleus (LC) significantly decreased abnormal follicular development, and the damaged animals did not show elevated serum testosterone or estradiol levels. Thus, the authors suggested that LC-mediated sympathetic
activity, which is noradrenaline (NE) stimulation to the ovary in particular, results in the development of PCOS-like phenotypes in this experimental model.

12. 1.415-436. Group all naturally occurring PCOS-like animal studies together. They are currently inappropriately split between chemical causes and natural causes. All could be caused by environmental exposures (captive and free-living) or none could be caused by chemical exposures.

→ The authors appreciate the constructive comment. The paragraph has been grouped together with chemically induced PCO phenotypes in other animals paragraph. And the changes have been updated in Table of Contents.

13. Table 3. Cystic ovaries in cattle are not related to PCOS and the underlying mechanisms have been well described by Wiltbank. Morphological presentation is not polycystic ovary-like.

→ The authors appreciate the constructive comment. The title of the Table 3 has been modified as Table 3. Alterations of ovarian phenotype in the other animals.

14. 1.483-484. The authors inappropriately group together cystic and polycystic ovaries, and thus arrive at a potentially erroneous conclusion that polycystic ovaries and hyperandrogenism arise separately. The authors need to carefully define ovarian morphology (or AMH physiology) emulating PCOS ovaries and morphology that does not, i.e., cystic or hemorrhagic ovaries. They eventually engage AMH in this manner by l.511-520, but it needs to be connected throughout.

→ The authors agree to the comments and the inappopriate paragraph has been removed.

15. 1.489. In humans, in stark contrast to rodents (and possibly other non primates), increased lipid accumulation or BMI inhibits LH release, hence why LH levels in obese women with PCOS can be closer to normal than in lean/overweight women with PCOS. The authors need to be careful of this when trying to dissect out LH-neuroendocrine vs insulin-adiposity mechanisms.

→ The paragraph has been removed. The authors appreciate this comment.

16. 1.496-497. Ovary specific estrogen receptor knockout mice can have elevated testosterone but not pituitary LH levels since negative feedback loops controlling this system are regulated by estradiol and progesterone.
The authors appreciate the constructive comment by the reviewer. The sentence has been positioned at first paragraph of 2.4.1. Physiological insights from the animal models with PCOS-like symptom.

17. Figure 1. At least naturally occurring PCOS-like monkeys in Table 2 are shown as having high LH levels so there needs to be some arrow connection between those two boxes here to reflect Table 2.

The authors deeply appreciate the constructive comment. Figure 1 has been updated as recommend and the new figure is attached as follows.

18. 1.530-532. Altered thyroid hormone release is a basis for exclusion from PCOS in women. The authors must make the same distinction clear in dealing with animal model considerations. The mechanistic underpinnings are not the same.

The authors appreciate this comment. The paragraph has been deleted.

The effects of adrenal corticosterone and thyroid hormone should be considered when using environmentally (stress) induced animal PCOS models.

19. 1.531. The authors confuse elevated cortisol levels with stress. Altered adrenal glucocorticoid release can occur for a variety of reasons, including altered steroidogenic enzymatic function and regulation that are not based in stress.

The authors appreciate the constructive comment by the reviewer. This part has been deleted and modified as follows.
A study by Abbott et al. (2017) showed that female rhesus monkeys with high testosterone levels (about 16% of the total observed monkeys). Thus, animals vulnerable to stress may be prone to developing a PCOS-like phenotypes and infertility.

20. 1.567-569. Elevated insulin levels are indeed associated with ovarian hyperandrogenism in women with female patients with either type 1 or type 2 diabetes. Their hyperandrogenism is usually mild in comparison to PCOS. Escobar-Morreale has studied and reviewed this in women and points out not to confuse such causes of female hyperandrogenism with PCOS. The authors appreciate this constructive comment. The sentence has been removed and rephrased with the recommended reference. New sentence reads as follows. Elevated insulin levels are indeed associated with ovarian hyperandrogenism in women with female patients with either type 1 or type 2 diabetes. Their hyperandrogenism is usually mild in comparison to PCOS [127].