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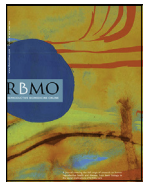

COMMENTARY

A primate perspective on oocytes and transgenerational PCOS

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ABSTRACT

'Androgenized' rodent models are widely used to explore the pathophysiology underlying human polycystic ovary syndrome (PCOS), including reproductive and metabolic dysfunction. Based on a recent study using a dihydrotestosterone (DHT)-treated murine model, it has been proposed that prenatal androgen excess alone can predispose to transgenerational transmission of PCOS. From RNA sequencing analysis of metaphase II (MII) oocytes of androgenized lineages, the authors speculated that oocyte factors, including up-regulation of cytotoxic granulosa-associated RNA binding protein-like 1 (*TiaL1*), are sufficient to promote disease transfer across generations. Although this is an intriguing concept, it was not considered in the context of earlier publications in which the transcriptomes of human MII oocytes from PCOS women undergoing IVF were compared with women without PCOS. In one of these papers, a number of differentially expressed genes in PCOS MII oocytes (*TIAL1* was not differentially expressed) were found to have putative response elements in their promoters for androgen receptors and peroxisome proliferating receptor gamma, providing a mechanism for how excess androgens and/or metabolic defects associated with PCOS might affect female germ cells.

Rodent models that examine the effects of prenatal androgen exposure (i.e. prenatal androgenization) are commonly used to explore the pathophysiology underlying human polycystic ovary syndrome (PCOS), including reproductive and metabolic dysfunction. Based on a recent study using a dihydrotestosterone (DHT)-treated murine model, it has been proposed that prenatal androgen excess alone can predispose to transgenerational transmission of PCOS, perhaps through oocyte factors, including *Tial1* up-regulation, in mature oocytes of an androgenized lineage (*Picton and Balen, 2019*; *Risal et al., 2019*). We congratulate the authors on this paper, but also suggest that their findings, while intriguing, should be interpreted in the context of earlier publications, including those by *Wood et al. (2007)* and *Liu et al. (2016)*, which were not cited by the authors (*Risal et al., 2019*) or *Picton and Balen (2019)*. Here, we wish to integrate the findings of *Risal et al. (2019)* with other publications regarding important aspects of PCOS oocyte pathophysiology.

Risal et al. (2019) reported 410 differentially expressed genes (DEG) in the metaphase II (MII) oocytes of androgenized mice and 231 DEG from an obese mouse lineage. In our previous work (*Wood et al., 2007*), we examined the MII oocyte transcriptome of women with and without PCOS undergoing assisted reproduction. Comparing apparently healthy MII oocytes of women diagnosed with PCOS according to the National Institutes of Health consensus criteria, which emphasize hyperandrogenism, versus women with structural infertility ('endocrinologically normal' women), we found 374 DEG in PCOS versus normal oocytes as detected by Affymetrix U133 microarrays (ATLAS Biolabs GmbH, Berlin, Germany).

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TABLE 1 COMPARISON OF DIFFERENTIALLY EXPRESSED GENES BETWEEN *RISAL ET AL. (2019)* AND *WOOD ET AL. (2007)*

Exact match	Family member matches	
Risal *et al.* (1) Wood *et al.* (3)	Risal *et al.* (1)	Wood *et al.* (3)
ATP9a	*Abcf2*	*ABCF3*
DARS	*Atp6v0d1*	*ATP6V1C2*
DR1	*Cbx1*	*CBX5*
DSTN	*Dcbld1*	*DCBLD2*
FABP5	*Eml6*	*EML1*
NUDCD1	*Fgfr1op*	*FGFR1OP2*
	Fkbp14	*FKBP4*
	Ipo7	*IPO8*
	Med12	*MED19* *MED4*
	Otx1	*OTX2*
	Rgs10 *Rgs3*	*RGS7*
	Slc9a7	*SLC9A6*
	Snx30	*SNX26*
	Tnrc6a	*TNRC6*
	Ube2n	*UBE2E2* *UBE2V2*
	Exoc2	*EXOC1* *EXOC5*

Differential expression of selected genes was confirmed by PCR (*Wood et al., 2007*). Approximately 80% of the differentially expressed transcripts were increased in abundance in human PCOS oocytes, representing several functional categories including signal transduction, cellular metabolism, DNA transcription and RNA processing (*Wood et al., 2007*). Fifteen of the differentially expressed transcripts we identified were associated with meiosis, and included genes involved in spindle dynamics, homologous recombination/chromosome alignment, cell-cycle checkpoints and centrosome function.

Subsequently, *Liu et al. (2016)* found that 839 genes were differentially expressed in PCOS oocytes as determined by single-cell RNA sequencing. In addition to highlighting genes involving meiosis, Liu and colleagues reported DEG involving gap junction, hormone receptor signalling, DNA repair and genes encoding oocyte-secreted factors (*Liu et al., 2016*). Discrepancies between differentially expressed transcripts identified in these two reports could represent differences in study populations, ovarian stimulation protocols, RNA extraction and analysis, and/or small numbers of PCOS and 'normal' oocytes analysed.

Comparison of the oocyte DEG identified in our study (*Wood et al., 2007*) with those in the androgenized mouse MII oocytes reported by *Risal et al. (2019)* revealed six genes that were an exact match, and 20 genes that belong to the same family (TABLE 1). Importantly, cytotoxic granulosa-associated RNA binding protein-like 1 (*TiaL1*), as a proposed oocyte DEG marker of an androgenized lineage in mice, was not one of them. Moreover, the gene families in which there is overlap do not include loci that have been associated with PCOS in genome-wide association studies.

Although *Risal et al. (2019)* postulate that differential expression of mouse oocyte genes is triggered by androgens, androgen responsiveness of the DEG identified in their analyses was not evaluated. However, we did indeed identify putative androgen response elements in some DEG of MII PCOS oocytes. Specifically, 68 of the DEG in PCOS oocytes contained putative androgen receptor and/or peroxisome proliferating receptor gamma binding sites, implying that androgens and other metabolic activators of nuclear receptors could affect differential gene expression in the PCOS oocyte and, therefore, impact its developmental competence (*Wood et al., 2007*). In support of this concept, maternal metabolic syndrome in mice induces mitochondrial changes in F1–F3 offspring muscle via mitochondrial changes in F1–F2 oocytes, suggesting that mitochondrial dysfunction of oocyte origin, mediated through metabolic alterations rather than androgens alone, could be transmitted throughout the entire organism for consecutive generations (*Jaeger et al., 2017*). It is not known whether the mitochondrial abnormalities described by *Risal and colleagues (2019)* were a consequence of altered expression of nuclear genes, genes encoded by the mitochondrial genome, both, or a mechanism other than altered gene expression. In this regard, prenatally testosterone-treated female rhesus monkeys exhibit a permanent PCOS-like phenotype, characterized by LH hypersecretion, ovarian/adrenal hyperandrogenism, ovulatory dysfunction and impaired glucose–insulin homeostasis (*Abbott et al., 2019*). When rhesus monkeys exposed to testosterone in early-to-mid gestation undergo ovarian stimulation for IVF, impaired oocyte developmental competence accompanies LH hypersecretion and relative insulin excess, implicating complex reproductive–metabolic interactions with altered post-implantation embryo development (*Dumesic et al., 2002*).

Although our findings on human oocytes and those of *Liu et al. (2016)* could be viewed as being consistent with the notion that PCOS-induced alterations in oocyte gene expression promote a transgenerational phenotype, one must be cautious in extrapolating data from murine experiments to human studies. That differential expression of MII oocyte transcripts of DHT-treated mice was not substantially similar to that of PCOS women raises questions regarding the fidelity with which rodent models of PCOS replicate PCOS *per se* (*Abbott et al., 2019*). Moreover, it is not yet possible to determine whether altered oocyte gene expression in PCOS is 'imprinted' through the female germ line across generations, given the complexities of epigenetic reprogramming during gamete and

embryo development among species (*Zacchini et al., 2019*).

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