Research Square

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The neurodevelopment of neonates born to women with polycystic ovary syndrome: evidences from China

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Abstract

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. Mothers with PCOS are at increased risk of various gestational complications and adverse outcomes of offspring. The health problems, including neurobehavioral phenotypes, of PCOS offspring has attracted wide attention and been associated with intrauterine environment changes due to maternal PCOS. Using neonatal behavioral neurological assessment (NBNA), we conducted measurement of neonate's neurodevelopment on the third day after birth. Total score of NBNA and score of behavioral capacity, orientation response-animate visual and auditory, were significantly lower in PCOS group than those in control group. Concerning sex difference, we found significantly lower total score of NBNA and score of behavioral capacity, orientation response-inanimate visual, in male neonates of PCOS group compared to those of control group, while this did not exist in female neonates. In conclusion, our data indicated an association between maternal PCOS and offspring neurobehavior at the beginning of neonatal period in a sex-specific manner. Further evidence of intrauterine environment is required to clarify the potential mechanism underlying impacts of maternal PCOS on neurodevelopment.

What is known

Mothers with PCOS are at increased risk of gestational diabetes, hypertension, endothelial dysfunction and pre-eclampsia, which can alter the fetal endocrine environment, leading to adverse outcomes in offspring. The babies born to PCOS mothers had a significantly higher risk of admission to a neonatal intensive care unit and a higher perinatal mortality, unrelated to multiple births. A significantly higher prevalence of obesity, diabetes, cardiovascular disease was observed in offspring of women with PCOS, which could not be completely attributed to pregnancy complications, and seems to be more related to maternal PCOS condition, which our studies also lend support to. Concerning neurobehavioral phenotypes, maternal PCOS has been reported to respectively increase the odds of offspring attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) by 42% and 59%. Consistently, the previous work of the author's group has suggested a different pattern of brain metrics in PCOS offspring in utero, and alterations of neurobehavioral phenotypes in female offspring of both women with PCOS and PCOS model rats which might involve learning and memory.

What is new: Neonatal behavioral neurological assessment (NBNA) was used as the measurement of neonates neurodevelopment in the current study, which was administered on the third day after birth. NBNA was invented by Bao et al based on the method of Brazelton and Amiel-Tison for behavioral neurological measurement in neonates as well as the clinical experiences in Chinese new-borns. The NBNA was tested with distinct stability and reliability by several large cohorts in China, and showed high consistency over various geographic locations .

In the current study, total score of NBNA and score of behavioral capacity, orientation response-animate visual and auditory, were significantly lower in PCOS group than those in control group. Moreover, male neonates in PCOS group showed significantly lower total score of NBNA and score of behavioral capacity, especially orientation response-inanimate visual, than those in controls, while the female PCOS neonates did not. This result indicated that PCOS offspring probably showed social attention impairments, and that the male neonates of women with PCOS might have lower visual sensory ability of nonsocial information, which appeared as early as the beginning of the neonatal period. The possible explanation for sex difference in neurobehavior of PCOS offspring is that intrauterine androgen exposure in male PCOS offspring is most enhanced resulting from both maternal PCOS condition and fetal testicular function.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. Women with PCOS exhibit heterogeneous features which involve reproductive, metabolic and psychological function [1, 2]. The specific gene mutations affecting androgen synthesis, insulin secretion and insulin activity explain most of the endocrine and metabolic symptoms, while environmental risk factors (during either prenatal or postnatal life), seem to convert an occult PCOS into a clinically manifest syndrome[3].

Mothers with PCOS are at increased risk of gestational diabetes, hypertension, endothelial dysfunction and pre-eclampsia, which can alter the fetal endocrine environment, leading to adverse outcomes in offspring[4]. The babies born to PCOS mothers had a significantly higher risk of admission to a neonatal intensive care unit and a higher perinatal mortality, unrelated to multiple births[5]. A significantly higher prevalence of obesity, diabetes, cardiovascular disease was observed in offspring of women with PCOS, which could not be completely attributed to pregnancy complications, and seems to be more related to maternal PCOS condition[6-9], which our studies also lend support to[10-12]. Concerning neurobehavioral phenotypes, maternal PCOS has been reported to respectively increase the odds of offspring attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) by 42% and 59%[13-16]. Consistently, the previous work of the author's group has suggested a different pattern of brain metrics in PCOS offspring in utero[17], and alterations of neurobehavioral phenotypes in female offspring of both women with PCOS[18] and PCOS model rats[19] which might involve learning and memory. Hence, further study is needed to confirm the role of maternal PCOS in early offspring neurodevelopment for both boys and girls.

METHODS

The recruitment of pairs of mother and neonate and collection of clinical information

This study was conducted between December 2016 and June 2017 in two hospitals in China, including Hangzhou Red Cross Hospital and Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University. Nineteen neonates born to mothers with PCOS and 38 neonates (as controls) born to the healthy mothers were recruited. Written informed consent was obtained from all the women. The study was approved by the Ethics Committee of Hangzhou Red Cross Hospital, Hangzhou, China. All mothers with PCOS were diagnosed according to the Rotterdam Consensus (European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine criteria)[20]. During recruitment, the mother's age, body weight, height, pregnancy complication, parity, educational background and conception mode were noted. Birth mode, gestational week, gender, birth weight, serum bilirubin and Apagar score of neonates were recorded after birth.

Assessment of growth and neurodevelopment status of neonates

Neonatal behavioral neurological assessment (NBNA) was used as the measurement of neonates neurodevelopment in the current study, which was administered on the third day after birth. NBNA was invented by Bao et al[21] based on the method of Brazelton and Amiel-Tison for behavioral neurological measurement in neonates as well as the clinical experiences in Chinese new-borns. The NBNA was tested with distinct stability and reliability by several large cohorts in China, and showed high consistency over various geographic locations [22, 23].

The NBNA assesses functional abilities, most reflexes and responses, and stability of behavioural status during the examination. It involves five scales: behavior (six items), passive tone (four items), active tone (four items), primary reflexes (three items), and general assessment (three items). Each above item has three dimensions of score (0, 1 and 2). Twenty items are summarized in the summary score with a maximum score of 40. Neonates with summary score more than 37 were considered to be well developed, and those with summary score more than 35 and lower than 37

were considered to be acceptable, otherwise neonates would probably be associated with poor clinical outcomes. Examiners were blinded with regard to exposure status when NBNA were carried out.

Statistical analysis

All data were analyzed with SPSS Version 21.0. Independent sample t-test was used to compare variables approximately normally distributed, and a nonparametric test (Mann-Whitney U test) was used for variables with distributions other than normal. Numeration data were analyzed using chi-square test and Fisher's exact test when the cell count was <5. $P<0.05$ was considered statistically significant.

RESULTS

Baseline clinical characteristics

Concerning the mothers: age, BMI, duration of pregnancy, pregnancy complication, educational background and mode of delivery did not differ between two groups (all $P>0.05$, Table 1). However, mode of conception and parity differed significantly between two groups ($P=0.010$ and $P=0.003$, respectively, Table 1). Concerning the offspring: gender, birth weight, and Apgar score were comparable between two groups (all $P>0.05$, Table 1). After divided into subgroups based on neonatal gender, we observed comparable baseline clinical characteristics of both mothers and neonates (all $P>0.05$, Table 2) except mothers' parity in subgroup of male offspring and mode of conception in subgroup of female offspring ($P=0.010$ and $P=0.023$, respectively, Table 2).

Table 1 Baseline clinical characteristics of pairs of mothers and neonates

Characteristics	PCOS(n=19)	Control(n=38)	P value
Mother			
Age(years)	31.16±4.03	31.73±4.10	0.621
BMI(kg/m^2)	26.42±3.54	27.36±2.75	0.291
Mode of conception			
Natural	15(78.95)	38(100.00)	0.010*
Ovulation induction	2(10.53)	0	
IVF/ICSI	2(10.53)	0	
Gestational week	39.65±1.15	39.33±0.94	0.274
Pregnancy complication			
Gestational diabetes mellitus	2(10.50)	6(15.80)	0.590
Thyroid dysfunction	4(21.10)	4(10.50)	0.500
Group B streptococcal infection	2(10.50)	1(2.60)	0.529
Parity	1.22±0.55	1.70±0.52	0.003#
Educational background**			
University	11(57.90)	10(27.00)	0.063
High school	6(31.60)	15(40.50)	
Less than high school	2(10.50)	12(32.40)	
Mode of delivery			
Vaginal delivery	10(52.60)	26(68.40)	0.244
Cesarean section delivery	9(47.40)	12(31.60)	
Offspring			
Neonatal gender			
Male	10(52.60)	18(47.40)	0.708
Female	9(47.40)	20(52.60)	
Birth weight(g)	3392.11±424.02	3426.32±321.26	0.735
Apgar score	10.00±0.00	10.00±0.00	-

Note: Data were presented as mean±SD or number (percentage). *P<0.05, compared with control group; #P<0.01, compared with control group. **Missing data for one subject.

Table 2 Baseline clinical characteristics of pairs of mothers and neonates in subgroups based on neonatal gender

Characteristics	Male offspring(n=28)			Female offspring(n=29)		
	PCOS(n=10)	Control(n=18)	P value	PCOS(n=9)	Control(n=20)	P value
Mother						
Age(years)	30.60±4.09	30.67±3.97	0.967	31.78±4.12	32.74±4.07	0.567
BMI(kg/m^2)	26.85±3.25	27.95±2.46	0.332	25.94±4.01	26.81±2.96	0.538
Mode of conception						
Natural	9(90.00)	18(100.00)	0.357	6(66.67)	20(100.00)	0.023*
Ovulation induction	0	0		2(22.22)	0	
IVF/ICSI	1(10.00)	0		1(11.11)	0	
Gestational week	39.60±1.40	39.60±0.67	0.988	39.70±0.86	39.09±1.09	0.150
Pregnancy complication						
Gestational diabetes mellitus	0	4(22.22)	0.265	2(22.22)	2(10.00)	0.568
Thyroid dysfunction	3(30.00)	1(5.56)	0.116	1(11.11)	3(15.00)	1.000
Group B streptococcal infection	1(10.00)	1(5.56)	1.000	1(11.11)	0	0.310
Parity	1.20±0.42	1.71±0.47	0.010*	1.25±0.71	1.70±0.57	0.090
Educational background**						
University	4(40.00)	3(16.67)	0.280	7(77.78)	7(36.84)	0.176
High school	5(50.00)	9(50.00)		1(11.11)	6(31.58)	
Less than high school	1(10.00)	6(33.33)		1(11.11)	6(31.58)	
Mode of delivery						
Vaginal delivery	4(40.00)	8(44.44)	1.000	6(66.67)	18(90.00)	0.287
Cesarean section delivery	6(60.00)	10(55.56)		3(33.33)	2(10.00)	
Offspring						
Birth weight(g)	3565.00±323.22	3541.67±338.79	0.861	3200.00±456.21	3322.50±272.65	0.374

Apgar score	10.00±0.00	10.00±0.00	-	10.00±0.00	10.00±0.00	-

Note: Data were presented as mean±SD or number (percentage). *P<0.05, compared with control group. ** Missing data for one subject.

Neonatal neurodevelopment

Total score of NBNA and score of behavioral capacity (especially in orientation response-animate visual and auditory) were significantly lower in PCOS group than those in control group (all P<0.05). In active muscle tension, passive muscle tension, primitive reflex and general status, NBNA score were comparable between two groups (all P>0.05, Table 3).

To investigate the gender difference, we compared neonatal neurodevelopment between girls and boys in both PCOS and control groups. On one hand, male neonates in PCOS group showed significantly lower total score of NBNA and score of behavioral capacity, especially orientation response-inanimate visual, than those in control group (all P<0.05), but there was no statistical difference between two groups in active muscle tension, passive muscle tension, primitive reflex and general status. On the other hand, female neonates were comparable in all items of NBNA score between PCOS and control groups (all P>0.05, Table 4). In addition, we found no statistical differences in all items of NBNA scores between female neonates and male neonates in both PCOS and control groups (all P>0.05, Table 4).

Table 3 Comparison of NBNA between PCOS group and control group

Item	PCOS(n=19)	Control(n=38)	P value
Total NBNA	36.68±1.11	37.76±1.08	0.001#
BC	8.84±1.12	9.92±1.08	0.001#
AMT	7.95±0.23	7.97±0.16	0.618
PMT	8.00±0.00	7.95±0.23	0.317
PR	5.89±0.32	5.92±0.27	0.746
GS	6.00±0.00	6.00±0.00	-
Response decrement to light	2.00±0.00	2.00±0.00	-
Response decrement to rattle	1.84±0.37	1.97±0.16	0.158
Orientation response-inanimate auditory	1.26±0.45	1.53±0.51	0.054
Orientation response-inanimate visual	1.05±0.52	1.21±0.41	0.220
Orientation response-animate visual and auditory	0.89±0.66	1.24±0.43	0.022*
Consolability	1.79±0.42	1.97±0.16	0.079
Scarf sigh	2.00±0.00	2.00±0.00	-
Recoil of upper limbs	2.00±0.00	2.00±0.00	-
Recoil of lower limbs	2.00±0.00	2.00±0.00	-
Popliteal angle	1.95±0.23	1.97±0.16	0.618
Raise to sit	2.00±0.00	2.00±0.00	-
Hand grasp	2.00±0.00	1.95±0.23	0.160
Pull reaction	2.00±0.00	2.00±0.00	-
Righting reaction	2.00±0.00	2.00±0.00	-
Automatic walking	1.89±0.32	1.92±0.27	0.746
Moro reflex	2.00±0.00	2.00±0.00	-
Sucking	2.00±0.00	2.00±0.00	-
Alertness	2.00±0.00	2.00±0.00	-
Crying	2.00±0.00	2.00±0.00	-
Activity level	2.00±0.00	2.00±0.00	-

Note: Data were presented as mean±SD. *P<0.05, compared with control group; #P<0.01, compared with control group. AMT=active muscle tension, BC=behavioral capacity, GS=general status, NBNA=neonatal behavioral neurological assessment, PMT=passive muscle tension, PR=primitive reflex.

Table 4 Comparison of NBNA between PCOS group and control group based on neonatal gender

Item	PCOS(n=19)		Control(n=38)		P value			
	Female (n=9)	Male (n=10)	Female (n=20)	Male (n=18)	PF vs. CF	PM vs. CM	PF vs. PM	CF vs. CM
Total NBNA	36.67±1.50	36.70±0.67	37.65±1.04	37.89±1.13	0.050	0.006#	0.950	0.502
BC	9.00±1.50	8.70±0.67	9.80±1.11	10.06±1.06	0.118	0.001#	0.574	0.472
AMT	7.89±0.33	8.00±0.00	8.00±0.00	7.94±0.24	0.347	0.466	0.347	0.331
PMT	8.00±0.00	8.00±0.00	7.95±0.22	7.94±0.24	0.512	0.466	-	0.941
PR	5.78±0.44	6.00±0.00	5.90±0.31	5.94±0.24	0.395	0.466	0.169	0.623
GS	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	-	-	-	-
Response decrement to light	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Response decrement to rattle	1.89±0.33	1.80±0.42	2.00±0.00	1.94±0.24	0.347	0.337	0.620	0.331
Orientation response-inanimate auditory	1.33±0.50	1.20±0.42	1.50±0.51	1.56±0.51	0.422	0.061	0.537	0.740
Orientation response-inanimate visual	1.22±0.67	0.90±0.32	1.20±0.41	1.22±0.43	0.913	0.048*	0.213	0.871
Orientation response-animate visual and auditory	0.78±0.67	1.00±0.67	1.15±0.37	1.33±0.49	0.146	0.140	0.478	0.202
Consolability	1.78±0.44	1.80±0.42	1.95±0.22	2.00±0.00	0.294	0.168	0.912	0.350
Scarf sigh	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Recoil of upper limbs	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Recoil of lower limbs	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Popliteal angle	1.89±0.33	2.00±0.00	2.00±0.00	1.94±0.24	0.347	0.466	0.347	0.331
Raise to sit	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Hand grasp	2.00±0.00	2.00±0.00	1.95±0.22	1.94±0.24	0.512	0.466	-	0.941
Pull reaction	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Righting reaction	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Automatic walking	1.78±0.44	2.00±0.00	1.90±0.31	1.94±0.24	0.395	0.466	0.169	0.623
Moro reflex	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-

Sucking	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Alertness	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Crying	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-
Activity level	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	-	-	-	-

Note: Data were presented as mean±SD. PF=PCOS female group, PM=PCOS male group, CF=control female group, CM=control male group, AMT=active muscle tension, BC=behavioral capacity, GS=general status, NBNA=neonatal behavioral neurological assessment, PMT=passive muscle tension, PR=primitive reflex. *P<0.05, significant difference; $^{\#}$P<0.01, significant difference.

Discussion

Little is known about the effects of maternal PCOS on fetal development[23]. During pregnancy, PCOS women have been shown to have increased blood androgen concentrations compared to control women[24]. This increase could affect the intrauterine androgen exposure of offspring. Female infants born to women with PCOS were found to have umbilical testosterone concentrations not only higher than control females but comparable to control males[25]. A positive association was found between elevated fetal testosterone in amniotic fluid and autistic features[26]. Elevated fetal testosterone in the mixture of arterial and venous umbilical cord blood also showed a high risk of language delay, particularly in male children[27]. Recent studies support the hypothesis that prenatal exposure to testosterone contributes to cognitive function development[28]. This would suggest that maternal PCOS with excess androgens, would increase the risk of ASD in the offspring[29].

ASD is a set of heterogenous neurodevelopment conditions characterised by deficits in social communication, as well as severe anxiety and repetitive, restricted behaviour and interests[30-32], with worldwide prevalence of 1% to 2%[33-37]. The aetiology of autism is not fully understood, but genetics and early environmental factors can be the main causes of affecting the neurodevelopment[38-41]. Possible risk factors include advanced parental age[42, 43], exposure to chemicals[40, 44-47] and hormones[48-50] during pregnancy and perinatal and neonatal complications[39]. High prenatal fetal testosterone levels could affect the development of sexually dimorphic brain structures, thus lead to sex differences and autistic traits in individuals[51]. This idea is supported by the ‘extreme male brain of autism’ theory[52] that extend the ‘empathising-systemising theory’ in typical sex differences[53]. These theories argue that individuals with ASD have male-typical ‘systemising’ cognitive abilities, considered as the drive to analyse and construct a system and less female-typical ‘empathising’ profiles[52, 53]. Nevertheless, they were challenged by controversial evidence. Kung *et al*. showed no evidence in their study testing autistic traits in children with congenital adrenal hyperplasia and typically developing children with amniotic fluid[54]. Considerable research has found that children with autism orient less frequently to both social and nonsocial stimuli than their peers[55]. ASD children showed distinct visual working memory and sensory processing[56].

In the current study, total score of NBNA and score of behavioral capacity, orientation response-animate visual and auditory, were significantly lower in PCOS group than those in control group. Moreover, male neonates in PCOS group showed significantly lower total score of NBNA and score of behavioral capacity, especially orientation response-inanimate visual, than those in controls, while the female PCOS neonates did not. This result indicated that PCOS offspring probably showed social attention impairments, and that the male neonates of women with PCOS might have lower visual sensory ability of nonsocial information, which appeared as early as the beginning of the neonatal period. The possible explanation for sex difference in neurobehavior of PCOS offspring is that intrauterine androgen

exposure in male PCOS offspring is most enhanced resulting from both maternal PCOS condition and fetal testicular function.

Limitations in the present study point to the direction of our future work. Firstly, increasing sample size is crucial to provide statistical power. This can be achieved by recruiting both more PCOS mothers and controls with an equal ratio of sons and daughters. Secondly, intrauterine androgen exposure of PCOS offspring should be further clarified. Currently, there is no established method that is both ethical and practical that can measure prenatal hormone exposure[57]. An ideal method could be through fetal blood collected during the critical period of neurodevelopment, but this is not feasible because of ethical reasons. Amniotic fluid is an alternative approach which probably reflects testosterone in fetal blood, but the sampling procedure (amniocentesis) is only performed for prenatal diagnosis for high-risk pregnancies[58]. In addition, intrauterine exposure of other endocrine factors besides androgen are also promising to give interpretation for the altered neurodevelopment of PCOS offspring[59].

To sum up, the current study suggested an association between maternal PCOS and offspring neurobehavior at the beginning of neonatal period in a sex-specific manner. Our findings provide a clue for the mechanism underlying that prenatal androgen exposure impacts the neurodevelopment of offspring. However, our study is too small to answer whether early attention or intervention is required for children of PCOS mothers. It should be noted that children of PCOS mothers showed neurobehavior changes might related to autistic traits, but the risk of having children with ASD for PCOS women is still rare[13, 60, 61]. Therefore, the association between two conditions should not be overstated to avoid unnecessary stress on mothers with PCOS. Further research is required to understand the mechanisms of PCOS and ASD, as well as the involvement in fetal, maternal hormone levels and other conditions to optimise clinical interventions.

abbreviations

Abbreviations	Full Name
ADHD	attention-deficit/hyperactivity disorder
AMT	active muscle tension
ASD	autism spectrum disorders
BC	behavioral capacity
CF	control female group
CM	control male group
GS	general status
NBNA	neonatal behavioral neurological assessment
PCOS	polycystic ovarian syndrome
PF	PCOS female group
PM	PCOS male group
PMT	passive muscle tension
PR	primitive reflex

Declarations

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Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Hangzhou Red Cross Hospital, Hangzhou, China.(Date 2016.12.16/No [2016] 07)

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Consent to publish: Patients signed informed consent regarding publishing their data.

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References

1. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet 2007;370(9588):685-97.https://doi.org/10.1016/s0140-6736(07)61345-2
2. Rosenfield RL, Ehrmann DA. The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. Endocr Rev 2016;37(5):467-520.https://doi.org/10.1210/er.2015-1104
3. Crosignani PG, Nicolosi AE. Polycystic ovarian disease: heritability and heterogeneity. Hum Reprod Update 2001;7(1):3-7.https://doi.org/10.1093/humupd/7.1.3
4. Puttabyatappa M, Cardoso RC, Padmanabhan V. Effect of maternal PCOS and PCOS-like phenotype on the offspring's health. Mol Cell Endocrinol 2016;435:29-39.https://doi.org/10.1016/j.mce.2015.11.030
5. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. Hum Reprod Update 2006;12(6):673-83.https://doi.org/10.1093/humupd/dml036
6. Sir-Petermann T, Hitchsfeld C, Maliqueo M, Codner E, Echiburú B, Gazitúa R, et al. Birth weight in offspring of mothers with polycystic ovarian syndrome. Hum Reprod 2005;20(8):2122-6.https://doi.org/10.1093/humrep/dei009
7. Chen X, Koivuaho E, Piltonen TT, Gissler M, Lavebratt C. Association of maternal polycystic ovary syndrome or anovulatory infertility with obesity and diabetes in offspring: a population-based cohort study. Hum Reprod 2021;36(8):2345-57.https://doi.org/10.1093/humrep/deab112
8. Gunning MN, Sir Petermann T, Crisosto N, van Rijn BB, de Wilde MA, Christ JP, et al. Cardiometabolic health in offspring of women with PCOS compared to healthy controls: a systematic review and individual participant data meta-analysis. Hum Reprod Update 2020;26(1):103-17.https://doi.org/10.1093/humupd/dmz036

9. Schmidt AB, Lund M, Wohlfahrt J, Melbye M. Polycystic ovary syndrome and offspring risk of congenital heart defects: a nationwide cohort study. Hum Reprod 2020;35(10):2348-55.https://doi.org/10.1093/humrep/deaa168
10. Zhang F, Ying L, Zhang Q, Wang F, Qu F. Association between maternal polycystic ovary syndrome and early childhood growth: a continuous observation from 3 months to 6 years of age. J Assist Reprod Genet 2022;39(2):461-71.https://doi.org/10.1007/s10815-021-02378-9
11. Wang Y, Guo L, Jiang J, Wang F, Hardiman PJ, Qu F. Development of 1-2 years Offspring Born to Mothers with Polycystic Ovary Syndrome. J Coll Physicians Surg Pak 2021;31(10):1186-90.https://doi.org/10.29271/jcpsp.2021.10.1186
12. Zhang FF, Zhang Q, Wang YL, Wang FF, Hardiman PJ, Qu F. Intergenerational Influences between Maternal Polycystic Ovary Syndrome and Offspring: An Updated Overview. J Pediatr 2021;232:272-81.https://doi.org/10.1016/j.jpeds.2021.01.018
13. Kosidou K, Dalman C, Widman L, Arver S, Lee BK, Magnusson C, et al. Maternal polycystic ovary syndrome and the risk of autism spectrum disorders in the offspring: a population-based nationwide study in Sweden. Molecular psychiatry 2016;21(10):1441-8.https://doi.org/10.1038/mp.2015.183
14. Kosidou K, Dalman C, Widman L, Arver S, Lee BK, Magnusson C, et al. Maternal Polycystic Ovary Syndrome and Risk for Attention-Deficit/Hyperactivity Disorder in the Offspring. Biological psychiatry 2017;82(9):651-9.https://doi.org/10.1016/j.biopsych.2016.09.022
15. Katsigianni M, Karageorgiou V, Lambrinoudaki I, Siristatidis C. Maternal polycystic ovarian syndrome in autism spectrum disorder: a systematic review and meta-analysis. Molecular psychiatry 2019;24(12):1787-97.https://doi.org/10.1038/s41380-019-0398-0
16. Dalgaard CM, Andersen MS, Jensen RC, Larsen PV, Find LG, Boye H, et al. Maternal polycystic ovary syndrome and attention deficit hyperactivity disorder in offspring at 3 years of age: Odense Child Cohort. Acta obstetricia et gynecologica Scandinavica 2021;100(11):2053-65.https://doi.org/10.1111/aogs.14259
17. Bao Z, Zhang Q, Pan M, Xi X, Wang Y, Zhang F, et al. Alterations of brain metrics in fetuses of women with polycystic ovary syndrome : a retrospective study based on fetal magnetic resonance imaging. BMC Pregnancy Childbirth 2021;21(1):557.https://doi.org/10.1186/s12884-021-04015-w
18. Wang F, Xie N, Zhou J, Dai M, Zhang Q, Hardiman PJ, et al. Molecular mechanisms underlying altered neurobehavioural development of female offspring of mothers with polycystic ovary syndrome: FOS-mediated regulation of neurotrophins in placenta. EBioMedicine 2020;60:102993.https://doi.org/10.1016/j.ebiom.2020.102993
19. Zhang X, You L, Zhang X, Wang F, Wang Y, Zhou J, et al. Neurobehavioral alternations of the female offspring born to polycystic ovary syndrome model rats administered by Chinese herbal medicine. Chin Med 2021;16(1):97.https://doi.org/10.1186/s13020-021-00512-4
20. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19(1):41-7.https://doi.org/10.1093/humrep/deh098
21. Bao XL, Yu RJ, Li ZS, Zhang BL. Twenty-item behavioral neurological assessment for normal newborns in 12 cities of China. Chin Med J (Engl) 1991;104(9):742-6,
22. Bao XL, Yu RJ, Li ZS. 20-item neonatal behavioral neurological assessment used in predicting prognosis of asphyxiated newborn. Chin Med J (Engl) 1993;106(3):211-5,
23. Yu XD, Yan CH, Shen XM, Tian Y, Cao LL, Yu XG, et al. Prenatal exposure to multiple toxic heavy metals and neonatal neurobehavioral development in Shanghai, China. Neurotoxicol Teratol 2011;33(4):437-43.https://doi.org/10.1016/j.ntt.2011.05.010

24. Sir-Petermann T, Maliqueo M, Angel B, Lara HE, Pérez-Bravo F, Recabarren SE. Maternal serum androgens in pregnant women with polycystic ovarian syndrome: possible implications in prenatal androgenization. Hum Reprod 2002;17(10):2573-9.https://doi.org/10.1093/humrep/17.10.2573
25. Barry JA, Kay AR, Navaratnarajah R, Iqbal S, Bamfo JE, David AL, et al. Umbilical vein testosterone in female infants born to mothers with polycystic ovary syndrome is elevated to male levels. J Obstet Gynaecol 2010;30(5):444-6.https://doi.org/10.3109/01443615.2010.485254
26. Auyeung B, Taylor K, Hackett G, Baron-Cohen S. Foetal testosterone and autistic traits in 18 to 24-month-old children. Mol Autism 2010;1(1):11.https://doi.org/10.1186/2040-2392-1-11
27. Whitehouse AJ, Mattes E, Maybery MT, Sawyer MG, Jacoby P, Keelan JA, et al. Sex-specific associations between umbilical cord blood testosterone levels and language delay in early childhood. J Child Psychol Psychiatry 2012;53(7):726-34.https://doi.org/10.1111/j.1469-7610.2011.02523.x
28. Manson JE. Prenatal exposure to sex steroid hormones and behavioral/cognitive outcomes. Metabolism 2008;57 Suppl 2:S16-21.https://doi.org/10.1016/j.metabol.2008.07.010
29. Baron-Cohen S, Knickmeyer RC, Belmonte MK. Sex differences in the brain: implications for explaining autism. Science 2005;310(5749):819-23.https://doi.org/10.1126/science.1115455
30. Association AP. Diagnostic and statistical manual of mental disorders: DSM-5™, 5th ed. Arlington, VA, US: American Psychiatric Publishing, Inc.; 2013. doi: 10.1176/appi.books.9780890425596.
31. Boucher J. Research review: structural language in autistic spectrum disorder - characteristics and causes. J Child Psychol Psychiatry 2012;53(3):219-33.https://doi.org/10.1111/j.1469-7610.2011.02508.x
32. Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet 2014;383(9920):896-910.https://doi.org/10.1016/s0140-6736(13)61539-1
33. Fombonne É QS, Hagen A. Epidemiology of Pervasive Developmental Disorders. Autism Spectrum Disorders. Oxford University Press; 2011, p. 99-111.
34. Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcín C, et al. Global prevalence of autism and other pervasive developmental disorders. Autism Res 2012;5(3):160-79.https://doi.org/10.1002/aur.239
35. Russell G, Rodgers LR, Ukoumunne OC, Ford T. Prevalence of parent-reported ASD and ADHD in the UK: findings from the Millennium Cohort Study. J Autism Dev Disord 2014;44(1):31-40.https://doi.org/10.1007/s10803-013-1849-0
36. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, et al. Prevalence of autism spectrum disorders in a total population sample. Am J Psychiatry 2011;168(9):904-12.https://doi.org/10.1176/appi.ajp.2011.10101532
37. Baron-Cohen S, Scott FJ, Allison C, Williams J, Bolton P, Matthews FE, et al. Prevalence of autism-spectrum conditions: UK school-based population study. Br J Psychiatry 2009;194(6):500-9.https://doi.org/10.1192/bjp.bp.108.059345
38. Geschwind DH. Genetics of autism spectrum disorders. Trends Cogn Sci 2011;15(9):409-16.https://doi.org/10.1016/j.tics.2011.07.003
39. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics 2011;128(2):344-55.https://doi.org/10.1542/peds.2010-1036
40. Rodier PM. Environmental Exposures That Increase the Risk of Autism Spectrum Disorders. Autism Spectrum Disorders. Oxford University Press; 2011, p. 863-74.
41. Corrales MA, Herbert MR. Autism and Environmental Genomics: Synergistic Systems Approaches to Autism Complexity. In: Amaral D, Geschwind D, Dawson G, editors. Autism Spectrum Disorders. Oxford University Press; 2011, p. 875-92. doi: 10.1093/med/9780195371826.003.0056.

42. Hultman CM, Sandin S, Levine SZ, Lichtenstein P, Reichenberg A. Advancing paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies. Molecular psychiatry 2011;16(12):1203-12.https://doi.org/10.1038/mp.2010.121
43. Lampi KM, Hinkka-Yli-Salomäki S, Lehti V, Helenius H, Gissler M, Brown AS, et al. Parental age and risk of autism spectrum disorders in a Finnish national birth cohort. J Autism Dev Disord 2013;43(11):2526-35.https://doi.org/10.1007/s10803-013-1801-3
44. Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, McConnell R. Traffic-related air pollution, particulate matter, and autism. JAMA Psychiatry 2013;70(1):71-7.https://doi.org/10.1001/jamapsychiatry.2013.266
45. Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environ Health Perspect 2007;115(10):1482-9.https://doi.org/10.1289/ehp.10168
46. Christensen J, Grønborg TK, Sørensen MJ, Schendel D, Parner ET, Pedersen LH, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. Jama 2013;309(16):1696-703.https://doi.org/10.1001/jama.2013.2270
47. Rai D, Lee BK, Dalman C, Golding J, Lewis G, Magnusson C. Parental depression, maternal antidepressant use during pregnancy, and risk of autism spectrum disorders: population based case-control study. Bmj 2013;346:f2059.https://doi.org/10.1136/bmj.f2059
48. Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G. Fetal testosterone and autistic traits. Br J Psychol 2009;100(Pt 1):1-22.https://doi.org/10.1348/000712608x311731
49. de Cock M, Maas YG, van de Bor M. Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? Review. Acta Paediatr 2012;101(8):811-8.https://doi.org/10.1111/j.1651-2227.2012.02693.x
50. Baron-Cohen S, Auyeung B, Nørgaard-Pedersen B, Hougaard DM, Abdallah MW, Melgaard L, et al. Elevated fetal steroidogenic activity in autism. Molecular psychiatry 2015;20(3):369-76.https://doi.org/10.1038/mp.2014.48
51. Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R. Why are autism spectrum conditions more prevalent in males? PLoS Biol 2011;9(6):e1001081.https://doi.org/10.1371/journal.pbio.1001081
52. Baron-Cohen S. The extreme male brain theory of autism. Trends Cogn Sci 2002;6(6):248-54.https://doi.org/10.1016/s1364-6613(02)01904-6
53. Baron-Cohen S. Empathizing, systemizing, and the extreme male brain theory of autism. Prog Brain Res 2010;186:167-75.https://doi.org/10.1016/b978-0-444-53630-3.00011-7
54. Kung KT, Spencer D, Pasterski V, Neufeld S, Glover V, O'Connor TG, et al. No relationship between prenatal androgen exposure and autistic traits: convergent evidence from studies of children with congenital adrenal hyperplasia and of amniotic testosterone concentrations in typically developing children. J Child Psychol Psychiatry 2016;57(12):1455-62.https://doi.org/10.1111/jcpp.12602
55. Dawson G, Toth K, Abbott R, Osterling J, Munson J, Estes A, et al. Early social attention impairments in autism: social orienting, joint attention, and attention to distress. Dev Psychol 2004;40(2):271-83.https://doi.org/10.1037/0012-1649.40.2.271
56. Stevenson RA, Ruppel J, Sun SZ, Segers M, Zapparoli BL, Bebko JM, et al. Visual working memory and sensory processing in autistic children. Sci Rep 2021;11(1):3648.https://doi.org/10.1038/s41598-021-82777-1
57. Hollier LP, Keelan JA, Hickey M, Maybery MT, Whitehouse AJ. Measurement of androgen and estrogen concentrations in cord blood: accuracy, biological interpretation, and applications to understanding human

behavioral development. Front Endocrinol (Lausanne) 2014;5:64.https://doi.org/10.3389/fendo.2014.00064

58. Nagamani M, McDonough PG, Ellegood JO, Mahesh VB. Maternal and amniotic fluid steroids throughout human pregnancy. Am J Obstet Gynecol 1979;134(6):674-80.https://doi.org/10.1016/0002-9378(79)90649-5
59. Vacher CM, Lacaille H, O'Reilly JJ, Salzbank J, Bakalar D, Sebaoui S, et al. Placental endocrine function shapes cerebellar development and social behavior. Nat Neurosci 2021;24(10):1392-401.https://doi.org/10.1038/s41593-021-00896-4
60. Cherskov A, Pohl A, Allison C, Zhang H, Payne RA, Baron-Cohen S. Polycystic ovary syndrome and autism: A test of the prenatal sex steroid theory. Transl Psychiatry 2018;8(1):136.https://doi.org/10.1038/s41398-018-0186-7
61. Berni TR, Morgan CL, Berni ER, Rees DA. Polycystic Ovary Syndrome Is Associated With Adverse Mental Health and Neurodevelopmental Outcomes. J Clin Endocrinol Metab 2018;103(6):2116-25.https://doi.org/10.1210/jc.2017-02667