An Overview of Research Programmes on PCOS at NIRRH

Dr. Smita Mahale
NIRRH Mandate

Reproductive Health

Research

Services

Basic

Clinical

Human Resource Development

Operational & Socio behavioural
Polycystic Ovary Syndrome

- Multifactorial, multigenic disorder of unknown etiology
- Complex endocrinopathy with global incidence of 5-10%

Long term health complications:
- Anovulatory infertility
- Type 2 Diabetes Mellitus
- Dyslipidemia
- Cardiovascular Disorders
- Endometrial cancer
PCOS: Research at NIRRH

- Clinical
- Basic
- Bioinformatics
- Epidemiological
NIRRH Infertility Clinic Data

Female factors (n=383)

- PCOS: 64%
- Tubal factor: 9.92%
- POF: 6%
- Thyroid disorder: 4%
- Hyperprolactinemia: 4%
- FGTB: 6%
- Endometriosis: 6%
- Unexplained: 6.53%

BMI (n=98)

- < 19: 6.3%
- 19.1-23: 27%
- 23.1-24.9: 11.6%
- Obese 25 and above: 55.2%

64% of the infertile women studies showed symptoms of PCOS
Community Based Study of PCOS: Prevalence among Adolescents and Young Girls in Mumbai

- Systematic multistage random sampling method
  - Zone - Ward - Health post area: 80,000 population
- Inclusion Criteria: Age 15-24 yrs, 2 years post menarche and unmarried
- A sample size of 900 was calculated assuming a prevalence of 10% and precision of 2% at 95% confidence level
- Considering a non-response rate of 10%, the total was estimated to be about 1000 girls
- Eligible girls in selected study area: approx. 10,600 (census data)
- Household list obtained
- Every 10th household approached
- History at household after informed consent
- Medical examination at health post
- Blood collection on 3rd to 7th day of normal cycle
- USG abdominal - collective activity
- All enrolled cases screened - probable cases – Diagnosis of exclusion - Controls – Reference Joshi et al., IJEM 2014
Prevalence of PCOS among Adolescents and Young Girls in Mumbai

- Enrolled Participants (n=1000)
- After five rounds of home based health educative sessions with validated written information brochures
- Completed Investigations (n=600)
  - Completed investigations
  - Partially (n=178)

Probable PCOS
- Rotterdam = 149 (24.8%)
- AES = 79 (13.2%)

Probable PCOS
- Rotterdam = 20 (11.2%)
- AES = 76 (10.7%)

Common Phenotypes
- Mild – 52.6%
- Frank – 27.4%
- Classic – 13.3%
- Ovulatory – 6.7%

- Specificity - 93.9%
- Sensitivity - 83.3%

- Metabolic syndrome -0.8%
- Mean 2hrs post glucose and LDL values significantly higher among obese and overweight

- 19% hyperinsulinemic
- History of oligomenorrhea
  - Specificity - 93.9%
  - Sensitivity - 83.3%
- 26.4% of PCOS cases were overweight/obese

Salient findings

- Matched controls reference values

- Ovulatory – 6.7%
Outreach Activities for the Society

- Local schools
- Parents Teachers Associations
- Mumbai Municipal Corporation
- Adolescent Friendly Health Services
- General Practitioners
- Support groups
Basic Research on PCOS: (Genomics and Proteomics)

Genetics
- Candidate Gene Approach
  - INSR, PPARG, PON1, INSL3

Proteomics
- Quantitative proteomic analysis of follicular fluid
- Global and candidate gene methylation profiles

Epigenetics
- Tissue specific epigenetic susceptibility loci are associated with PCOS

Angiogenesis
- Angiogenesis in follicle of women with PCOS
- Expression of angiogenic markers and angiogenic activity in Granulosa cells and FF

Polymorphisms in above candidate genes influence PCOS pathophysiology and its associated metabolic and androgen traits

Several dysregulated processes identified may contribute to PCOS pathophysiology

Tissue specific epigenetic susceptibility loci are associated with PCOS
Genetic Studies in PCOS

- PCOS is a complex Multigenic Disorder where gene and environmental factors interact with each other to produce PCOS phenotype
- The mode of inheritance remains unclear
- Candidate gene association studies look for differences in the frequency of genetic variants between disease cases and healthy controls
- Investigated the association of candidate genes related to insulin resistance, oxidative stress, and steroidogenesis with PCOS in a population of Indian women

Genes

- INSR
- PPARγ
- PON1
- INSL3
Candidate Gene Studies in PCOS

- Genetic pathogenic mechanism of PCOS may differ between lean and obese women
- In PCOS research, sub grouping of study participants according to phenotype is important
- Such studies would help to develop predictive genetic predisposition profiles for early identification and therapeutic intervention for women at risk of PCOS development
Lean vs Obese PCOS: Biochemical and Molecular Differences

**Metabolic and Hormonal Profiles**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lean PCOS (n=136)</th>
<th>Obese PCOS (n=199)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg/dl)</td>
<td>87 ± 8.63</td>
<td>91.86 ± 14.46</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.57 ± 1.58</td>
<td>4.28 ± 2.58*</td>
</tr>
<tr>
<td>LH:FSH</td>
<td>1.8 ± 1</td>
<td>1.38 ± 0.86</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.61 ± 0.34</td>
<td>0.59 ± 0.32</td>
</tr>
<tr>
<td>FAI</td>
<td>4.52 ± 4.32</td>
<td>6.2 ± 4.75</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>150.25 ± 32.41</td>
<td>158.32 ± 32.47</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>80.51 ± 39.49</td>
<td>107.59 ± 46.32*</td>
</tr>
<tr>
<td>ApoB:ApoA-1</td>
<td>0.64 ± 0.28</td>
<td>0.75 ± 0.29*</td>
</tr>
</tbody>
</table>

**Genotype Association**

- L55M showed association with lean PCOS

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Lean and obese PCOS women are biochemically and genetically distinct

CT+TT genotype associated with lean PCOS

L55M showed association with lean PCOS
**Follicular Fluid Proteomics: from Physiology to Pathophysiology of PCOS**

**Follicular fluid: a useful alternative for ovarian tissue**

**FF catalog**
- Total protein identified: 480
- Novel protein in FF: 323
- Novel proteins in ovary: 19

**Significance of study**
- Better understanding of the ovarian pathophysiology of PCOS
- Reference proteome for screening of suitable biomarkers for oocyte quality, IVF outcome
- Suggests alteration of COC matrix functioning, follicular angiogenesis and compromised oocyte development in PCOS
Structural and Functional Alteration of COC Matrix in PCOS

Follicular fluid from PCOS had less capacity to support murine COC expansion than control.

COCs from women with PCOS were smaller than control.

Expansion and mucification related genes (TNFAIP6, HAS2), growth factor AREG were significantly reduced in granulosa cells from PCOS.
Angiogenesis is essential for follicle development, oocyte growth and corpus luteum formation. Follicular fluid (FF) and granulosa cells (GCs) play an important role in the development of follicle vasculature. A proteomics study has revealed differential expression of several angiogenic proteins in FF in PCOS (Ambekar et al 2015). Expression of angiogenic markers in melanocytic granulosa cells (MGCs) and FF of women with PCOS needs to be investigated.

**Sample collection**

- **Control Women:** Healthy, regularly menstruating women of reproductive age undergoing IVF due to male factor or oocyte donor.
- **Women with PCOS:** Women with PCOS undergoing IVF (Diagnosis based on Rotterdam Criteria 2003).
Understanding Follicular Angiogenesis in PCOS

- Angiogenesis is required for follicle development and subsequent corpus luteum (CL) formation.

- Proteomics study revealed differential expression of several angiogenic proteins in follicular fluid (FF) in PCOS (Ambekar et al., JCEM 2015).

- Comparison of angiogenic markers in mural granulosa cells (MGCs) and FF of women with PCOS.

**Follicular Fluid Levels**

**Gene Expression in MGCs**

Decreased concentration of bFGF in FF along with down regulation of ECM related genes involved in angiogenesis in MGCs suggests impairment of follicular angiogenesis in PCOS.
Endocrine Disruptors:

- BPA is found in reusable plastic containers, food and beverage can liners, thermal receipt papers, baby bottles, detergents, soaps, cosmetics, dental sealants, etc.
- Phthalates are used in polyvinyl chloride plastics, beauty and infant products, medical devices, and the enteric coating of some medications.
# Plasma Levels of Endocrine Disrupting Chemicals

<table>
<thead>
<tr>
<th>Endocrine Disruptor</th>
<th>Fertile Women (ng/ml)</th>
<th>PCOS (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>2.64</td>
<td>5.80</td>
</tr>
<tr>
<td>MBzP</td>
<td>5.72</td>
<td>25.71</td>
</tr>
<tr>
<td>MEHHP</td>
<td>1.71</td>
<td>8.76</td>
</tr>
</tbody>
</table>

**Methodology:**
Gas Chromatography Mass Spectrometry (GC-MS) for simultaneous estimation of Bisphenol A & Phthalate metabolites in plasma

(Vanage et al.)
basic research: future perspectives

- genome wide association study (GWAS) to identify susceptibility loci and associations of genetic variants across the entire genome in Indian women with PCOS
- assessment of follicular fluid proteins as potential biomarkers for oocyte quality prediction
- need to study differential glycoproteome between control and PCOS
- role of endocrine disruptors in the pathogenesis of PCOS
Bioinformatics Approach

http://www.pcoskb.bicnirrh.res.in/
Polycystic ovary syndrome (PCOS) is a very common endocrine disorder reported in women worldwide and is one of the major causes of female subfertility infertility. Numerous genes, SNPs and diseases are known to be associated with PCOS.

PCOSKB is a manually curated knowledgebase on PCOS. PCOS-related information available through scientific literature is cross-linked with molecular, biochemical and clinical databases. Information on associated genes, SNPs, diseases, gene ontologies and pathways along with supporting reference literature is collated and integrated in PCOSKB. PCOSKB will be useful for scientists and clinicians. The database currently holds information on 241 genes associated with PCOS.

Citation:

<table>
<thead>
<tr>
<th>Section</th>
<th>Information present</th>
<th>Cross-references to public databases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene information</td>
<td>Gene symbol, gene name, chromosomal location, gene summary</td>
<td>NCBI Gene, nucleotide databases, Ensembl database</td>
</tr>
<tr>
<td>Information</td>
<td>SNPs, upstream and downstream sequence, literature reference</td>
<td>NCBI SNP database, PubMed database</td>
</tr>
<tr>
<td>Gene Ontology</td>
<td>Ontology category, name, evidence and references</td>
<td>GO database, PubMed database</td>
</tr>
<tr>
<td>Associated diseases</td>
<td>Associated diseases and their references</td>
<td>PubMed database</td>
</tr>
</tbody>
</table>
### PCOSKB: Browse Genes

#### Browse Genes

- **Genes**
- **SNPs**
- **Associated Diseases**
- **Pathways**
- **Ontology terms**

#### 1 to 10 of 241 Genes

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Aliases</th>
<th>Gene Name</th>
<th>Chromosomal Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>ABC-1, ABC1, CERP, HDLDT1, TGD</td>
<td>ATP-binding cassette, sub-family A (ABC1), member 1</td>
<td>9q31</td>
</tr>
<tr>
<td>ACE</td>
<td>ACE1, CD143, DCP, DCP1, ICH, MVCD3</td>
<td>Angiotensin I converting enzyme</td>
<td>17q23.3</td>
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<tr>
<td>ACVR1</td>
<td>ACTRIA, ACVRLK2, ALK2, FOP, SKR1, TSRI, ACVR1</td>
<td>Activin A receptor, type I</td>
<td>2q23-q24</td>
</tr>
<tr>
<td>ADIPOQ</td>
<td>ACDC, ACRP30, ADIPQTL1, ADPN, APM-1, APM1, GBP28</td>
<td>Adiponectin, C1Q and collagen domain containing</td>
<td>3q27</td>
</tr>
<tr>
<td>ADIPOR1</td>
<td>ACDCR1, CGI-45, CGI45, PAQR1, TESBP1A</td>
<td>Adiponectin receptor 1</td>
<td>1q32.1</td>
</tr>
<tr>
<td>ADIPOR2</td>
<td>ACDCR2, PAQR2</td>
<td>Adiponectin receptor 2</td>
<td>12p13</td>
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<tr>
<td>ADM</td>
<td>AM, PAMP</td>
<td>Adrenomedullin</td>
<td>11p15.4</td>
</tr>
<tr>
<td>ADRB2</td>
<td>ADRB2R, ADRBR, B2AR, BAR, BETA2AR</td>
<td>Adrenoceptor beta 2, surface</td>
<td>5q31-q32</td>
</tr>
</tbody>
</table>

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**Details...**
## PCOSKB: Browse Associated Diseases

### 1 to 10 of 500 Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene (Gene ID)</th>
<th>Genes (Gene ID)</th>
<th>Statistics</th>
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<tbody>
<tr>
<td>46 XY disorder of sex development</td>
<td>NR3C1</td>
<td></td>
<td>19246354</td>
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<tr>
<td>Abnormal endometrial function</td>
<td>PNPLA2</td>
<td></td>
<td>3188971</td>
</tr>
<tr>
<td>Abnormal follicular development</td>
<td>ESR1</td>
<td></td>
<td>12466349</td>
</tr>
<tr>
<td>Abnormal folliculogenesis</td>
<td>IGF2R</td>
<td></td>
<td>22951915</td>
</tr>
<tr>
<td>Abnormal glucose tolerance</td>
<td>CHI3L1</td>
<td></td>
<td>22583189</td>
</tr>
<tr>
<td>Abortion</td>
<td>LIF</td>
<td></td>
<td>16640878, 11930689</td>
</tr>
<tr>
<td>Acantholysis</td>
<td>IL1A</td>
<td></td>
<td>12930304, 17624241, 10082594</td>
</tr>
<tr>
<td>Acanthosis nigricans</td>
<td>CYP21A2</td>
<td></td>
<td>16052863</td>
</tr>
</tbody>
</table>
Diseases Associated with PCOS Candidate Genes

- Blood Disorders: 13%
- Bone disorders: 6%
- Cancer: 5%
- Cardiac diseases: 13%
- Endocrine disorders: 4%
- Eye disorders: 1%
- Gastrointestinal diseases: 11%
- Immune disorders: 2%
- Liver disorders: 2%
- Metabolic disorders: 1%
- Muscular disorders: 8%
- Psychiatric/Brain disorders: 3%
- Renal disorders: 3%
- Reproductive disorders: 21%
- Skin disorders: 7%
- Tumor: 2%
- Respiratory disorders: 1%
- Unclassified: 1%
Need of the Hour

Multidisciplinary approach to address PCOS
Expert Group Meeting (Dec 2015) for Initiating Multidisciplinary PCOS Clinic at NIRRH

Team composition, Case Record Form and Logistics finalised
# NIRRH Multidisciplinary PCOS Clinic Team

## Mentors
- Dr. Rama Vaidya
- Dr. Smita Mahale
- Dr. Sanjay Chauhan

## NIRRH Core Team
- **Dr. Anushree D Patil**
  - Gynaecologist
- **Dr. Rahul Gajbhiye**
  - Basic scientist & Public health specialist
- **Dr. Beena Joshi**
  - Public Health Specialist
- **Dr. Suchitra Surve**
  - Paediatrician
- **Dr. Susan Thomas**
  - Bioinformatics
- **Dr. Srabani Mukherjee**
  - Basic Scientist
- **Dr. Shahina Begum**
  - Statistical Expert

## Social Workers
- Mrs Pratibha Kokate
- Mrs Bhagyashree Kanje
- Mr Irrana Mashal
- Mrs. Ruhi Pednekar
- Mrs Shobha Banga
- Mrs Sunita Kale
- Mrs Rachana Dalvi
- Mr. Ram Barai
- Mr. Prashant Tapse
- Mr. Vaibhav Shinde

## Multidisciplinary Team
- **Dr. Sukhpreet Patel**
  - Infertility Specialist
- **Dr. Vidya Kharkar**
  - Dermatologist (KEMH)
- **Dr. Ajita Nayak**
  - Psychiatrist (KEMH)
- **Dr. Satish Pathak**
  - Yoga Expert
  - Kaivalyadhham Inst
- **Mrs Shobha Udipi**
  - Nutritionist
- **Mrs Veena Yardi**
  - Nutritionist
  - Nirmala Niketan

## Staff Nurses
- Mrs. Pratibha Kokate
- Mrs Bhagyashree Kanje
- Mr Irrana Mashal
- Mrs. Shobha Banga
- Mr. Prashant Tapse
- Mr. Vaibhav Shinde

## Clinic Staff
- **Mr. Ram Barai**
  - Bioinformatics
- **Mr. Prashant Tapse**
  - Computer Programmer
- **Mr. Vaibhav Shinde**
  - Photographer
Multidisciplinary Model of Care for PCOS

Objectives:

 To address complete set of medical problems that come in the way of women suffering from PCOS including psychological issues
 To develop a database of clinical, biochemical and ultrasound profile of patients with PCOS in different age groups
 To develop simple management algorithms
 To develop a cohort for research on PCOS
Inauguration of NIRRH Multidisciplinary PCOS Clinic

Secretary, Department of Health Research and Director General, ICMR, Dr. Soumya Swaminathan inaugurated the clinic on 30th April 2016.
Yoga in Management of PCOS

Asanas Beneficial in PCOS

- Nadisodhan Pranayama
- Bhramri Pranayama
- Meditation
- Bhadrasana (Butterfly pose)
- Sun Salutation (Surya Namaskar)
- Bhujangasana (Cobra pose)
- Naukasana (Boat pose)
- Dhanurasana (Bow pose)
- Warrior pose (Superman pose)
- Supta Badhakonasana
- Bharadvajasana
- Chakki Chalanasana (moving the grinding wheel)
- Shavasana (Corpse pose)
- Padmasana
ICMR Multicenter Task Force Study
Evaluation of prevalence, regional phenotypic variation, comorbidities, risk factors and the variations in response to different therapeutic modalities among Indian women with PCOS

Objectives:
• Estimate regional prevalence of PCOS in 6 different zones of the country
• Study phenotypic variations, if any, among PCOS women from these regions
• Estimate the burden of comorbidities
• Assess knowledge and management practices among service providers in public health sector

9 States – 9000 women
NIRRH, Mumbai
ICMR, Delhi
SKIMS Kashmir
If you want to go fast, go alone.
If you want to go far, go together.

— African Proverb
Thank You All ............

- Kasturba Health Society Medical Research Center
- ICMR - National Institute for Research in Reproductive Health
- Tata Institute of Social Sciences
- The PCOS Society of India