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A REVIEW AND A CRITICAL INTERPRETATION ON ANIMAL EXPERIMENTAL MODELS TO ANALYSE POLYCYSTIC OVARIAN DISEASE (PCOS)

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INTRODUCTION –

PCOS is identified as most common endocrinal disorder affecting reproductive span of women's life. PCOS is burden on social life, medical systems and also its economical drain. This data gives more focus in research fields also, basically to study any disease some pathways is need to define. In the form of preclinical study and the clinical studies. Animal studies plays an important role to study the pathophysiology of any disease due to its mimic systems like human. PCOS is also being studied with the help of various animals.

To study PCOS, common animal models of mice, rhesus monkeys, rats and ewes are used, induced with the route of subcutaneous injection or implantation of androgens, anti-progesterone, estrogens, and letrozole, prenatal coverage to excess androgens and by exposure to constant light. (1-6)

For the additional studies, some unprompted models for PCOS-like rodent models (JCR:LA-cp rats) and transgenic (Tg) mouse models (LHr-hIGF-I and bLHb-CTP Tg strains) are tried. After studying and implementing all these models, the rat model has been showed most convenient and beneficial to study PCOS. And hence the most widely used for PCOS, due to many benefits like the small body size, short lifespan, and high reproduction index. (7-9)

Information regarding models of PCOS is very vast and complicated, this literature review is aimed at basic models of PCOS induction and there application in different views of PCOS pathogenesis, depending upon its causative factors.

MATERIAL- Various scientific research and review articles published in last 10 years were identified through pubmed and Google scholar websites using MeSH terms regarding the animal models of PCOS. With the combination of PCOS, all these key words were used to specify the pointed results: animal model, sheep, rodent, mouse, rat and fish.

METHODOLOGY- Animal experimental models for PCOS are searched from more than 150 research articles and the review papers. These articles were grouped according to similar activity and the working mechanism and compiled accordingly.

RESULTS AND DISCUSSION

Etiology of PCOS is still exactly not unrevealed, but many of the survey studies mentions some major causing factors are sedentary life style, hormonal imbalance, genetic factors, environmental factors and some unknown factors. Considering all the events probable animal's models are generated. Most commonly used animals models according to major causative factors of PCOS are demonstrated in fig 1.

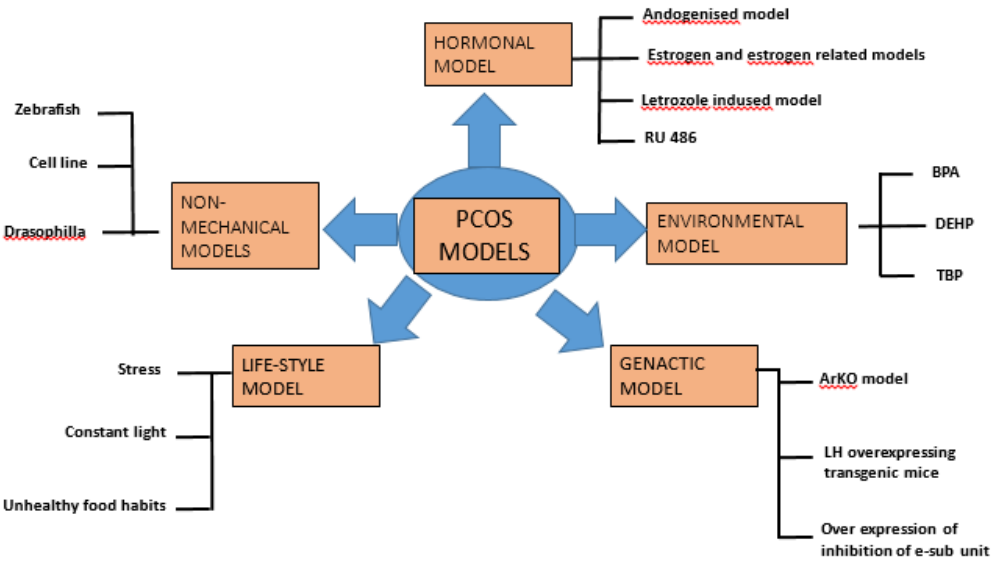

FIG 1. DIFFERENT ANIMAL MODELS BASED ON CAUSETIVE FACTORS.

LIFE STYLE MODELS-

Stress model - In the continuous stressful conditions and sympathetic activity was reported that 8 weeks of chronic cold stress (at 4 °C for 3 h/day)is capable to induce PCOS morphology and prolonged estrous in rats. (10)

Both hormones in serum estradiol and testosterone levels were significantly amplified. The LH and FSH levels were not statistically different when compared with the control group. While, 3 weeks of cold stress upregulated the expression of heat-shock protein 90 (Hsp 90) in the ovary and serum corticosterone levels.

In combination with the aforementioned 17NF mice, the direct effect of increased sympathetic nervous activity on the ovary is surely related to the progress of PCOS-like phenotypes in rodents. However, non-shivering (adaptive) thermogenesis will occur under cold conditions, which would be also presented in case of excessive calorie intake models, i.e., high-fat diet. Non-shivering thermogenesis is regulated by the thyroid hormone and NE under cold conditions. In this process, fatty acid oxidation occurs rapidly. (11) Thus, chronic metabolic burdens should not be neglected.

Constant light model- study focused on relation in between mammalian reproductive system and the photoperiods, concludes that in rodents, cyclic light –dark photoperiod is the controlling factor for gonadotropins and also inhibitory factors that trigger ovulation. (12)
To study this statement study has been carried out with Exposure of to 60 to 90 days, constantly 24 hours per day, fluorescent light with intensity of 350 to 400 Lux to 1m2 on base of cage, and this gives results with the induction of PCOS in rat. Pathogenically findings and probable mode of action is explained as constant Hyperandrogenemia and hyperestrogenemia can be caused by enzymatic changes occurring in the theca interna and interstitial cells of rat PCOS ovaries due to constant light. (13) In human studies it is observed that constant light affects female reproductive system and can develop in to PCOS with the features like including loss of body weight, lethargy, fur loss, and hyperanderogenemia. (14) Major advantages of this model is the induction of PCOS is being in hormone-free condition, though further researches are necessary to show applicability of this model in PCOS in sight of Pathogenesis and also the assessment of the similarity of PCOS pathogenesis and etiology.

Unhealthy food habits- Metabolic alteration is directly influences endocrinal levels and functions. Animal obese model for PCOS is consist of high fat and high sugar diet for a long term i.e. 14 days. (HDHS; high fat chow with 60% of the calories derived from fat and 32% sucrose solution as daily water) significantly elevated the serum testosterone and insulin levels in rats. (15) Induction of PCOS with high fat and high sugar diet cystic ovaries, increased fat mass, and irregular estrous cycle were observed. On the hormonal platform LH level was significantly decreased, but serum estradiol and AMH levels were same as normal diet group. Therefore, the impaired LH surge may result in the development of cystic ovaries in this model. When the same diet is given for 11 weeks in the same group manifested hyperinsulinemia but not hyperandrogenemia.(16) Use of 60% high-fat diet for 20 days, in mice and showed that increased serum testosterone levels and an irregular estrous cycle, though no follicular cysts were observed. (17)

HORMONAL MODELS –

Androgenised model-Androgen actions are helped via the androgen receptor (AR) and transgenic *Ar* knockout mouse models have recognised that AR-mediated androgen actions have a part in regulating female fertility and ovarian function. With the androgens in excess, working via the AR, play an important role in the origins of polycystic ovary syndrome (PCOS). This model helps in Identifying and confirming the locations of AR-mediated actions and the molecular mechanisms involved in the

development of PCOS. And it results that, there is significant evidence from human and animal studies demonstrating that excess androgens through the AR play a key role in the origin of PCOS. (18)

Estrogen and estrogen related models- In this model some drugs are introduce to present estrogenic effects. Again it is depends on at which phase of age it is being administered i.e. postnatal and puberty and the other one is in to adulthood. In postnatal and puberty models again acute effect and short term effects of estrogenic effect developing phenotypes like PCOS are executed. Acute means hour to a day, and short term intervention means 2days to 30 days. The study was performed with acute effect on the second postnatal day in thirty female Wistar rats, with the impression of androgen and estrogen introduction on the ovaries of adult female rats in the duration of their neonatal period in the gene expression of Lhr and Cyp17a1 are evaluated with the hormone levels, folliculogenesis and the theca-interstitial cell population. And results in to exposure to excess testosterone in early life increased the LH and testosterone serum levels, the LH/FSH ratio, ovarian theca-interstitial area and gene expression of Lhr and Cyp17a1 in adult rats. And increase in the ovarian theca-interstitial area, the secondary follicle population and gene expression of Lhr and Cyp17a1. (19)

Letrozole induced model-Letrozole is well-known non-steroidal aromatase inhibitor. It is being introduces by oral route. letrozole (1 mg/kg, once daily, for 21–28 days) in both pre pubertal and post pubertal female rats is able to induce PCOS. Letrozole, blocks the conversion of testosterone to estradiol and also induces PCOS in 6-week-old female rats.The letrozole model targets the study of aromotase deficiency–induced classic PCOS (20) and can used for studying the mechanism for the complex pathogenesis of PCOS. (21)**.** Twenty four female Wistar rats of regular estrus cycle were treated with letrozole (1 mg/kg) for 21 days for induction of PCOS.is effectively shows phenotypes of PCOS. (22) In the other method of PCOS induction with the help of letrozole in mice, five-week treatment of pre-pubertal mice are bring about in both metabolic and reproductive phenotypes (23) In the experiment of mice with pre-pubertal model of PCOS, from the age of 21 days, the animals were treated with continuous-release pellets containing 8 mg of letrozole for 90-day These animals dose not demonstrated any metabolic features of PCOS, but exhibited reproductive characters of PCOS and haemorrhagic cysts atypical of PCOS.(24)

Due to the reversibility of reproductive function after withdrawal of letrozole is noticed, hence this model has limitations. But many other models also reflect the same. Due to constant and similar features reported in different studies to human PCOS have increased this model application, and so in the recent years, many researchers are selected this model to exhibit PCOS.

RU 486-RU-486, first trade name for mifepristone, a synthetic steroid drug prescribed for inducing abortion during the early weeks of pregnancy. Administration of 4 mg of the antiprogestagen RU486 to 4-day-cyclic rats over 8 consecutive days starting on the day of estrus (Day 1) induced an anovulatory cystic ovarian condition with endocrine and morphological features similar to those exhibited in polycystic ovarian disease (PCO). Intervention of mifepristone shows Increases in Pituitary and ovary weight, number of formation of follicular cysts, size of the corpora lutea, and rates of follicular growth and atresia. (25)

In other study for the induction of PCOS with RU486 3 dosage forms were administered, 5.0mg, 7.5 mg and 10 mg in rats. It has been observed that no ovarian follicular growth and atresia are seen in to group of 5.0 mg and 7.5 mg, while 10 mg group shows this manifestation. And the study concludes that mifepristone (RU486) with subcutaneous administration induces PCOS in rats by creating decreased levels of progesterone with the 10 mg dose. (26)

ENVIRONMENTAL FACTORS –

BPA- With the many studies carried out on evaluation of pathogenesis yet the exact pathophysiological mechanisms of the syndrome is unknown, its heterogeneity recommends a multifactorial causal incidences. In the last two decades, numerous environmental chemicals, including Bisphenol-A (BPA) that is used in the synthesis of polycarbonate plastics, have been proposed as potential contributors to the aetiology of PCOS. BPA may constitute a consequence of the syndrome rather than a cause, but further research is still needed to clarify this. Continued efforts to study the early origins of PCOS, using prospective-designed studies, are required to identify the exact effect of BPA on women with PCOS. (27)

DEHP- Di(2-ethylhexyl) phthalate (DEHP) is a known endocrine-disrupting chemical, and toxic to reproductive system. DEHP can be easily absorbed by food and water from contact materials. (28) Also DEHP is capable to cross the placenta, as DEHP and its metabolites have been detected in amniotic fluid, which may result in coverage risk to the developing fetus. (29) Effects of direct DEHP contact on antral follicle growth in pre-pubescent mice by use of intraperitoneal injection, results revealed that the percentage of large antral follicles was significantly reduced when mice were exposed to 20 or 40 μg/kg DEHP every 5 days from postnatal day 0 (0 dpp) to 15 dpp. In 20 dpp, it has been performed microarray of these ovaries. And it results indicated that mRNA levels of apoptosis related genes were increased. DEHP induced the differential gene expression of *Hsp90ab1*, *Rhoa*, *Grina* and *Xdh* which may play an important role in this process. (30)

TBT- Tributyltin chloride (TBT) is endocrine disruptor chemical. With the oral administration in rats it increases testosterone levels and induces irregular estrous cycle. Although the TBT-treated rats did not show PCO morphology, increased fibrosis and apoptosis were observed in the ovary. (31)

NON MECHANICAL MODELS

Zebra fish model - Rapid embryonic development and Short life cycle made zebrafish a suitable model to investigate endocrine systems, reproduction, and transgenerational epigenetic effects. Testosterone and dihydrotestosterone (50, 500 or 1000 ng/L) were mixed in the egg water of zebrafish embryos early or late days post fertilization to induce a fish model of PCOS for a transgenerational epigenetic study. This model is were used to analyses pathogenesis of the epigenome by introduction to excess androgen during early embryonic development. The changes in glucose homeostasis and global methylation in the early but not late androgen exposed F0 generation and crossed F1 unexposed generation were observed. (32)

Drasophilla – It has been observed in all organisms that nutritional status has direct impact on formation of female gametes. Despites this the direct link between nutrition and ovarian development is comparatively not so clear about cellular and molecular mechanisms that underlie how dietary components modulate egg production, Drasophilla model helps to understand the status of PCOS with nutritional status. Because *Drosophila melanogaster*, with its dominant and far-reaching genetic tools as well as its well-recognising ovarian response to diet, has proven to be instrumental in addressing this issue. With this model it is known about the dietary control of oogenesis in *Drosophila* and the significant features of the fruit fly that make it a model for nutritional control of ovarian function. It shows Nutritional status effects reproduction at the level of gametogenesis, particularly ovarian function. (33)

Cell line- This study was designed to investigate the role and therapeutic potential of miR-324 in PCOS. With the quantitative real time-polymerase chain reaction (qRT-PCR) to assess expression. Then Cell viability was fixed by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay. Acridine orange/ethidium bromide (AO/EB) and annexin V/PI staining were performed to study apoptosis. Western blot analysis was used to determine protein expression. And it concludes that miR-324 regulates the proliferation of KGN cells in PCOs and be essential in the management of PCOS. (34)

CONCLUSION

Considering this fact that is difficult to develop a single animal model that mimics all aspects of multidimensional syndrome like PCOS, but, similarity of biological, anatomical, and/or biochemical features of animal model to the human PCOS phenotypes can increase its application. To achieve these purposes, these animal models are helpful in many aspects. Every model has its own limitations yet contribution to science is cannot be ignored.

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