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# Development and evaluation of novel rodent model of PCOS mimicking clinical phenotype in human disease

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## Abstract

**Background:** Polycystic ovary syndrome is a most common female reproductive disorder, involving endocrine and metabolic disorders with unclear etiology. Androgen-based rodent animal models like DHEA and DHT are most suitable for PCOS induction, but still, these models fail to produce non-lean PCOS phenotypes such as hyperandrogenism, hyperinsulinemia, elevated estrogen levels, and ovary weight. Excess fructose consumption leads to hyperandrogenism, hyperinsulinemia, and insulin resistance. The purpose of this study is to investigate, whether fructose consumption along with androgens in rats, could develop all metabolic and endocrine phenotypes of non-lean human PCOS disease.

**Methods:** Prepubertal SD rats were administered with DHT (83ug, s.c.) and fructose (20%, p.o.) for 90 days whereas DHEA (7 mg/kg, s.c) and fructose (20%, p.o.) for 30 days. During study duration, the blood glucose level for oral glucose tolerance test, estrus cyclicity, and ultrasonography was observed. Reproductive hormones LH, FSH, insulin, testosterone, and estradiol levels were assessed using ELISA. The ovary, uterus, abdominal fat, and subcutaneous fat were collected and weighed, and histopathology was done for any anomaly's findings.

**Results:** DHT + fructose-treated rats showed significant ($p < 0.05$) increase in serum testosterone, LH, estradiol, decreased FSH levels, and caused multiple cystic follicles. Abdominal fat, subcutaneous fat, ovary, and uterine weight were higher in DHT + F and DHEA + F when compared to control groups. OGTT reveals impaired insulin sensitivity and glucose tolerance in both model groups. Ovarian histopathology of DHT + F shows more cysts than the DHEA + F groups. No significant changes in uterine histology of DHT + F and DHEA + F-treated rats.

**Conclusion:** DHT + F-treated rats mimic all clinical phenotypes and could be used as novel rodent model for non-lean type PCOS.

**Keywords:** DHT, DHEA, Fructose, Hyperinsulinemia, Insulin resistance, Polycystic ovary syndrome

## Background

Animal models have an imperative role in the generation of new knowledge in medical sciences, including pharmacology. Experimental models have distinct advantages because they can reproduce in vivo, cellular characteristics, and reactions that occur in humans. Animal models in PCOS are particularly important in the development of the scientific basis for understanding its etiological processes. Polycystic ovary syndrome (PCOS) is a progressively common metabolic and reproductive disorder among women of reproductive age. According to the diagnostic criteria of National Institute of Health (NIH), 4–10% women of reproductive age are affected by non-lean type of PCOS all over the world [1]. Polycystic ovarian syndrome (PCOS) may clinically be manifested in young women of reproductive age as oligo-ovulation,

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biochemical or clinical hyperandrogenism, hirsutism, male pattern baldness, acne, acanthosis nigricans, and polycystic ovaries, additionally having a long prodroma with detectable abnormalities that present as the metabolic syndrome like hyperinsulinemia, obesity, dyslipidemia related to decrease in high-density lipoprotein cholesterol and hypertriglyceridemia, hypertension, atherosclerosis, increased cardiovascular risk, and type II diabetes [2]. To understand the etiology of PCOS and their treatment, several animal models have been adopted, and selecting the appropriate animal model each of these phenotypes can be analyzed individually. Various in vitro PCOS studies based on ovarian cells lack the complexity to examine all phenotypical features [3]. Currently existing animal models of rats, mice, and rhesus monkeys are used to study PCOS induced by subcutaneous injection or implantation of androgens, estrogens, anti-progesterone, and letrozole, prenatal exposure for excess androgens, or by exposure to constant light [4]. A well-used letrozole-induced PCOS, a nonsteroidal aromatase inhibitor that blocks the conversion of testosterone to estradiol, induces PCOS in 6-week-old female rats [5]. Endocrine disturbances like those in human PCOS are observed, but the metabolic characteristics of the syndrome are lacking. Androgen excess models shows endocrine disturbances like those in human PCOS were observed, but the metabolic disorder hyperinsulinemia and dyslipidemia of the syndrome were not well characterized and are not significantly comparable with the human phenotype [6]. Androgen model studies of DHT(5-alpha dihydrotestosterone) [7] shows that rodents of prenatal age when exposed to them, exhibit irregular reproductive cycles, LH hypersecretion, and impaired glucose tolerance but normal insulin sensitivity and body mass, and still considered to be the most suitable models mimicking the human PCOS disease most [8–10]. However, still they are not reported with elevated testosterone, estrogen levels, and hyperinsulinemia as generally seen in PCOS women. Hyperinsulinemia and insulin resistance have been suggested as the key triggers for the development of PCOS [1, 11]. Another androgen DHEA (dehydroepiandrosterone) model [12] when exposed to rats exhibit some human PCOS characteristics, including hyperandrogenism, acyclicity, anovulation, and polycystic ovaries [13] but still lack metabolic disturbances [14]. Thus, these animal models fails to provide adequate insight into the pathogenesis of PCOS for evaluation of treatment alternatives [15]. Improvement of these rat models exhibiting all phenotypes would be valuable and would enable further evaluation of new treatments for PCOS. It is unclear whether hyperandrogenism causes the PCOS morphology in the emblematic pathology of the disease or if it results secondarily to changes in ovarian morphology resulting from other metabolic disturbances [16]. Limited models have only focused on the analysis of predisposing conditions that increase the risk of PCOS, particularly genetic background, environmental factors and sedentary-diet lifestyle includes like high fructose consumption. Fructose is a monosaccharide which on consumption gets absorbed by the intestine via the GLUT 5 transporter conducted from enterocytes to the blood vessel leading to the liver by the GLUT 2 transporter where it enters the process of glycolysis, and there, it is used as a substrate for gluconeogenesis, glycogenolysis, and lipid synthesis. Many studies suggest that fructose consumption [17] in higher level leads to metabolic syndrome, which is usually characterized by obesity, insulin resistance, dyslipidemia, prediabetes, and inflammation [18]. Increased fructose intake leads to hyperglycemia, which stimulates the pancreatic beta cells causing hyper insulin secretion. Higher level of insulin results in stimulation of the theca cells, leading to increase in androgen production and could possibly be responsible for hirsutism and acne. Since no model solely represents all the PCOS clinical phenotypes [18], development of suitable animal models of PCOS has become a key area of research to understand the etiology and relationship of metabolic factors to chronic disease outcomes in the PCOS condition. In PCOS therapy, metformin an oral insulin sensitizer has been recommended for the treatment of anovulatory condition, where it reduces serum insulin levels and insulin resistance leading to improve in ovulatory function. Clomiphene citrate has been used to induce ovulation by alleviating hyperandrogenism condition. No sole drug treats all clinical manifestation of PCOS [19, 20]. Thus, these two drugs were chosen as standard drug for evaluation of the established model. The current study was intended to investigate whether the fructose consumption with coadministration of two prepubertal androgen DHT and DHEA-induced PCOS establish the metabolic phenotypes with endocrine abnormalities as seen in human PCOS disease.

## Methods

DHT and DHEA used in this study were obtained from TCI chemicals (Mumbai, India). Fructose crystalline powder form was obtained from Suvchem Pvt. Ltd. (Mumbai, India). ELISA kits of FSH, LH, estradiol, testosterone, and insulin were obtained from Shanghai Korean bioassay BT (Wuhan, China). Oral glucose tolerance test (OGTT) was performed using one touch glucometer product of Life scan medical devices private limited.

### Experimental animals

Sprague Dawley female rats of 3 weeks (21 days) [10] of age was obtained from in-house animal breeding facility

(jai research foundation, vapi). The animals were housed in cage under well-controlled conditions of temperature (22 ± 3℃), humidity (30–70%), and 12-h light: 12-h dark cycle. Animals had free access to standard rat feed and purified R.O water ad libitum.

## Experimental design

The rats were acclimatized for a week, randomized, and divided into eight groups ($n = 6$). DHT and DHEA were prepared freshly everyday by suspending the drugs in sunflower oil whereas clomiphene citrate, metformin, and fructose 20% solution were prepared using distilled water. Each group received following treatment; Group I served as control and received 0.5-ml sunflower oil. Group II received only DHT (83ug/day/kg.b. wt.; s.c) [21]. Group III received coadministration of DHT (83ug/day/kg.b. wt; s.c) + fructose (20%; p.o.). Group IV received only fructose (20%; p.o.). Group V received cotreatment of clomiphene citrate (100 mg /k.g. b.wt.; p.o), DHT (83ug/day/kg.b.wt; s.c), and fructose (20%; p.o.). Group VI received cotreatment of metformin (200 mg/k.g. b.wt.; p.o), DHT (83ug/day/kg.b.wt; s.c.), and fructose (20%; p.o.). Group VII received only DHEA (7 mg/kg. b. wt; s.c.). Group VIII received coadministration of DHEA (7 mg/kg. b. wt; s.c.) and fructose (20%; p.o.) [13].

The five groups exposed to DHT were dosed for 13 weeks, whereas the groups exposed to DHEA were dosed for a month. On the study terminal day, rats were fasted for 24 h and the blood was drawn by cardiac puncture under anesthesia condition for different reproductive hormone level assessments. Animals were sacrificed by cervical decapitation, and reproductive organs were dissected out, rinsed in ice-cold saline, and were stored for biochemical and histopathological evaluation.

## Experimental parameters

### *Animal body weight*

In groups, individual animal body weights were recorded on weekly basis starting from the day 0 till end of study day 30 and 90 for DHEA+F and DHT+F model, respectively.

### *Estrous cyclicity*

Vaginal smears were taken from 11th week of age of the animals to the end of experiment. Vaginal secretions were collected every day with a plastic pipette filled with normal saline (NaCl, 0.9 %) by inserting a clean tip into the rat vagina, not deeply. The vaginal fluid was placed on glass slides. One drop of crystal violet stain was added and observed under a light microscope, with 10× and 40× objective lenses. Cyclicity in rats was determined based on the presence/absence and proportion of epithelial cells, cornified cells, and leucocytes. Cyclicity was also compared by calculating the percentage days spent in diestrus stage. The calculation was done using the following: % days in diestrus = No. of days exhibiting diestrus stage/total no. of days × 100 [22].

### *Oral glucose tolerance test*

Animals were kept for fasting for 18 h before the test. The rats were given an oral dose of glucose at a dose of 2 g/kg body weight. The blood glucose levels were assessed at different time points, i.e., 0, 30, 60, 120, 180, and 240 min after the glucose meal using a glucometer strip for quantification. The fasting blood glucose (FBG), post-prandial blood glucose (PPBG), and AUC were determined for all the groups [23, 24]. OGTT was performed at different interval day; for DHT+F model, the blood was assessed on days 0, 60, and 90, whereas for DHEA+F, it was on days 0 and 30.

### *Ultrasonography*

In ultrasonography diagnosis, animal's hair was removed from the costal margin to the caudal abdomen using depilatory cream. The rats were anesthetized using thiopental sodium (40 mg/kg; i.p.) and placed in a supine position. Transducer with frequency of 12-5 MHz was used, which was moved along the vertical axis and horizontal axis (forward-to-back and side-to-side) by the hand. The ovarian size along with the appearance and size of the follicles were observed.

### *Estimation of reproductive hormones*

At terminal sacrifice, blood samples were collected by cardiac puncture. To obtain the serum, the samples were allowed to clot for 2 h at room temperature before centrifugation for 15 min at 1000×*g* at 2–8℃. The collected supernatant was stored at −20℃ and were assayed using ELISA kit for reproductive hormones levels.

### *Ovary and uterine weight*

Animals' ovary and uterus were collected at terminal sacrifice, weighed, and was stored in 10% formalin solution for histoslide preparation.

### *Histopathology evaluation*

The collected ovary and uterine horn were kept in 10% formalin solution overnight. For histoslide preparation, they were dehydrated, embedded in paraffin wax, and sectioned at 5-µm thickness followed by staining with hematoxylin and eosin (H&E) dye. The prepared slides were examined using light microscopy [25].

## Statistical analysis

All data were subjected to one-way or two-way ANOVA and Tukey's multiple comparison test using graph

pad prism 9. At $P<0.05$ was considered as difference of significance.

## Results

In this study, we firstly investigated whether consumption of fructose with DHT and DHEA influenced the body weight of animals. The DHT+ f group rats' body weight increased from 8 to 13 weeks. On day 90, the DHT+f group rats' body weight showed significantly increased when compared with that of the control, DHT, and fructose groups, shows "metabolic obesity" (Fig. 1). On day 30, the DHEA+f-treated rats were significantly heavier than the control group and DHEA group (Fig. 2). To elucidate, the body weight of day 0 and day 90 was compared (Fig. 3).

Estrous cyclicity phases were identified by observing the different cell morphology in vaginal smear. The DHT+f group and DHEA+f-treated rats showed rise

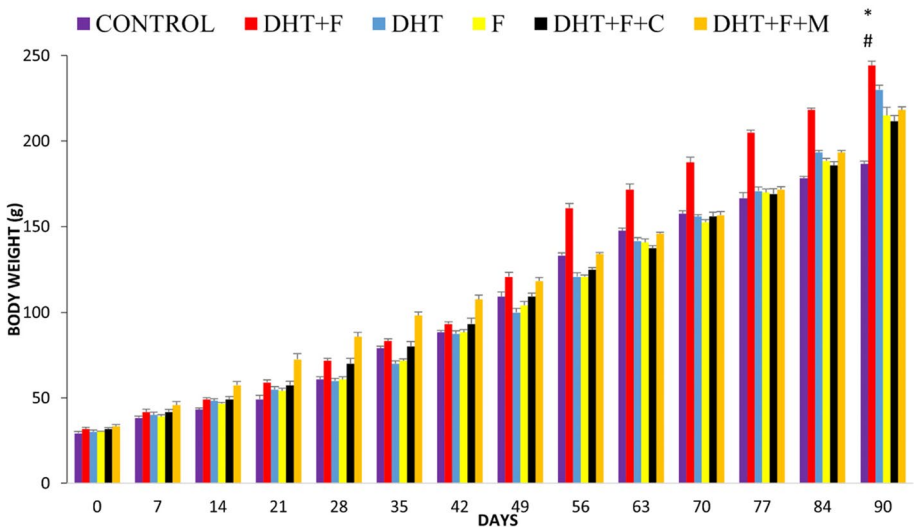


**Fig. 1** Comparison of body weight changes in different DHT groups and control group of rats. All data are expressed as mean ± SD ($n=6$ in each group) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *$p<0.05$ when compared to control, $^{\#}p<0.05$ when compared to DHT

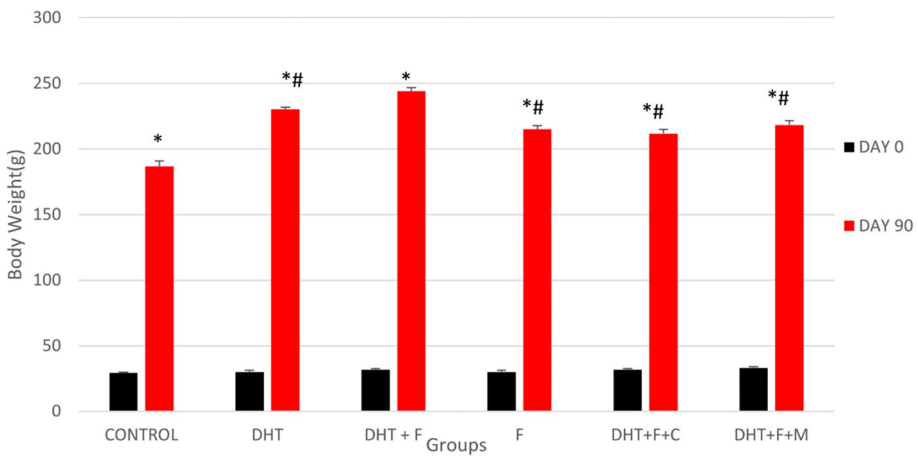


**Fig. 2** Body weight comparison of DHT-induced PCOS in rats day 0 vs day 90. All data are expressed as mean ± SD ($n=6$ in each group) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *$p<0.05$ when compared to the control group, $^{\#}p<0.05$ when compared to the DHT group

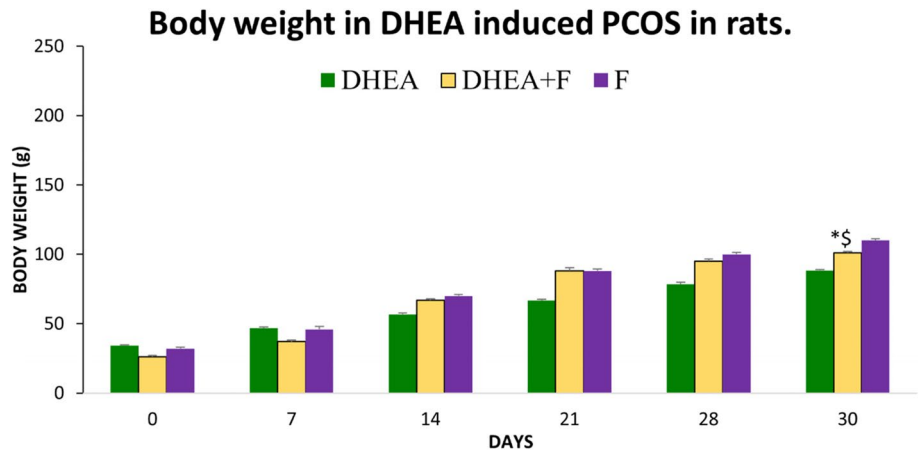


**Fig. 3** Body weight of DHEA-induced PCOS in rats. All data are expressed as mean ± SD ($n = 6$ in each group) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. $^{\$}p < 0.05$ when compared to the DHEA group

in % day diestrus spent when compared to the control groups showed normal estrus cyclicity (Fig. 4). In DHT and DHT+f, the pseudo-diestrus phase is prominently seen, indicating the disturbance of cyclicity as seen in human PCOS [26]. DHEA+f shows irregular estrous cycle, when compared with the control group indicates estrus phase arrest.

At terminal sacrifice, the reproductive organs were collected and weighed individually. The DHT+f group ovary weight was significantly higher when compared to control and DHT, while DHEA +f were significantly higher compared to the DHEA group. Metformin and Clomiphene-treated groups showed a significant decrease in their ovarian weight and hike in uterine weight as when linked with the DHT+f group and DHEA+f. Subcutaneous fat and abdominal fat deposition were significantly higher in DHT+f when compared to the DHT group [27]. Significant elevation of body fats was noticed in the DHT+f group and DHEA+f when compared with the DHT and DHEA groups indicating obesity. No significant difference was observed in the metformin treatment group, when compared with the control group (Fig. 5).

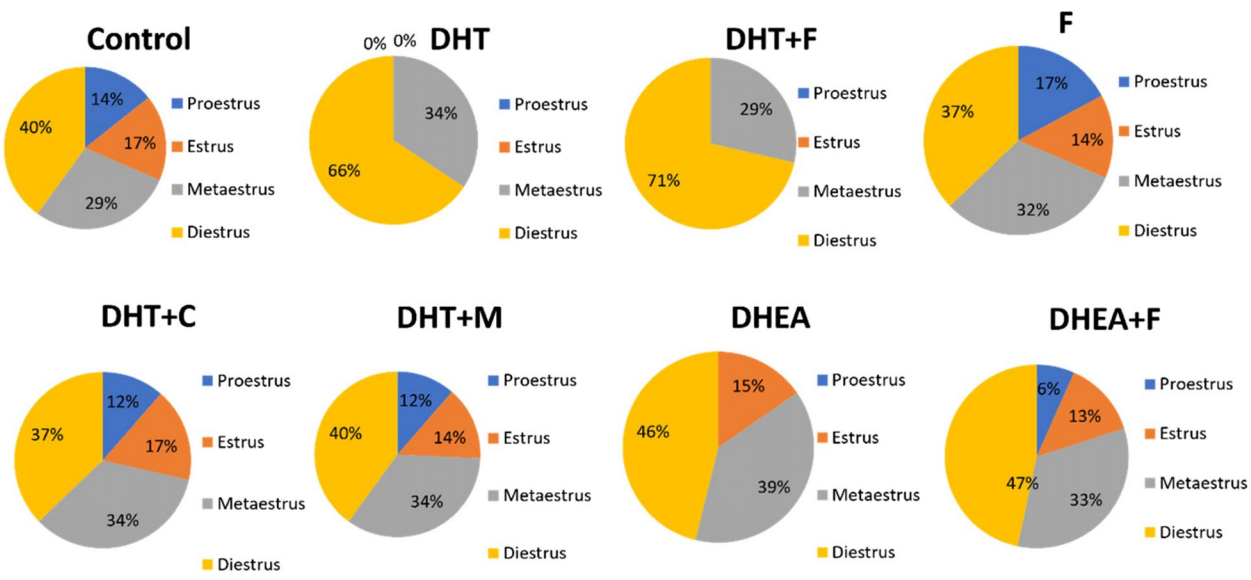


**Fig. 4** Percentage of the days spent in each phase of the estrous cycle by the animals from different groups

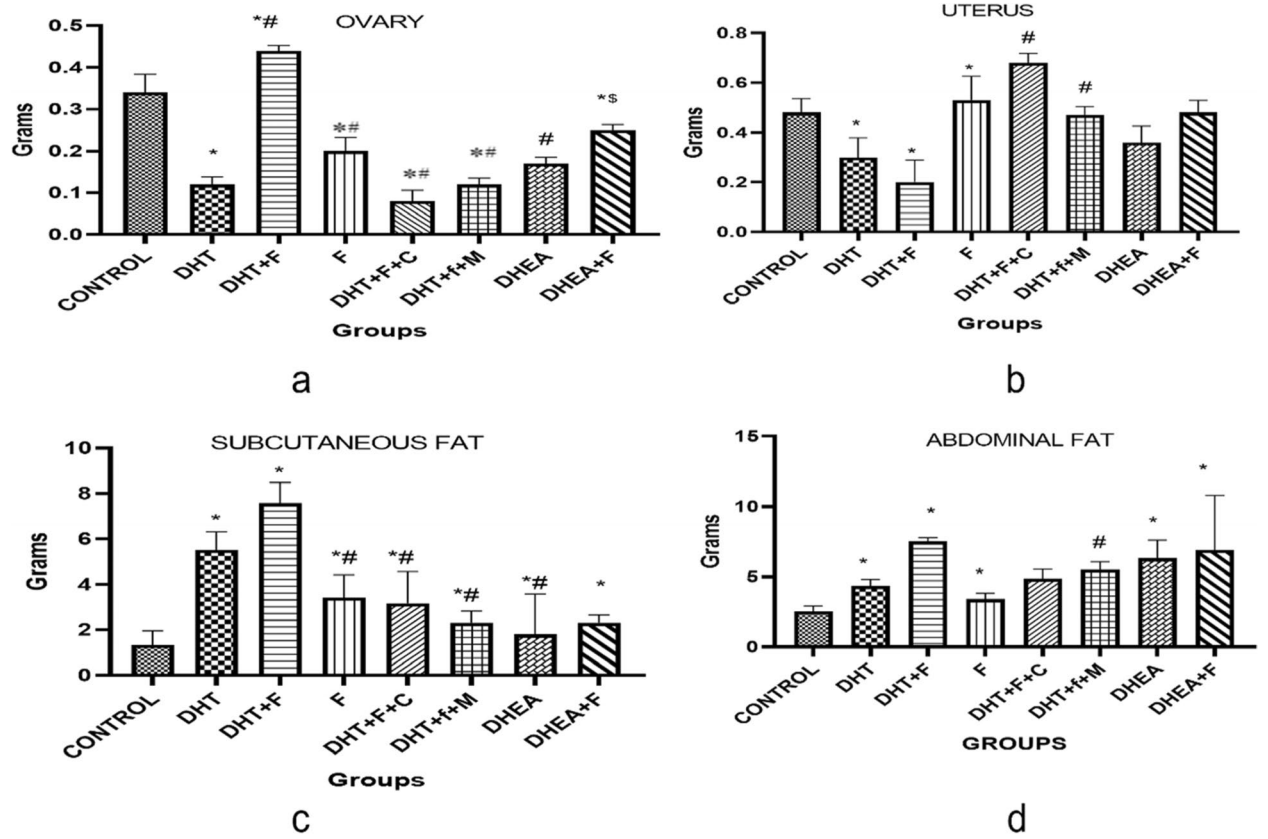


**Fig. 5** Reproductive organ and body fat weights. **a** Ovarian wt., **b** uterine wt., **c** s.c. fat, and **d** abdominal fat. All data are expressed as mean ± SD ($n = 6$ in each group) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. $^{*}p < 0.05$ when compared to the control group, $^{\#}p < 0.05$ when compared to the DHT group, and $^{\$}p < 0.05$ when compared to the DHEA group

Reproductive and insulin hormone levels were analyzed using ELISA from the blood serum which were stored earlier at −20 ℃ in a deep freezer. Serum FSH and LH levels shown in Fig. 6 show the DHT+f group and DHEA+f groups showed a significant increase in LH level, while significant lower FSH level when compared with the control group.

In Fig. 6, insulin levels in the DHT+f group and DHEA+f groups were seen significantly elevated when compared to the control, DHT, and DHEA groups stating hyperinsulinemia condition. Figure 7 indicates blood glucose levels of all groups. The AUC of blood glucose level of the DHT+f group and DHEA+f groups are showing a significant difference when compared to the control, DHT, and DHEA groups as shown in Fig. 8. Considering both results concludes insulin resistance in rats of both groups. Thus, in casing may be fructose is acting as positive regulator in inducing the hyperinsulinemia and insulin-resistance condition. The metformin treatment group shows a marked reduction in blood glucose levels making it a useful rationale as insulin sensitizers in PCOS.

Hyperandrogenism is an important feature for diagnosing of PCOS. Study findings show serum testosterone and estrogen levels in the DHT+f group and DHEA+f were significantly higher, when compared with the control group ($p<0.05$). No significant difference was seen in serum testosterone and estrogen levels between control group, when compared with the clomiphene and metformin treatment groups.

Ultrasonography examination was done in accordance with the earlier study [28]. USG reveals the multiple cystic formation in the ovary of rats and seems to be higher, enlarged oblong shaped in the DHT+f group, and lesser the n DHEA+f group, indicating the development of cystic ovaries. This was the first time that such type of attempt of USG technique was done for assessing cystic follicles in rat ovary as shown in Fig. 9.

The histopathology of the ovary in earlier studies using the letrozole and fructose PCOS model [29, 30] showed an increased number of cystic follicles. In this study, it was discovered that the DHT +f and DHEA +f groups had an increased number of cystic and atretic follicles comparing to other groups which could be resulted due

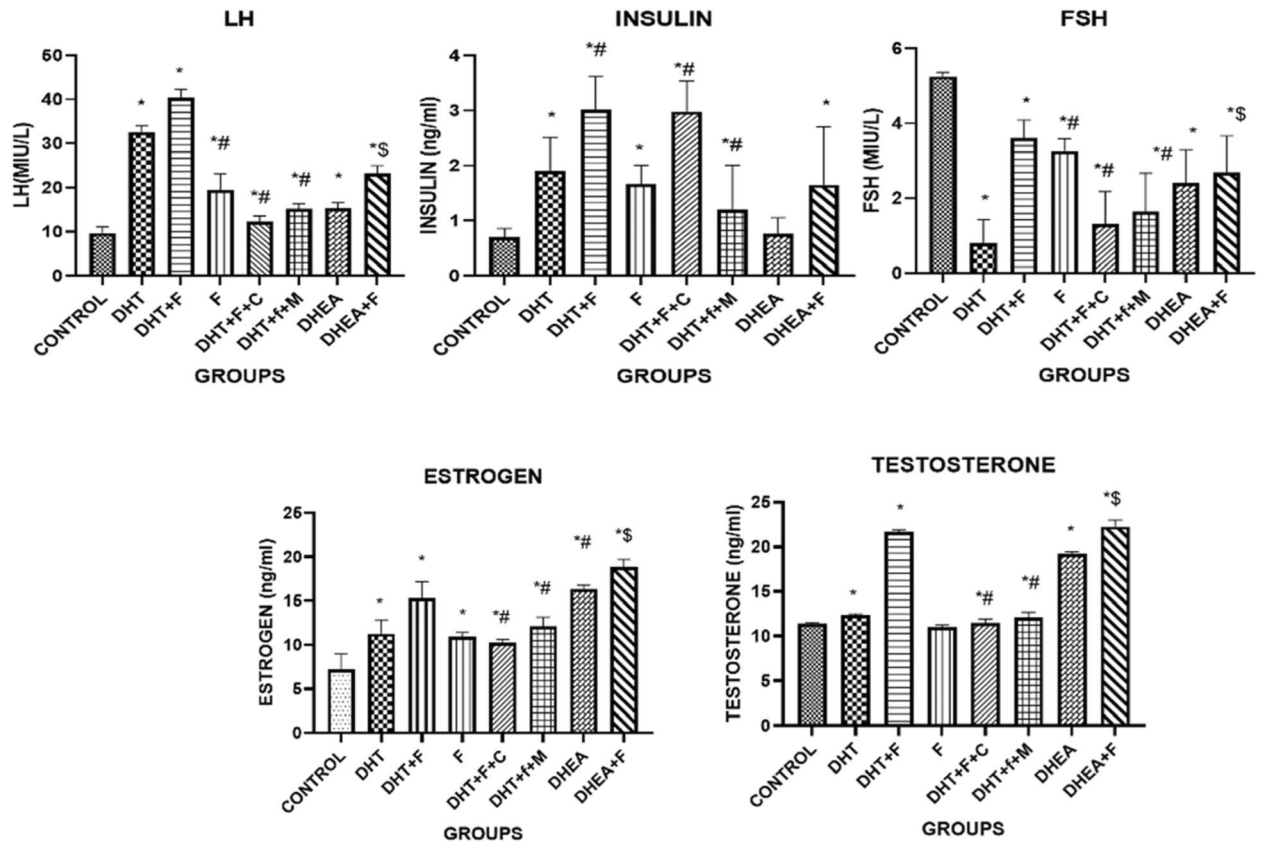


**Fig. 6** Reproductive hormone levels in different groups. All data are expressed as mean ± SD ($n = 6$ in each group) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *$p < 0.05$ when compared to the control group, $^{\#}p < 0.05$ when compared to the DHT group, and $^{\$}p < 0.05$ when compared to the DHEA group

to disruption of LH/FSH levels. Multiple cystic follicles, devoid of antral and corpus luteum, were higher in the DHT+f group compared to other groups shown in Fig. 10. Clomiphene and metformin have rescued cystic development. However, uterine histopathology does not attribute any significant findings except for the DHT and DHT + f groups showing a decreased endometrium layer thickness.

## Discussion

### Change in body weight

Obesity, which is more prevalent there than in the general population but not in lean-PCOS, is one of the etiological factors for PCOS. DHT specifically activated the androgen receptor, which led to an increase in body fat, intra-abdominal adipose tissue, and subcutaneous adipose tissue depots, which significantly increased the rats' body weight. Insulin resistance is not brought on by obesity [31]. There is a significant rise in central adiposity and hyperinsulinemia associated with insulin resistance and type 2 diabetes along with fructose. The increased body weight in DHEA rats was significant when compared to the control groups, in contrast to PCOS women, but was unrelated to changes in relative body fat mass. This might be due to less prolonged fructose consumption.

### Estrous cyclicity impairment

While the estrus cycle of the DHEA-exposed rats was arrested in the estrus stage and showed longer cycle duration when compared with control group, the androgen DHT induced rat PCOS model exhibits irregular cycles and a predominance of the "pseudo-diestrus" phase. According to our research, fructose causes hyperinsulinemia, increased fat deposition, and disruption of the estrus cycle which is consistent with other study [32]. Rats that were given both androgen and fructose showed impaired cyclicity and diestrus phase arrest.

### Oral glucose tolerance test and insulin resistance

Type 2 diabetes, insulin resistance, hyperinsulinemia, and glucose intolerance are more prevalent in women with PCOS. The results of the OGTT demonstrate that type 2 diabetes is present in both androgens and that chronic fructose consumption causes fasting blood

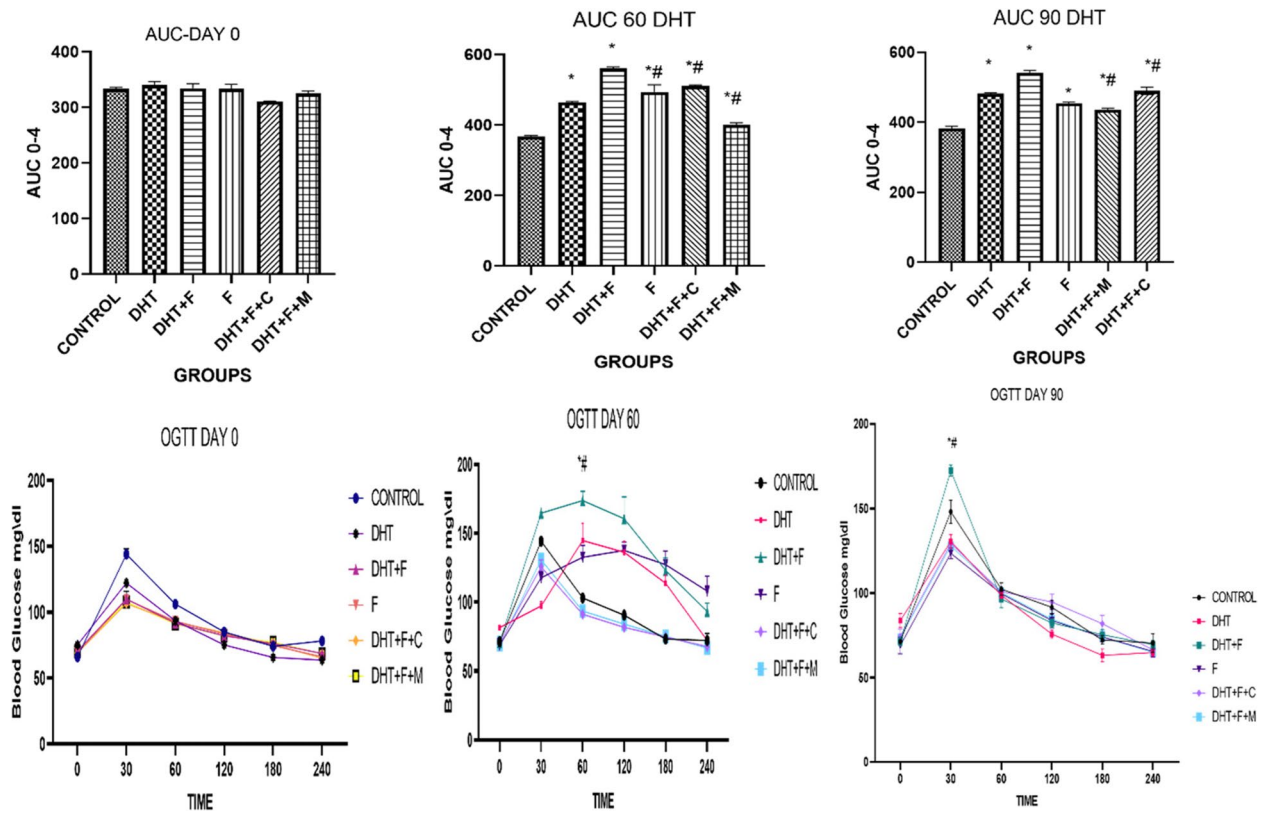


**Fig. 7** Oral glucose tolerance test and AUC in the DHT groups. All data are expressed as mean ± SD ($n = 6$ in each group) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *$p < 0.05$ when compared to to control group and $^{\#}p < 0.05$ when compared to the DHT group

glucose levels to be elevated. The level of serum insulin is crucial for regulating blood sugar. When compared to the DHT group, the DHT+ f group's insulin level was significantly higher. Additionally, the DHEA+F group demonstrated an increase in insulin level, though it was not statistically different from DHEA. In contrast to the DHT+f and DHEA+f animals, which displayed impaired glucose intolerance when compared to the androgen groups, the fructose-treated group demonstrated a normal profile towards glucose tolerance, according to the AUC of various day points. Blood sugar levels were higher in DHT+f and DHEA+f at the 30-, 60-, and 120-min time points, demonstrating each compound's unique contribution to decreased insulin sensitivity. Metformin, as anticipated, caused a statistically significant decrease in the fasting serum insulin levels in the rats given DHT+f. Concurrent fructose consumption may have led to structural changes in pancreatic cells, resulting in hyperinsulinemia and an inability to control serum blood glucose, which would have indicated insulin resistance [33, 34].

### Ultrasonography

Control rat USG ovarian scans reveal the presence of follicles with typical shapes. The USG scan of rats exposed to DHT and DHT+f, on the other hand, shows the formation of multiple follicles. It is interesting to note that fewer follicles are present in the metformin and clomiphene-treated animals than in the DHT+f animals. But compared to control animals, there were more follicles visible. The findings mentioned above indicate that androgen exposure causes cystic induction of PCOS in these animals, but fructose administration concurrently may have exacerbated the absence of cystic follicles. Although less than the DHT+f group, the DHEA+f group has displayed small, oblong, distorted follicles. More than measuring the effects of the treatment groups, the USG scan was intended to confirm the induction of PCOS.

### Reproductive hormones

Hyperandrogenism is an important feature for diagnosing of PCOS. Lower FSH levels and increase in LH,

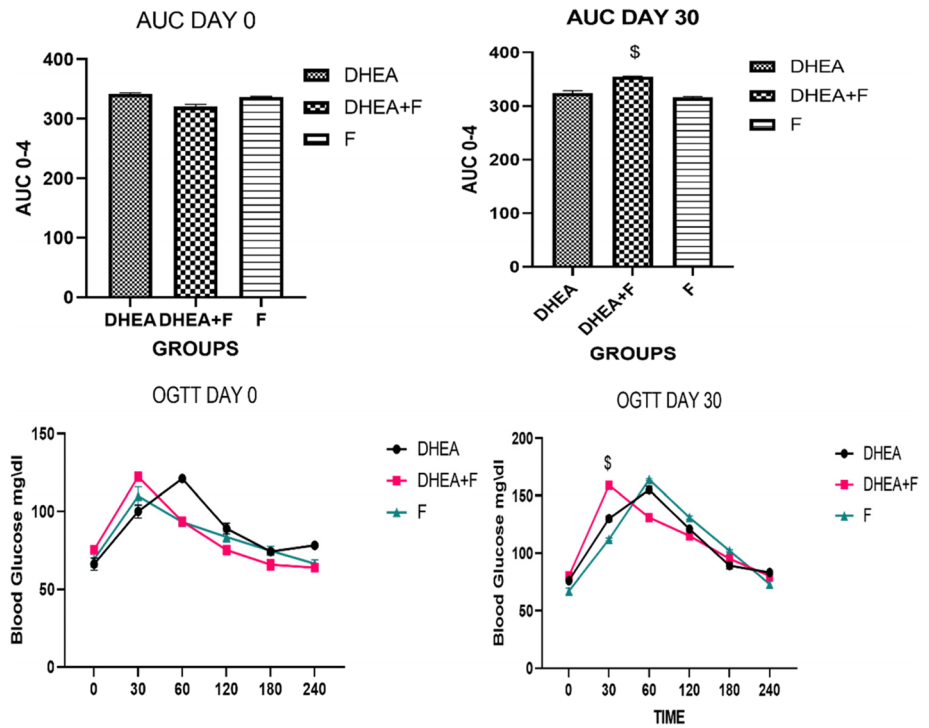


**Fig. 8** Oral glucose tolerance test and AUC in the DHEA groups. All data are expressed as mean ± SD ($n = 6$ in each group) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. *$p < 0.05$ when compared to the control group and $^{\$}p < 0.05$ when compared to the DHEA group

testosterone, and E2 in DHT+f group is significant compared with control and DHT group, whereas fructose alone did not much alter hormonal profile. However, FSH decreased, and LH and testosterone significantly increased in the DHEA+ f group compared to the control group. Besides, fructose-consumption significantly profound LH and testosterone levels in animals compared without fructose. Hypersecretion of luteinizing hormone and testosterone levels demonstrates the development of the PCOS hallmark feature that prevents fertilization. The increased production of testosterone precursors or dysregulation of the androgen synthesis process due to a rate-limited enzyme could both contribute to high levels of excess testosterone. In the present study, estradiol levels are significantly higher in DHT +f on compared with DHT and control group showing responsible cause lower FSH level causing anovulation in ovary.

### Reproductive organ and body fat weights

Due to excessive cyst formation brought on by abnormal folliculogenesis and steroidogenesis, the ovarian weight of the DHT+f and DHEA + f groups was significantly higher than that of the control DHT and DHEA groups. The development of the reproductive organs is, however, said to be stunted in female rats exposed to DHT due to altered hormonal balance. In the current study, we discovered that, when compared to the control group, the rats exposed to DHT for 90 days produced a significant decrease in ovarian weight. In contrast to the control group, the fructose group did not significantly reduce the weight of the ovaries. By likely normalizing folliculogenesis and steroidogenesis, metformin, and clomiphene significantly reduced ovary weight, indicating a promising role for treatment in PCOS women. In line with earlier studies on the DHT model, DHT increased the depots of abdominal fat. The assessment of abdominal and subcutaneous fats revealed profound obesity, a prominent phenotype seen in PCOS women, in the DHT+f group when compared to the control and DHT groups. Such reduced fats may be the result of hyperinsulinemia, steroidogenesis, and insulin resistance. On the other hand, DHEA +f was not found to be significant when compared to the DHEA group, failing to establish an obese state. This may be because fructose was administered to patients with DHEA for a shorter period of time.

### Histopathology

The control group's representation of the graafian follicle in Fig. 10a is very clear, showing an ovum that is immediately encircled by corona radiata. The zona granulosa

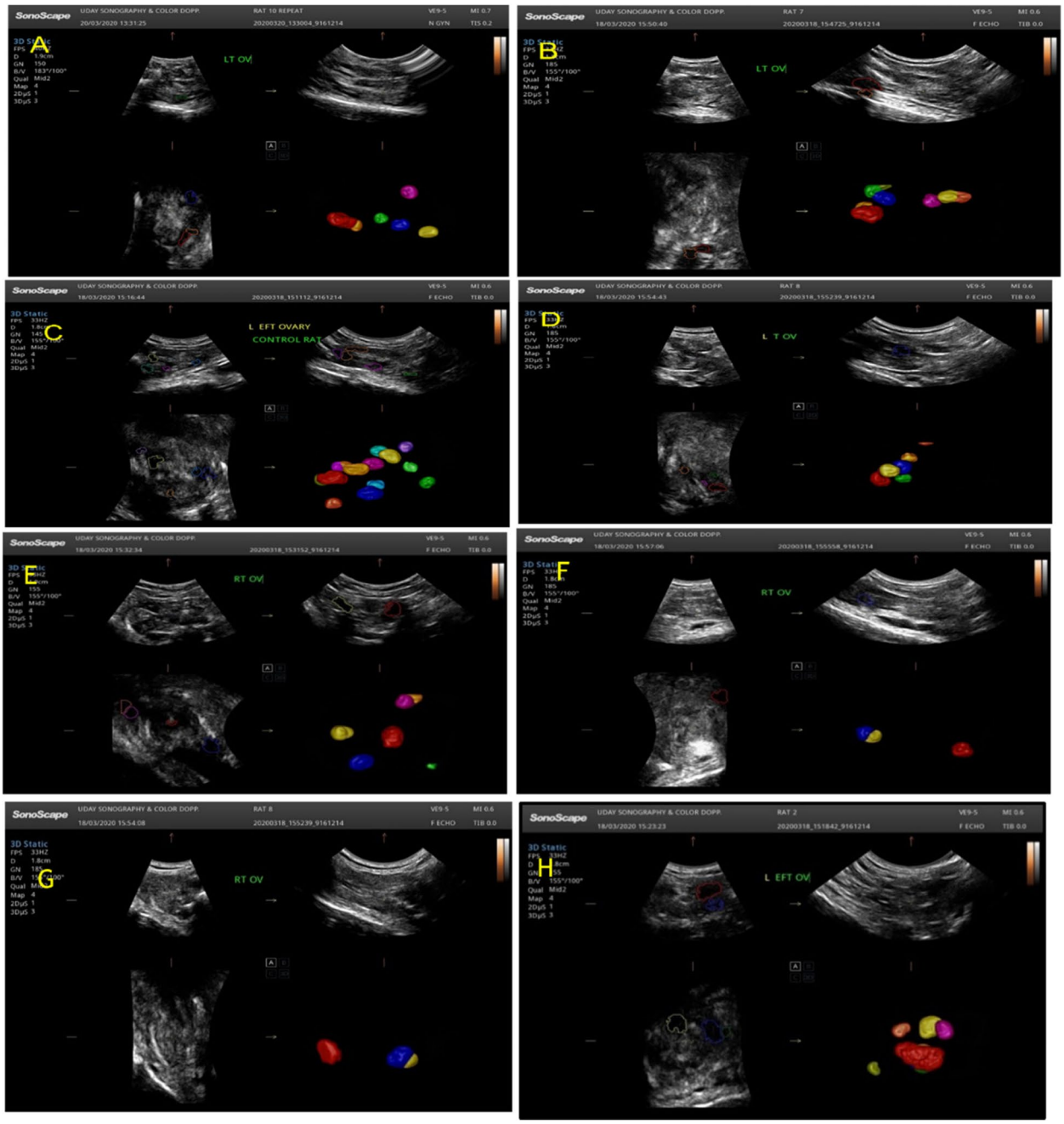


**Fig. 9** Ultrasonography analysis of ovary of experimental animals. **a** Control: showing normal shape number of follicles; **b** DHT: multiple follicles; **c** DHT + F: enlarged, oblonged, condensed multiple cystic follicles; **d** fructose: normal-shaped follicles; **e** DHT + F + C: reduced and detached normal-shaped follicles; **f** DHT + F + M: small, irregular reduced follicles; **g** DHEA: uneven, small-condensed multiple follicles; and **h** DHEA + F: oblonged enlarged multiple follicles

layer, which has nucleated granulosa cells, and the antrum (a fluid-filled space) are also parts of the graafian follicle. Figure 10b and c of the DHT and DHT+F groups show large antral follicles and a thickened theca interna cell layer, but no ovum or granulosa cells are present. These show that polycystic ovary is present. Additionally, atresia-prone follicles are seen. The animals that received DHT showed disruptive characteristics, such as the presence of numerous large follicles with large fluid-filled antral spaces, atretic and cystic follicles, a distinctive

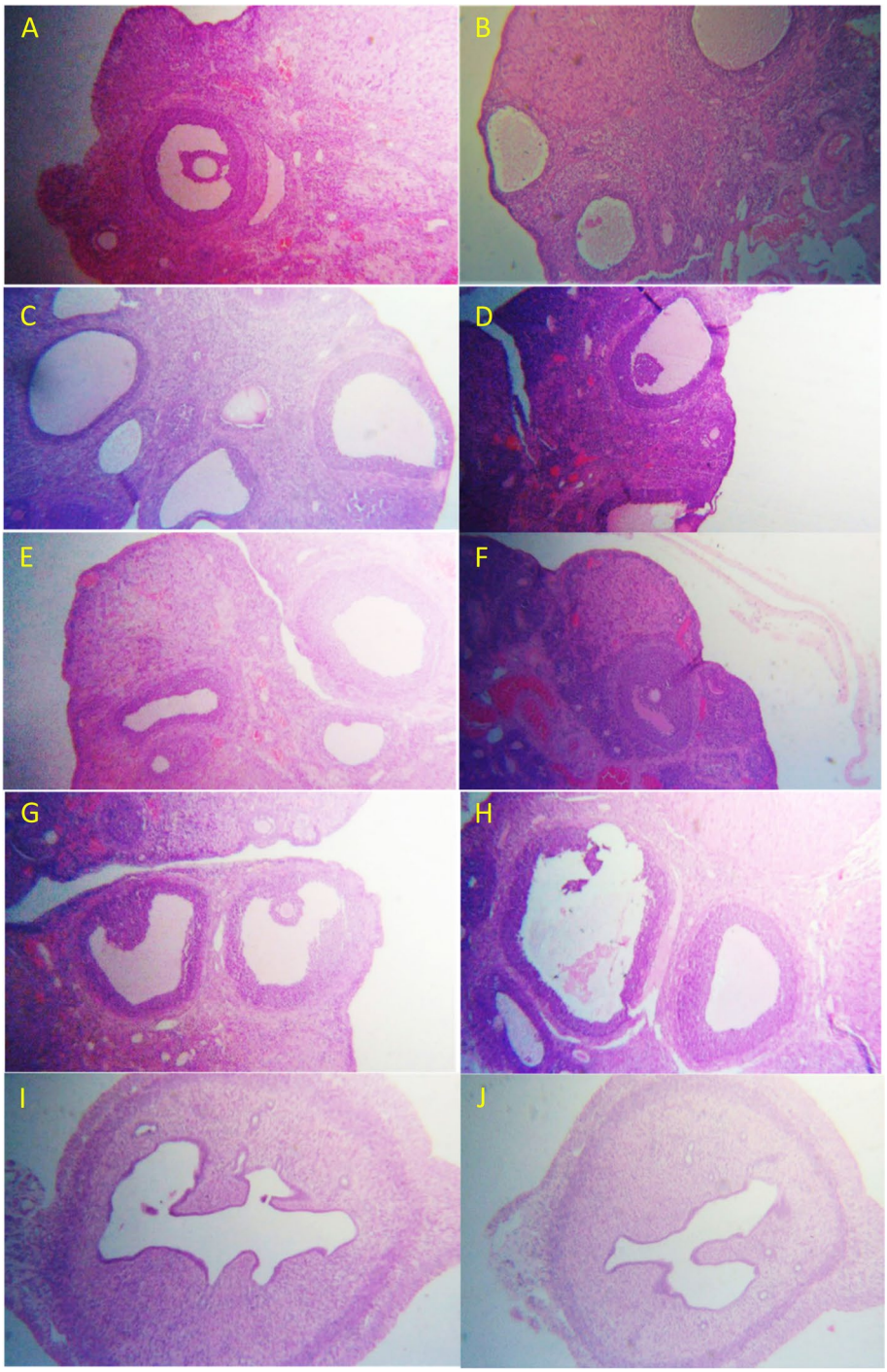


**Fig. 10** Histopathology analysis of ovary and uterine of experimental animals. **a** Control: normal ovarian depicts the presence of graafian follicle, ovum surrounded by corona radiata, graafian follicle, antrum (fluid filled space), and zona granulosa layer; **b** DHT: large antral follicles and thickened theca interna cell layer and atresia follicle; **c** DHT + F: multiple atresia cystic follicles; **d** Fructose: normal graafian follicles; **e** DHT + F + C: the presence of a developing follicle which contains the antrum and ovum which is surrounded by granulosa cells; **f** DHT + F + M: the presence of follicles with a typical presentation, exhibiting features like the presence of antral follicles at different stages of development and showing at least one graafian follicle with a mature ovum in it; **g** DHEA: the pre-antral follicle is observed which is surrounded by the layer of theca interna cells and theca externa. **h** DHEA + F: secondary and cystic follicles; **i** uterine DHT, thick endometrium; **j** uterine DHT + F, thinned endometrium (hematoxylin and eosin stain, × 10 magnification)

hyperthecosis of the internal layers, and thinning of the zona granulosa, but in DHEA+F, zona granulosa were thick, the presence of cystic follicles is due to the abnormal androgen levels like risen luteinizing hormone, testosterone levels, and decreased follicle-stimulating hormone levels. Metformin did not seem to have a major impact, though there were some follicles with a healthy granulosa layer and lesser number of cystic follicles as compared to animals exposed to DHT+F alone.

In the group that has been treated with clomiphene, there is evidence of a developing follicle with an antrum and an ovum that is encircled by granulosa cells. The presence of the corpus luteum signals ovulation. In the group that only received fructose alone, no noteworthy results were found.

## Conclusion

In rodents, when DHT and DHEA is given concurrently with fructose induces PCOS in rats. On comparison, the DHT+f model seems to represent all clinical features of PCOS and can be considered as a novel model for non-lean type PCOS. This model will allow to assess the therapeutic potential of various drugs, especially formulations which are having diversified approach regime in treatment of PCOS.

#### Abbreviations

NIH: National Institute of Health; GLUT-5: Glucose transporter-5; GnRH: Gonadotropin-releasing hormone; FBG: Fasting blood glucose; PPBG: Postprandial blood glucose; AUC: Area under curve; OGTT: Oral glucose tolerance test; DHEA: Dehydroepiandrosterone; DHT: 5 Alpha dihydrotestosterone; F: Fructose; ANOVA: Analysis of variance; ELISA: Enzyme-linked immunosorbent assay; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; PCOS: Polycystic ovary syndrome; USG: Ultrasonography.

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#### Authors' contributions

This study was designed by GSK and M.D. G.S.K. performed the experiments and statistical analysis and drafted the manuscript. P. T. assisted in the USG techniques and the preparation of histopathology slides. The authors have read and approved the final manuscript.

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#### Availability of data and materials

The supporting data or findings can be found if it is needed.

## Declarations

#### Ethics approval and consent to participate

All the experimental procedures were performed according to the institutional animal ethical committee of the ROFEL, Shri G.M. Bilakhia College of Pharmacy (Ethic protocol No: ROFEL/IAEC/2019/10).

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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