

## Hormonal imbalance and its effect on the pancreas in female rats with polycystic ovary syndrome

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### Abstract:

Polycystic ovary syndrome (PCOS) is a common endocrine disorder caused by hyperandrogenism, estrogen imbalance, and insulin resistance. It is a chronic condition that affects hormonal balance, but genetic and environmental factors are believed to play an important role in its development. There is no evidence that PCOS directly affects the pancreas and its enzymes. The aim was to understand the effect of PCOS on the pancreas from a physiological and histological perspective. Twenty-four female rats were divided into three groups (eight rats in each group). The first group was the control group, consisting of healthy rats that did not receive any medication. The second group was given 0.2 mg/kg of letrozole orally to induce PCOS. The third group was injected with 0.7 mg/kg of estradiol to induce PCOS. At the end of the experiment, blood samples were used to assess levels of hormones such as testosterone, estradiol, and insulin, as well as enzymes such as amylase. Ovarian and pancreatic organs were also taken for histological sectioning. The results showed a significant increase in testosterone concentrations in the letrozole-treated group and a significant decrease in estradiol concentrations, in contrast to the estradiol-treated group, where testosterone concentrations decreased and estradiol concentrations increased.

Regarding enzymes, amylase concentrations decreased in the letrozole-treated group, whereas they increased in the estradiol-treated group. Increased insulin concentrations were also observed in both groups. As for histological sections, ovarian sections from female mice in which polycystic ovary syndrome was induced using letrozole and estradiol showed numerous cystic follicles on the ovarian surface with a thin granular layer, dissolution of the corpus luteum, and the disappearance of primordial, primary, secondary, and Graafian follicles. In histological sections of the pancreas from female mice treated with letrozole, alpha cell proliferation and capillary dilation were observed. Pancreatic cells and islets of Langerhans were normal, and the effect of PCOS in this group was mild. Compared to the other group in which PCOS was induced with estradiol, we observed more pronounced changes, including islet cell hyperplasia, endothelial cell hyperplasia, capillary congestion, acinar cell necrosis, and beta cell degeneration. The study summarizes the effects of PCOS on the pancreas, leading to pathological changes and decreased activity.

**Keywords:** Polycystic ovary syndrome, Pancreas enzymes, Testosterone, Letrozole, Estrogen.

## Introduction

Polycystic ovary syndrome (PCOS) is a hormonal and clinical syndrome that is prevalent in the reproductive-age group of women. Named as the PCOD or PCOS, it was first characterized by Stein and Leventhal in 1935 [1]. Recommended for explanation, it is an ailment that causes anovulation, infertility, obesity, insulin resistance, and polycystic ovarian morphology. The dysfunctions related to PCOS are insulin resistance, type 2 diabetes mellitus, dyslipidemia, and obesity [1]. However, the origin of PCOS has not yet been established, and there is no broad plan or medication for handling patients with PCOS [3].

This recent study indicates that the PCOS rat model established by letrozole exhibits a phenotype very close to that of human PCOS, making it a suitable model for PCOS studies [4]. A PCOS rat model has been prepared using letrozole (LET), a nonsteroidal aromatase inhibitor [5]. Letrozole is a third-generation nonsteroidal aromatase inhibitor that has been widely used in clinical practice for ovulation induction. Its mode of action entails inhibiting estrogen levels in females by blocking the aromatization of androgens to estrogens, leading to reduced hypothalamic-pituitary hormone secretion and, consequently, decreased stimulation of ovulation [6].

The recent study indicates that the PCOS rat model established by letrozole exhibits a phenotype very close to that of human PCOS, making it a suitable model for PCOS studies [4]. A PCOS rat model has been prepared using letrozole (LET), a nonsteroidal aromatase inhibitor [5]. Letrozole is a third-generation nonsteroidal aromatase inhibitor that has been widely used in clinical practice for ovulation induction. Its mode of action entails inhibiting estrogen levels in females by blocking the aromatization of androgens to estrogens, leading to reduced hypothalamic-pituitary hormone secretion and, consequently, decreased stimulation of ovulation [6]. The second drug used to induce polycystic ovary syndrome is also estrogen in the form of Estradiol, which is a form of natural 17-estradiol ester, which is the naturally occurring human ovary estrogen hormone [7].

The pancreas originates from two buds, dorsal and ventral, that appear from one side and the other of the distal foregut endoderm, respectively. It encompasses a rather unique pattern of cell lineages. They have exocrine tissue, which comprises acinar cells that secrete digestive fluid, and a duct that drains the fluid into the intestine. The exocrine portion is scattered in groups of cells referred to as islets of Langerhans. It consists of several individual cells that secrete blood-borne hormones, at least five in the blood ( $\alpha$ -cells: glucagon;  $\beta$ -cells: insulin;  $\delta$ -cells: somatostatin;  $\epsilon$ -cells: ghrelin;  $\gamma$  [or PP] cells: pancreatic polypeptide). The fact that cells with such dissimilar roles in adults originate from the same progenitors is quite suggestive of a particular developmental course. Much of our understanding of the development of the organ in humans has depended on data inferred from studies on other animals, especially mice, which have been reviewed from time to time and comprehensively elsewhere [8]. The pancreas gland mainly functions as an exocrine gland. The exocrine secretory units are also known as the acini; the cells forming the acini are pyramidal and contain granules at the apices. The news of excretory ducts for the exocrine pancreas originates from the very middle of individual acini as pale-staining centroacinar cells, which extend into the brief intercalated ducts [9].

## Aim of the study

The aim of investigating the relationship between polycystic ovary syndrome (PCOS) and insulin resistance in affected female rats is to understand how this hormonal disorder affects cellular insulin sensitivity and to determine its effects on pancreatic function, particularly insulin secretion. This helps clarify the shared pathological mechanisms and supports the development of more effective treatments.

## Materials and Methods

### A) Animals

Therefore, 24 female Swiss white laboratory mice weighing 150-250 g were used in this study. These animals were confined in the animal house of the College of Education for Pure Sciences, University of Thi Qar, where these animals were maintained at a constant temperature of (22-25) degrees and a light and dark cycle lasting for twelve hours each throughout the entire preparatory experimental period of two weeks.

**B) Induction of polycystic ovary syndrome in laboratory rats**

In this experiment, two drugs were used: letrozole and estradiol to induce polycystic ovary syndrome. Letrozole 2.5 mg was dissolved in 8 ml of normal saline, and the animals in the second group were given the drug orally using a dosing device once daily. Estradiol 2 mg was dissolved in 8 ml of normal saline, and the animals in the third group received a single intramuscular dose once weekly. This continued for two months for both drugs.

**C) preparing design:**

The experiment included 24 female white rats, randomly divided into three groups, each group included eight females.

-control group (G1): eight normal female white rats, did not take any drug.

- Group Two (G2): was given an oral dose of letrozole 0.2mg/kg for two months, which induced polycystic ovary syndrome in this group.

- Group Three (G3): was injected with estradiol at a concentration of 0.7mg/kg mg for two months to induce cysts.

At the end of the experiment, the animal of each group was anesthetized with ether and sacrificed. The ovaries and liver of all groups were taken and preserved in 10% formalin for histological study.

**d) Histopathological study:**

Ovarian and pancreatic samples were dissected, fixed in formalin, dehydrated, and embedded in paraffin wax, then stained with hematoxylin and eosin and Mallory stain. Each slide was examined for histopathological changes under a light microscope based on [10].

**Statistical Analysis**

In all studies of the present results, these data were analyzed through the one-way covariance (ANOVA) test. All statistical analysis was performed using the computer software Statistical Package for the Social Sciences (SPSS V. 17 (SPSS Inc.)). The results were analyzed and presented as mean standard error (Mean  $\pm$  SE). (Inc,2006).

**Results**

The letrozole-treated group showed a non-significant decrease in estradiol levels ( $p=0.97$ ) and a significant increase in testosterone ( $p<0.05$ ). The other group treated with estradiol showed a significant increase in estradiol levels ( $p<0.05$ ), accompanied by a non-significant decrease in testosterone levels ( $p=0.93$ ) (Figure 1, 2). The current study showed no significant difference in insulin levels between the letrozole-treated and control groups ( $p=0.45$ ). At the same time, there were significant differences in insulin levels between the estradiol-treated and control groups ( $p<0.05$ ). (Figure 3).

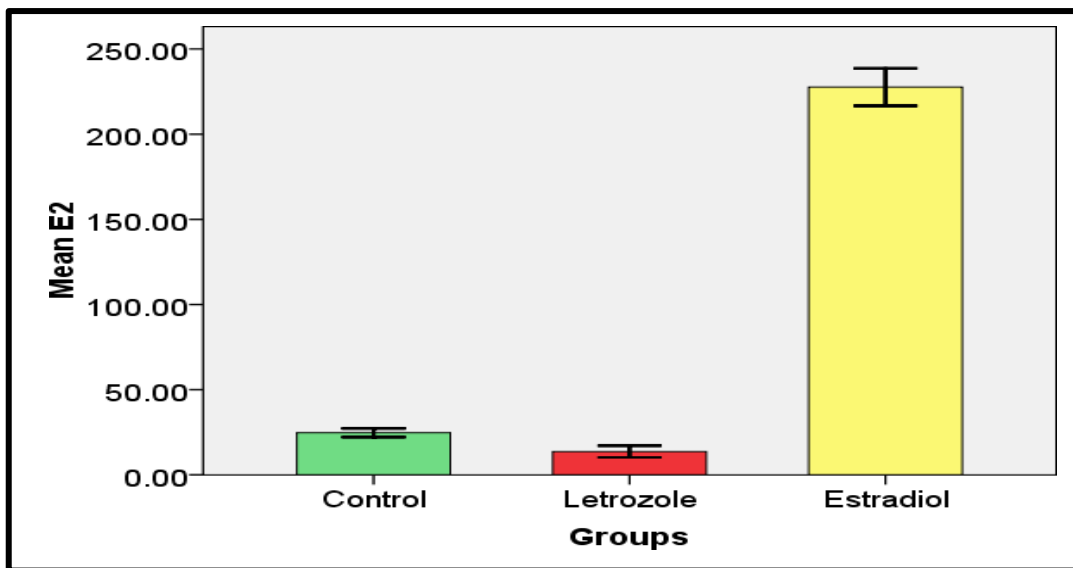


Figure 1: Comparisons Between Groups for Estradiol

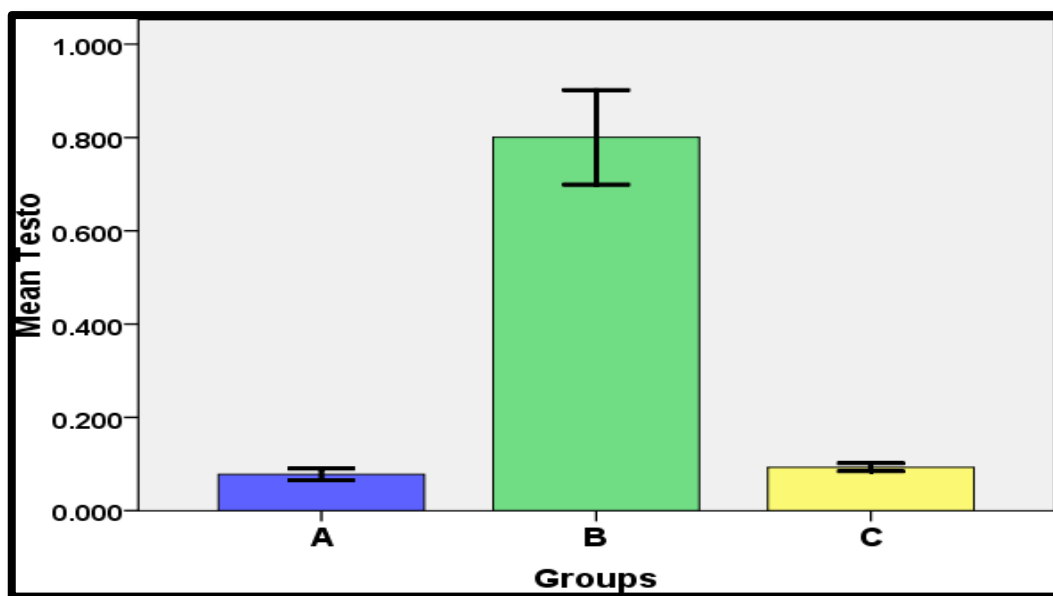
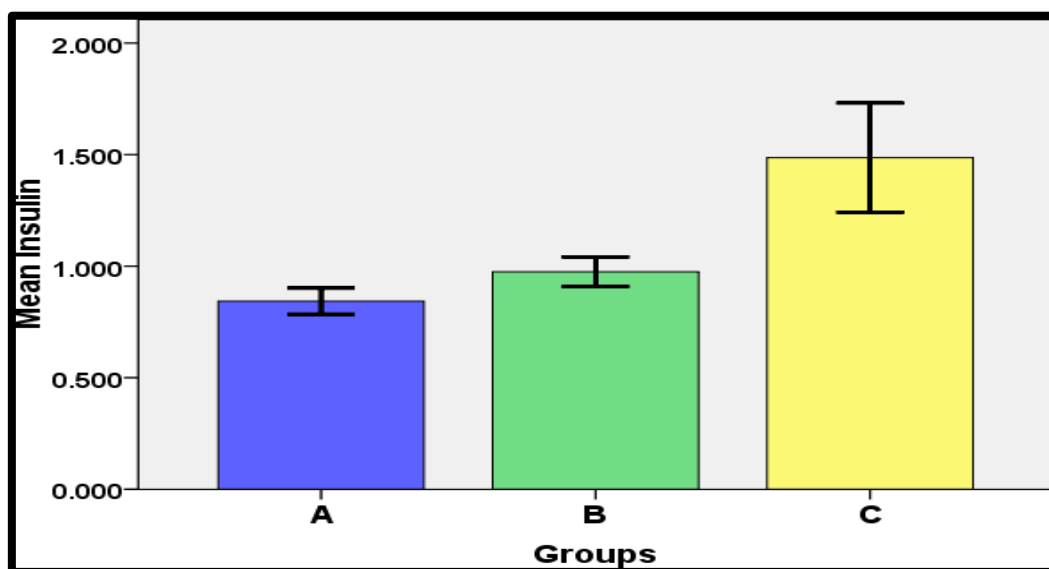


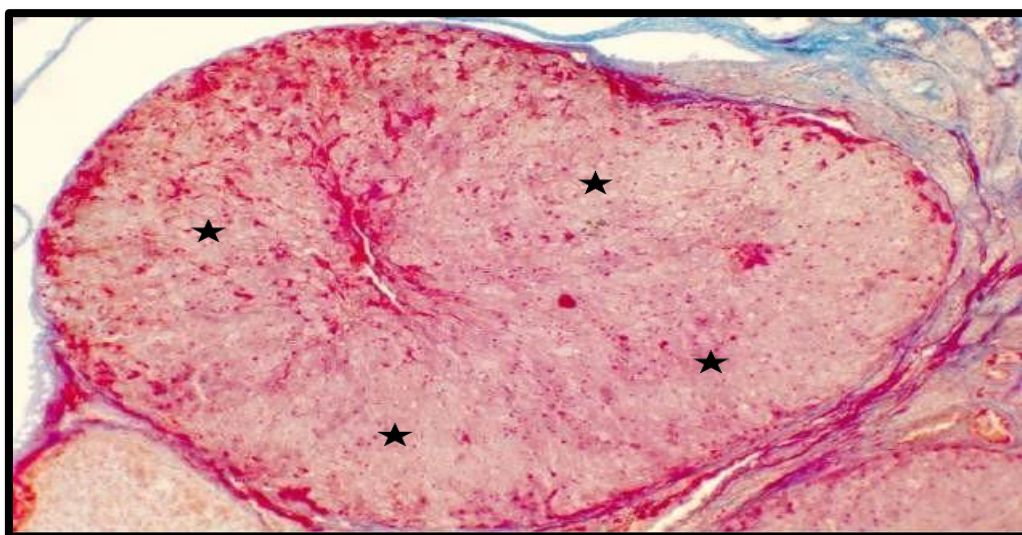
Figure 2: Comparisons Between Groups for testosterone



**Figure 3:** Comparisons Between Groups for Insulin

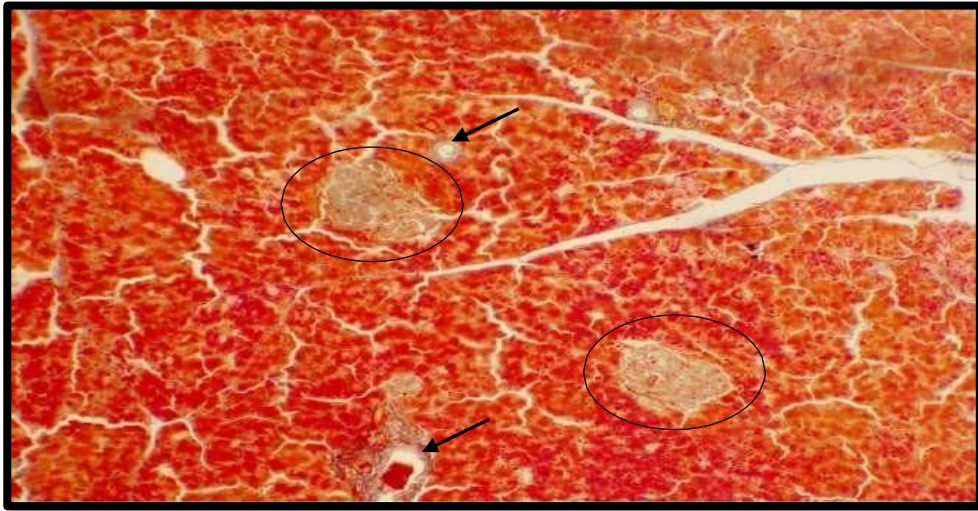
### Histological Study

In the control group, sections displayed normal histology of ovarian gonadal tissue, which included cortex and medulla sections with the different stages of follicles and corpus luteum (Figure 4). The control group had a normal histological structure of the pancreas without the alterations of groups of islets of Langerhans, beta cells, and alpha cells were normal, the same as acinar cells (Figure 5).



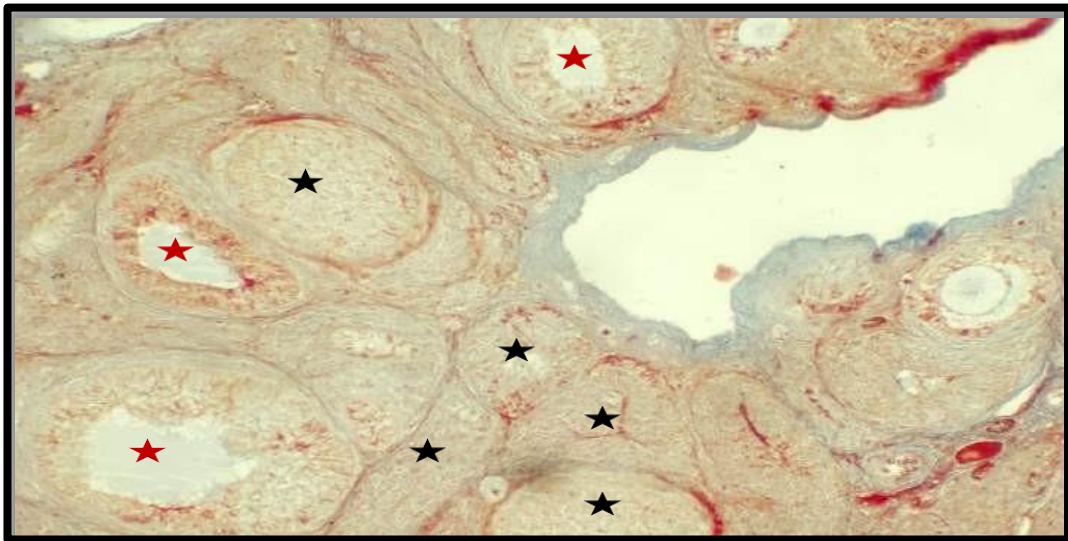
**Figure 3:** Oary normal tissue, normal corpus luteum (asterisk), Mallory, 10x



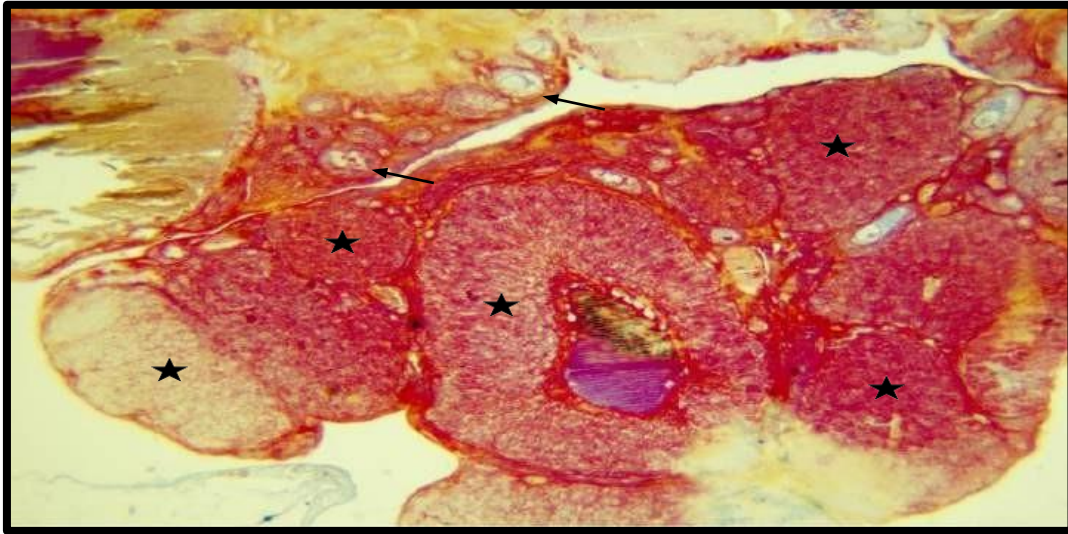


**Figure 4:** Pancreas normal tissue Langerhans Island (cycle), duct of pancreas(arrows), Mallory stain, 10x.

Sections of ovaries from female rats in which polycystic ovaries were induced with letrozole and estradiol showed the presence of numerous cystic follicles on the ovarian surface with a thin granular layer, dissolution of the corpus luteum, and disappearance of primordial, primary, secondary, and Graafian follicles. Group letrozole (Figure 5), Group estradiol (Figure 6).



**Figure 5:** ovary, lysis of most follicular (red asterisk) and disappearance of other follicular primordial, primary, secondary, and Graafian follicular with multiple corpus luteum (black asterisk), Mallory, 4x.



**Figure 6:** Ovary, multiple corpus luteum (asterisk) with lysis of follicular (arrows), Mallory, 4X.

Histological sections of the pancreas of female rats induced with polycystic ovary syndrome (PCOS) by letrozole show changes such as alpha cell proliferation and capillary dilation. The pancreatic cells and islets of Langerhans are normal. The effect of polycystic ovary syndrome in this group was slight (Figure 7). Compared to the other group in which polycystic ovary syndrome was induced by estradiol, we observed more changes and effects, such as hyperplasia of islet cells, hyperplasia of endothelial cells, capillary congestion, necrosis of acinar cells, and degeneration of beta cells Figure 8.



**Figure 7:** pancreas, normal cells and island (arrows) in pancreas, Mallory, 40x.





**Figure 8:** pancreas, hypertrophy of islet cells (cycle), Mallory, 10X.

## Discussion

The results showed that letrozole treatment did not significantly affect estradiol levels compared to the control group. This is consistent with letrozole's known role as an aromatase inhibitor, which prevents the conversion of androgens to estrogen. Therefore, it is expected that this treatment would not increase estradiol levels. However, in the estradiol-treated group, estradiol levels increased significantly compared to the other two groups. This is a normal response to direct exposure to estradiol from an external source. These results are consistent with the study by Wang et al. [11], which confirmed that letrozole inhibits estradiol synthesis, while estradiol treatment directly increases estradiol levels in the body.

In the letrozole-induced PCOS model, testosterone levels rise as a result of aromatase inhibition, disrupting pancreatic function, particularly in beta cells, and stimulating hyperinsulinemia and increased amylase. This is consistent with Skarra et al. [12], who demonstrated that androgen excess causes early hyperinsulinemia before the development of insulin resistance or obesity.

Histological findings in the ovaries and pancreas in the control group indicate normal structural and functional organization, reflecting an efficient ovarian cycle and proper glucose regulation. This is consistent with Zhao et al. [13], who demonstrated that standard ovarian architecture includes mature follicles and a functional corpus luteum, indicating regular ovulation. In contrast, the changes observed in PCOS models are consistent with those documented by Elsayed et al. [14], including an increase in the number of follicles and hyperplasia of the tunica albuginea, reflecting impaired follicular maturation. Li et al. [15] further supported that these structural changes are directly related to a dysfunctional hypothalamic-pituitary-ovarian axis.

Insulin is a hormone secreted by the beta cells in the islets of Langerhans in the pancreas. Our study revealed higher insulin concentrations in both the letrozole and estradiol groups compared to the control group, but the increase was more pronounced in the estradiol group. This suggests varying degrees of insulin resistance depending on the type of hormonal agent used to induce the PCOS model. The elevated insulin levels in the estradiol group may be explained by the fact that high doses of estradiol disrupt insulin receptor signalling, particularly in the liver and muscle, leading to insulin resistance. This explanation is supported by the study by Goodarzi et al. [16], which showed that estrogens may negatively affect insulin sensitivity at subphysiological levels. Wang et al. [17] also suggested that estradiol increases insulin resistance by activating inflammatory pathways linked to estrogen receptor alpha in peripheral tissues.



In the letrozole group, insulin elevation is expected due to elevated testosterone levels resulting from aromatase inhibition. Several studies, such as those by Diamanti-Kandarakis et al. [18], have shown that testosterone reduces cellular insulin response and increases insulin levels. However, the insulin elevation in this group was lower than that with estradiol, which may be attributed to the relatively slower metabolic effect of letrozole and its association with progressive hyperandrogenism. This is consistent with the results of our study and those of Rizk et al. [19], who observed a moderate insulin elevation with letrozole in animal models of PCOS.

In contrast, some studies have not reported apparent differences in insulin with letrozole, such as the study by Sahin et al. [20], which attributed this to variations in doses and exposure periods. This highlights the advantage of our study, which used a balanced design and a direct comparison of two hormonal stimulants under standardised conditions. Therefore, these results confirm that estradiol causes a greater metabolism than letrozole. They also highlight the variability in the effect of the two stimulants used to generate the PCOS model, a strength of our study design that distinguishes it from previous studies.

Amylase is a key biomarker of pancreatic function. Increased levels of this enzyme in the blood indicate disturbances in pancreatic cells, often caused by inflammation or metabolic stress. The study showed elevated amylase levels in both the letrozole and estradiol-treated groups compared to the control group. However, the nature of this increase differed between the two groups; the amylase increase in the letrozole group was not statistically significant, while the increase in the estradiol group was. This discrepancy in effect may be explained by the different hormonal mechanisms induced by each of the two drugs.

In the estradiol-treated group, however, the increase in amylase was significant, indicating an apparent direct effect on pancreatic function. It is believed that high doses of estrogen, especially with prolonged exposure, may negatively affect the pancreas through several mechanisms, including stimulating intracellular lipid accumulation, increasing bile viscosity, and stimulating an inflammatory response. This view is supported by the findings of Vega et al. [21], who reported that exposure to high doses of estrogen increases the risk of pancreatitis, as reflected in significant elevations in enzymes such as amylase. The same researchers also suggest that estrogen may interfere with the nervous and secretory regulation of the pancreas, leading to secretory hyperactivity or functional stress.

Histological sections of the pancreas of rats in the letrozole group showed a significant increase in the number of alpha cells within the islets of Langerhans, suggesting a potential compensatory response to eliminate the inhibitory effect of estrogen on these cells. This interpretation supports previous studies on the role of estrogen in regulating glucagon secretion from alpha cells. For example, Gao et al. [22] demonstrated that estrogen receptors are expressed in alpha cells and that estrogen inhibits glucagon secretion. Therefore, inhibiting aromatase with letrozole reduces estrogen levels, which may increase alpha cell activity and proliferation as a physiological response to compensate for this deficiency. In the estradiol-treated group, the current study revealed multiple histological changes in the rat pancreas, the severity of which varied according to the treatment type. Microscopic examination revealed that estradiol had a more severe and varied effect on pancreatic tissue than letrozole.

In the estradiol group, marked degeneration of the  $\beta$ -cells within the islets of Langerhans, which are responsible for insulin secretion, was observed. This degeneration indicates a loss of the typical structure and function of these cells, which may lead to impaired glucose regulation. Histological images also showed enlarged endothelial cell nuclei and capillary congestion, indicating a disturbance in blood flow within the pancreas. These changes suggest a direct or indirect effect of estradiol on the microvasculature. Previous studies (Tiano et al. [23]) have indicated that estradiol can protect beta cells from cell death through its interaction with estrogen receptors, especially  $ER\alpha$ , but high doses or chronic exposure may lead to harmful effects, such as oxidative stress or perfusion disturbances.

In contrast, the letrozole group showed one central histological change, necrosis of acinar cells, the cells responsible for secreting digestive enzymes. Necrosis indicates acute cell death, possibly due to indirect toxic effects arising from hormonal imbalance or disrupted blood supply. Despite this significant change, the effect of letrozole was limited compared to that of estradiol, and no changes were observed in the islets of Langerhans or endothelial cells. This

comparison demonstrates that estradiol induces multiple histological changes in endocrine and vascular cells, reflecting a more severe and widespread effect on the pancreas compared to letrozole, which caused limited, localised changes in exocrine cells.

## Conclusion

Letrozole-induced hyperandrogenism leads to insulin resistance and lipid accumulation, whereas estradiol-induced hyperestrogenism exacerbates disruption of the pancreatic cellular environment and increases its susceptibility to injury. Bioassays suggest that oxidative stress and inflammation play a critical role. Effects of PCOS on the pancreas include the induction of insulin resistance, which places a functional burden on beta cells and subsequently leads to dysfunction or injury. These changes are early indicators of type 2 diabetes and illustrate that hormonal imbalances in PCOS are directly linked to pancreatic and metabolic derangements. These findings reflect the complexity of PCOS as a multisystem syndrome and highlight the importance of linking hormonal, metabolic, and inflammatory functions to understand long-term effects on a vital organ such as the pancreas..

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