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Sex steroid hormones and insulin sensitivity: the role of KATP sensitive channels

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Abstract: Association of sex steroid hormones and insulin secretion has been reported in a number of studies, however, the mechanism behind the effects of sex steroid hormones on insulin secretion is still unclear. The present study investigated the effects of KATP sensitive channels blocker (verapamil) or opener (diazoxide) on insulin sensitivity in male and female rats. In this experimental-laboratory study, rats were divided to control (no treatment) and gonadectomized male and female rats, and male rats treated with testosterone enanthate, diazoxide and veramapil, and female rats treated with estradiol valerate, progesterone, diazoxide and veramapil. Serum glucose and insulin level were measured 4 weeks after surgery or treatment. Data were analyzed by one-way analysis of variance (ANOVA). Ttestosterone, verapamil, and co-administration of testosterone and verapamil resulted in a significant decrease in insulin sensitivity in male rats. Treatment of female rats with progesterone, diazoxide, and co-administration of progesterone and diazoxide or verapamil, led to significant increase in insulin sensitivity, however, estradiol and verapamil treatment resulted in a significant decrease in insulin sensitivity. Our findings indicated that testosterone and estradiol were insulin sensitivity reducers and progesterone was insulin sensitivity enhancer in rats.

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Despite progesterone, testosterone did not significantly affect K_{ATP} channels blocker (verapamil) or opener (diazoxide) action on insulin sensitivity.

Keywords: Sex steroid hormones, Verapamil, Diazoxide, Insulin sensitivity

1 Introduction

Type 2 diabetes is a progressive disease that has a complex pathophysiology (DeFronzo, 2009). Type 2 diabetes is a condition in which the body becomes resistant to the normal effects of insulin and gradually loses the capacity to make enough insulin in the pancreas. The condition has strong genetic and family-related (non-modifiable) risk factors and is also often associated with modifiable lifestyle risk factor. According to the International Diabetes Federation, the number of people with diabetes in the world in 2045 will reach 700 million a year (IDF, 2019). Various factors may play a role in the development of diabetes, such as insulin resistance (DeFronzo, 2009). Globally, the prevalence of insulin resistance varies between 15.5 and 46.5% among adults (Fahed et al., 2020).

Energy metabolism and reproduction are closely related. Insulin secretion is affected by gonadal steroids (Mauvais-Jarvis, 2016). Imbalance in sex hormones, especially due to involvement of visceral adipose tissue, has a significant effect on the incidence of type 2 diabetes (T2DM). The results of some studies indicate that hyperandrogenism in women and hypogonadism in men are risk factors for type 2 diabetes (Gambineri and Pelusi, 2019). Also, the results of some research indicate the prescription of male sex hormones can cause glucose intolerance and increase insulin is in the serum (Beck, 1973; Sechi et al., 1992; Shoupe and Lobo, 1984). In addition, it is reported that lower testosterone levels in men and higher testosterone levels in women are associated with diabetes (Ding et al., 2006). According to other studies, decreased testosterone levels in men are associated with an increased risk of T2DM. However, higher testosterone levels in women are associated with a higher risk for T2DM (Gyawali et al., 2018). Androgens play an important role in both men and women by acting on adipose tissue and skeletal muscle. In addition, according to some reviews, androgens increase insulin sensitivity by having a direct effect on insulin signaling (Schiffer et al., 2017). Conversely, it has been suggested that androgens, by some unknown mechanisms, might induce insulin resistance. On the other hand, it should be noted that ATP-sensitive K^+ channel in the pancreatic beta cells membrane are the most important mediators of insulin secretion. As ATP channels open, beta cells become hyperpolarized and insulin secretion is reduced (Koster et al., 2005). Among medicines, diazoxide is the most important opener and verapamil is the most common compounds for blocking membrane ATP-sensitive potassium channels of beta cells ((Shoupe and Lobo, 1984; Landon et al., 1963; George and McCrimmon, 2012; Ninomiya et al., 2003; Ahmadi et al., 2004).

Information about the exact relationship between male or female sex hormones with sensitivity to insulin is limited and further studies in this area are needed. Studies on the relationship between sex hormones and insulin secretion have been mainly focused on the

effects of insulin on hyperandrogenicity and few studies have investigated the effects of sex hormones on insulin secretion; according to which, the present study investigated the effects of male and female sex hormones on insulin secretion. In this study, the effects of unilateral and bilateral orchidectomy, ovariectomy, administration of testosterone (in male rats) and of progesterone and estradiol (in female rats) on insulin sensitivity were investigated. Also, co-administration of testosterone or progesterone with diazoxide or verapamil was studied to investigate the effects of testosterone and progesterone on ATP-sensitive K+ channel and insulin secretion.

2 Materials and Methods

2. 1. Animals and research protocol

Wistar rats weighting 200 ± 30 g were purchased from the Pasteur Institute. Animals were kept at 23 °C. Lighting was diurnal, 12:12-h light:dark (lights on 0600 to 1800). Testosterone enanthate, estradiol valerate, progesterone, diazoxide and veramapil in pure powder were provided by Abu Reihan Chemical Pharmaceutical Company. Table 1 and 2 show the protocol of study.

Table1. Protocol of study in male rats (ORCX: orchidectomy).

Experimental subgroups		Hormone or drug treatment (mg/kg/day)		
		Testosterone (T)	Diazoxide (D)	Verapamil (V)
1	Control	-	-	-
2	Vehicle treated	-	-	-
3	Bi-ORCX	-	-	-
4	Bi-ORCX + T	50	-	-
5	Uni-ORCX	-	-	-
6	Sham-ORCX	-	-	-
7	T treated	10	-	-
8	D treated	-	30	-
9	V treated	-	-	100
10	D + T treated	10	30	-
11	V + T treated	10	-	100

Table 2. Protocol of study in female rats (OVX: ovariectomy).

Experimental subgroups		Hormone or drug treatment (mg/kg/day)			
		Estradiol (E)	Progesterone (P)	Diazoxide (D)	Verapamil (V)
1	Control	-	-	-	-
2	Vehicle treated	-	-	-	-
3	Bi-OVX	-	-	-	-
4	Uni-OVX	-	-	-	-
5	Sham-OVX	-	-	-	-
6	Bi-OVX + E	0.2	-	-	-
7	Bi-OVX + P	-	20	-	-
8	D treated	-	-	30	-
9	V treated	-	-	-	100
10	E treated	0.2	-	-	-
11	P treated	-	20	-	-
12	P + D treated	-	20	30	-
13	P + V treated	-	20	-	100

2. 2. Insulin assay

A conventional RIA laboratory kit and laboratory glucose oxidase kit were used to measure serum insulin and glucose level, respectively. Blood samples were obtained 4 weeks after surgery or treatments. Fasting serum glucose preparation and serum insulin concentration were measured and the ratio of serum glucose to insulin level was calculated as an indicator of insulin sensitivity.

2. 3. Orchidectomy and ovariectomy

For surgery, the animals were first anesthetized using 120-100 mg/kg hydrochloride and 24 mg/kg xylene hydrochloride. To remove the ovary or ovaries, a 1 to 2 cm incision of the dorsal skin was made around the 13th animal rib. After a muscle incision, the ovary or ovaries were removed. For castration, after making a small incision in the middle of the scrotum wall and the testis or testes were removed. Finally, the incisions were repaired (Waynforth, 1988) (Figures 1, 2, 3, 4 and 5). Peritoneal injection of testosterone, estradiol and progesterone started from the third day after surgery and continued for 4 weeks.

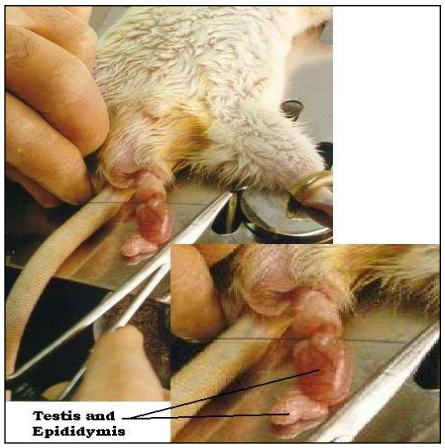

Figure 1: removing of the testis.

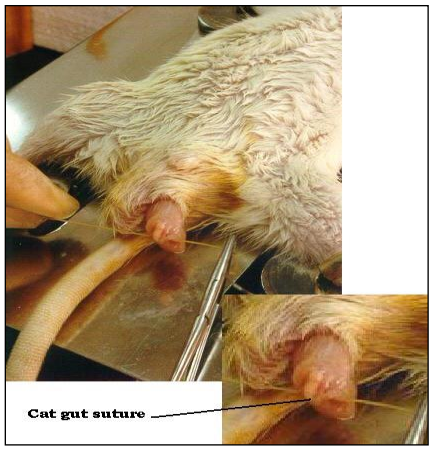

Figure 2: Catgut suture of muscle incision.

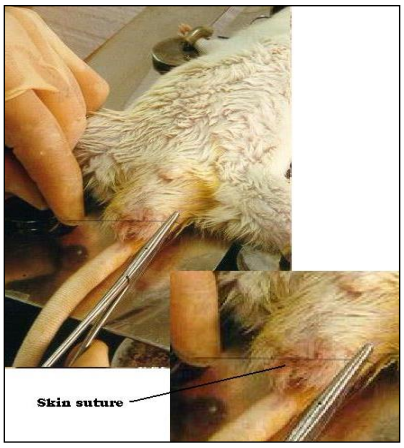

Figure 3: Nylon thread suture of skin incision.

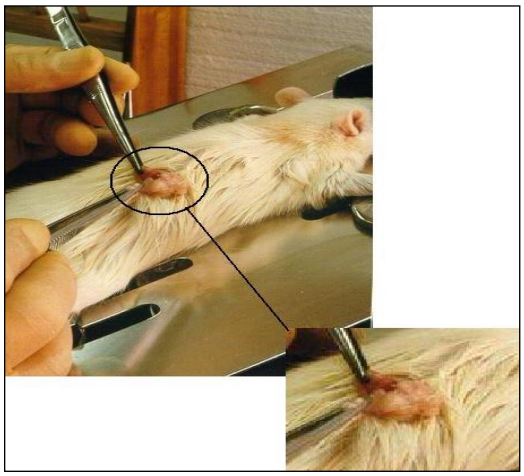

Figure 4: Removing of ovaries.

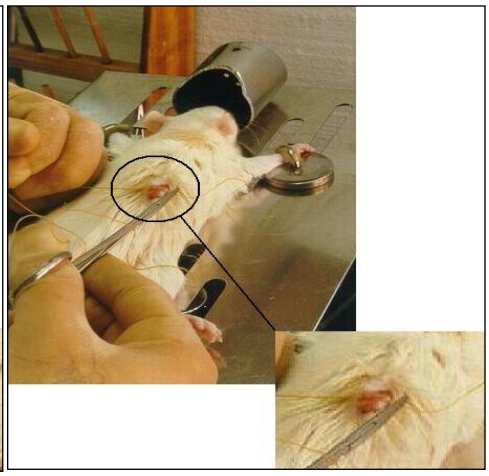

Figure 5: Catgut suture of muscle incision.

2. 4. Serum glucose

Blood samples were obtained by cardiac puncture technique and stored at laboratory temperature for 15 minutes (Morimoto et al., 2001). In order to prepare the serum, blood samples were centrifuged at 2500 rpm and after separating the serum, the serum samples required for insulin measurement were kept at a temperature of -20 °C (Liu and Bachmann, 1998). Serum glucose was measured immediately after preparation of serum samples by glucose oxidase method. Serum insulin concentration was determined using radioactive assay (RIA).

2. 5. Statistical analysis

Data were analyzed using SPSS software and student's t-test and one-way analysis of variance (ANOVA). In analysis of variance, the significance of the differences between the groups was determined using Fisher's exact test (FSD- LSD).

3 Results and Discussions

3. 1. Male groups

3. 1. 1. The effects of uni-orchidectomy and bi-orchidectomy on insulin sensitivity

Fasting serum glucose significantly decreased in uni-orcx ($p < 0.2$) and bi-orcx rats ($p < 0.01$) compared with control animals. Serum glucose in bi-orcx rats was significantly lower than uni-orcx rats ($p < 0.001$). Serum insulin significantly increased in uni-orcx rats ($p < 0.05$) and significantly decreased in bi-orcx animals ($p < 0.001$) compared with control rats. The results also indicated that insulin sensitivity (glucose / insulin ratio) significantly decreased in uni-orcx rats ($p < 0.05$) and increased in bi-orcx rats ($p < 0.05$) compared with control group (Figure 6).

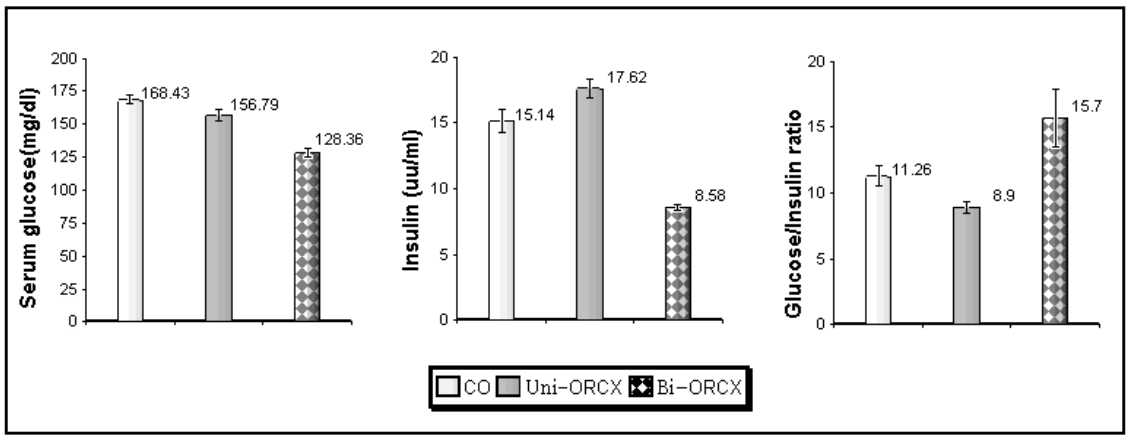

Figure 6: Serum glucose, insulin and glucose/ insulin ratio in intact control (CO), Uni- orchidectomised (Uni-ORCX) and Bi-orchidectomised (Bi-ORCX) male rats. Values are mean ± S.E.M of 5 rats.

3. 1. 2. The effects of testosterone replacement on insulin sensitivity in bi-orchidectomised rats

Serum glucose increased significantly in bi-orcx rats receiving daily dose of testosterone (50 mg/ kg/ day) compared with non-treated bi-orcx animals ($p < 0.05$). In testosterone treated bi-orcx group, serum glucose level was significantly lower than control rats ($p < 0.2$).

Serum insulin level increased significantly in testosterone treated bi-orcx rats compared with non-treated bi-orcx group ($p < 0.001$). Additionally, serum insulin level of testosterone was significantly higher in treated bi-orcx rats than control group ($p < 0.01$).

Insulin sensitivity (glucose/insulin ratio) significantly decreased in testosterone treated bi-orcx group compared with bi-orcx ($p < 0.001$) and control groups ($p < 0.01$) (Figure 7).

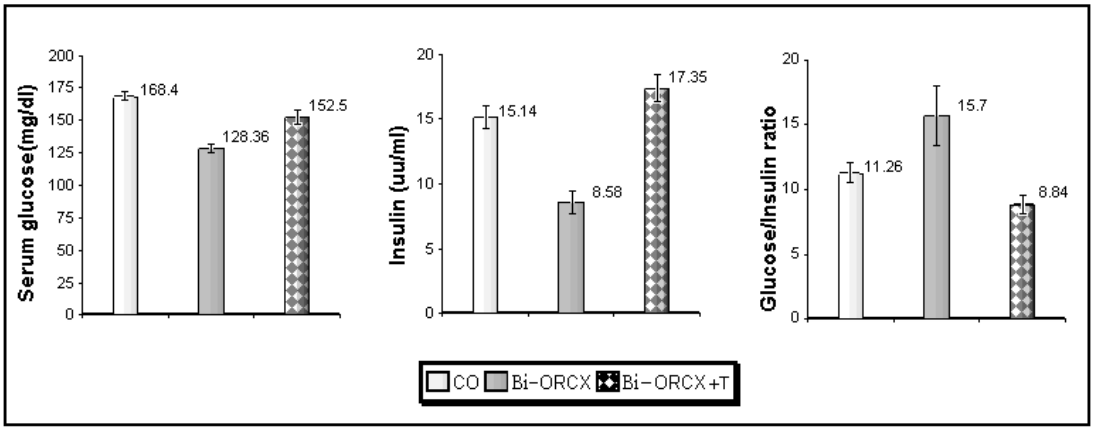

Figure 7: Serum glucose, insulin and glucose/ insulin ratio in intact control (CO), Bi-orchidectomised (Bi-ORCX) and Bi-orchidectomised testosterone treated (50 mg/ kg/ day)(Bi-ORCX + T) male rats. Values are mean ± S.E.M of 5 rats.

3. 1. 3. The effects of testosterone administration on insulin sensitivity in male rats

In testosterone treated (10 mg/ kg/ day) male rats, serum glucose ($p < 0.05$) and insulin ($p < 0.01$) increased significantly compared with control animals and insulin sensitivity (glucose / insulin ratio) significantly decreased ($p < 0.05$) 4 weeks after treatment (Figure 8).

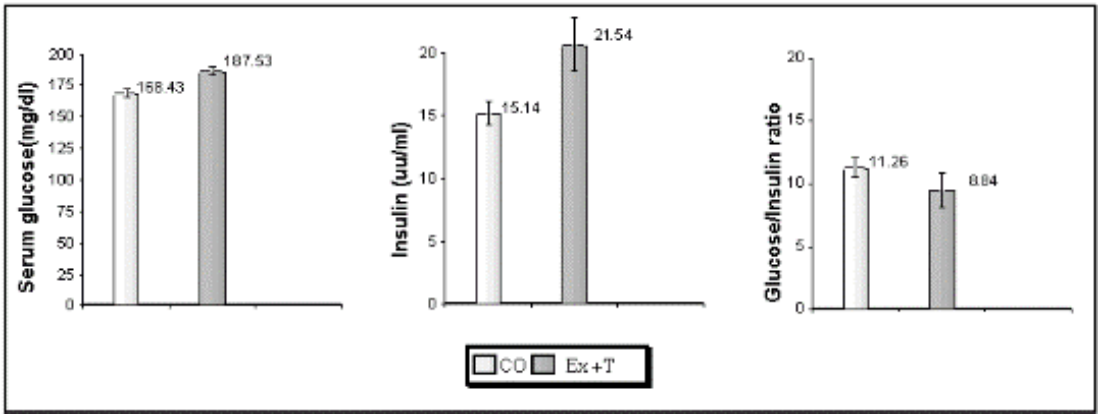

Figure 8: Serum glucose, insulin and glucose/ insulin ratio in intact control (CO), and testosterone treated (10 mg/ kg/ day)(Ex + T) male rats. Values are mean ± S.E.M of 5 rats.

3. 1. 4. The effects of diazoxide and "diazoxide + testosterone" on insulin sensitivity in male rats

In diazoxide (30 mg/kg/day) treated and "diazoxide (30 mg/kg/day) + testosterone (10mg/kg/day)" treated male rats, serum glucose level significantly decreased ($p < 0.01$ and $p < 0.05$, respectively) compared with control animals. Serum glucose level was more decreased in"diazoxide + testosterone" treated rats than diazoxide receiving group ($p < 0.01$).

Serum insulin level was also decreased in diazoxide ($p < 0.01$) and "diazoxide + testosterone" treated rats compared with control group ($p < 0.001$). However, decreasing of insulin level in "diazoxide + testosterone" receiving rats was not statistically significant compared with diazoxide receiving animals.

Insulin sensitivity (glucose/insulin ratio) increased significantly in both diazoxide and "diazoxide + testosterone" receiving groups compared with control group ($p < 0.01$). There was not statistically significant difference between "diazoxede + testosterone" treated and diazoxide receiving rats (Figure 9).

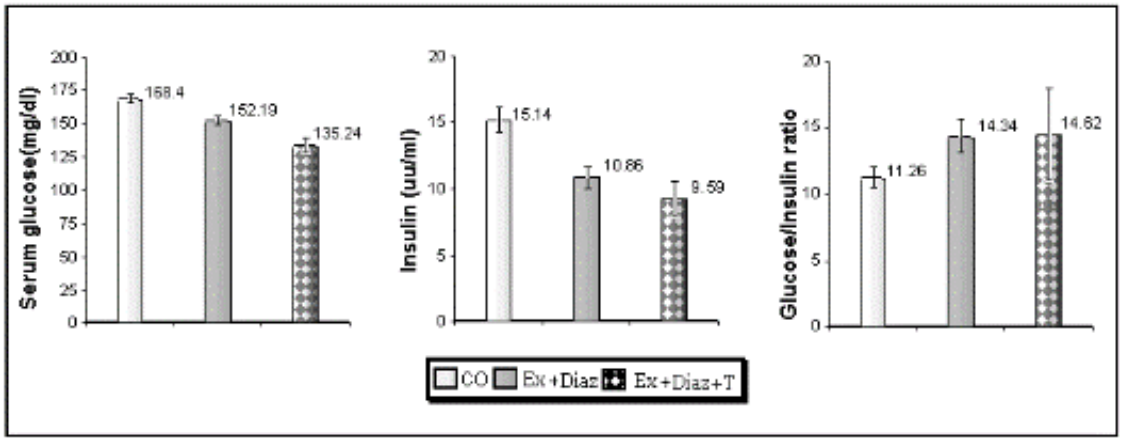

Figure 9: Serum glucose, insulin and glucose/ insulin ratio in intact control (CO), diazoxide (30 mg/kg/day) treated (Ex + Diaz) and "diazoxide (30 mg/kg/day) + testosterone (10 mg/kg/day) treated" (Ex + Diaz + T) male rats. Values are mean ± S.E.M of 5 rats.

3. 1. 5. The effects of verapamil and "verapamil + testosterone" on insulin sensitivity in male rats

Fasting serum glucose and insulin level in verapamil (100 mg/ kg/ day) and "verapamil (100 mg/kg/day) + testosterone (10 mg/kg/day)" receiving group significantly increased compared with control group ($p < 0.05$). However, there was not significant difference between serum glucose level of verapamil and "verapamil + testosterone" receiving groups.

Serum insulin level increased in verapamil and "verapamil + testosterone" treated rats ($p < 0.001$) compared with control animals. In addition, there was not significant difference between serum insulin levels of verapamil and "verapamil + testosterone" treated rats.

Insulin sensitivity (glucose/ insulin ratio) in verapamil and "verapamil + testosterone" treated groups significantly decreased compared with control group ($p \leq 0.05$) and there was no significant difference between insulin sensitivity of verapamil and "verapamil + testosterone" treated male rats (Figure 10).

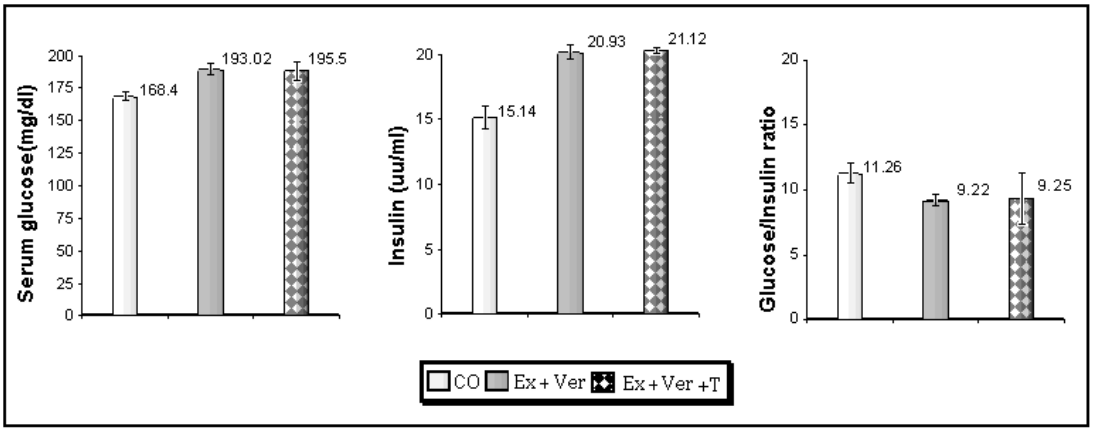

Figure 10: Serum glucose, insulin and glucose/ insulin ratio in intact control (CO), verapamil (100 mg/kg/day) treated (Ex + Ver) and "verapamil (100 mg/kg/day) + testosterone (10 mg/kg/day) treated" (Ex + Ver + T) male rats. Values are mean ± S.E.M of 5 rats.

3. 2. Female groups

3. 2. 1. The effects of uni-ovariectomy and bi-ovariectomy on insulin sensitivity

Serum glucose significantly decreased in uni-ovariectomised and ovariectomised rats compared with intact control animals ($p < 0.01$), but there was not statistically difference between uni- ovx and bi-ovx rats. Serum insulin level significantly decreased in uni-ovx ($p < 0.01$) and bi-ovx ($p < 0.001$) rats compared with control animals. In addition, serum insulin level was lower in bi-ovx than uni-ovx rats ($p < 0.01$). Insulin sensitivity (glucose/ insulin ratio) significantly increased in uni-ovx ($p < 0.01$) and bi-ovx ($p < 0.01$) rats compared with control animals. Insulin sensitivity was higher in bi-ovx than uni-ovx rats ($p < 0.05$) (Figure 11).

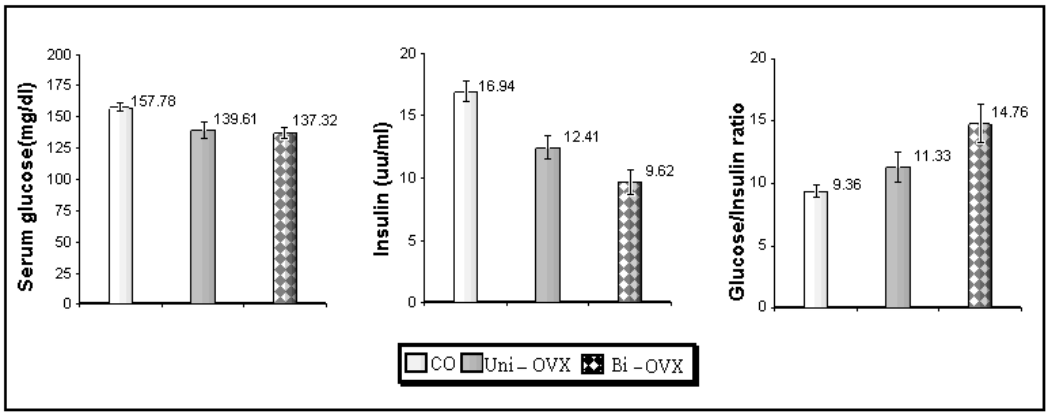

Figure 11: Serum glucose, insulin and glucose/ insulin ratio in intact control (CO), Uni-ovariectomised (Uni + OVX) and Bi-ovariectomised (Bi + OVX) female rats. Values are mean ± S.E.M of 5 rats.

3. 2. 2. The effects of estradiol and progesterone on insulin sensitivity in bi-ovariecomised rats

Serum glucose concentration increased significantly in estradiol (200 μ g/kg/day) treated ($p < 0.01$) but non-significantly in progesterone (20 mg/kg/day) treated bi-ovx rats compared with

non-treated bi-ovx rats. Serum insulin, increased significantly in estradiol treated ($p < 0.01$), however, decreased non-significantly in progesterone treated bi-ovx rats compared with non-treated bi-ovx animals. Insulin sensitivity (glucose/ insulin ratio) significantly decreased in estradiol treated ($p < 0.05$) but increased in progesterone treated bi-ovx rats ($p < 0.05$) compared with non-treated bi-ovx rats (Figure 12).

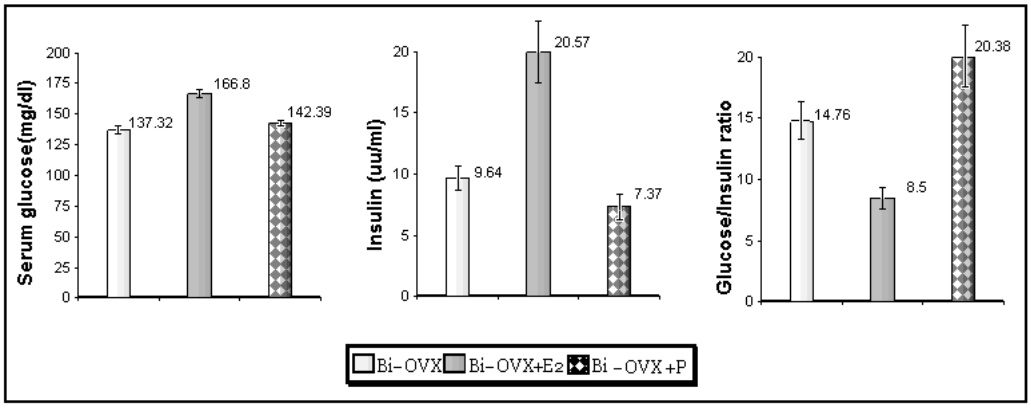

Figure 12: Serum glucose, insulin and glucose/ insulin ratio in Bi-ovariectomised (Bi + OVX), "Bi-ovariectomised + estradiol (200 µg/kg/day) treated" (Bi + OVX + E_2) and "Bi-ovariectomised + progesterone (20 mg/kg/day) treated" (Bi + OVX + P) female rats. Values are mean ± S.E.M of 5 rats.

3. 2. 3. The effects of estradiol and progesterone administration on insulin sensitivity in female rats

Serum glucose level increased significantly in estradiol treated (200 µg/kg/day) but decreased non-significantly in progesterone treated (20 mg/kg/day) female rats compared with control animals. Serum insulin level significantly increased in estradiol treated group ($p < 0.001$) but decreased in progesterone treated ($p < 0.001$) rats compared with control animals. Insulin sensitivity (glucose/insulin ratio) significantly decreased in estradiol treated ($p < 0.01$) and increased in progesterone treated ($p < 0.01$) female rats compared with control animals (Figure 13).

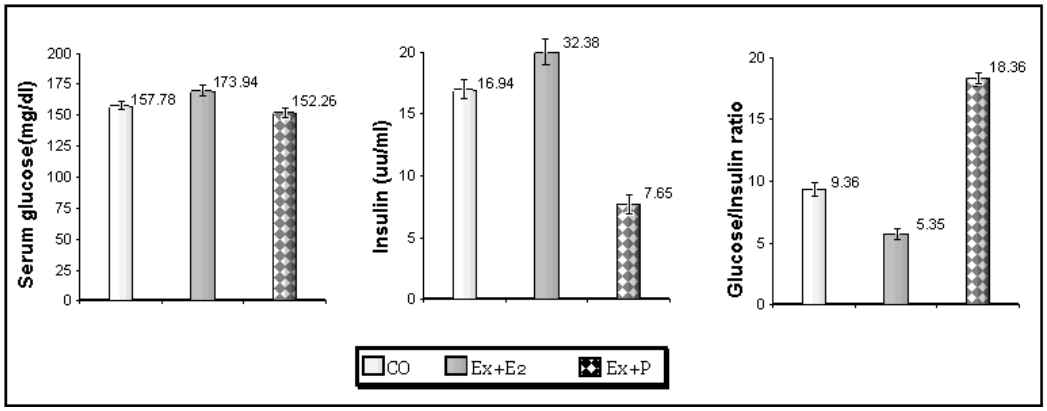

Figure 13: Serum glucose, insulin and glucose/ insulin ratio in control (CO), estradiol (200 µg/ kg/ day) treated (Ex + E_2) and progesterone (20 mg/ kg/ day) treated (Ex + P) female rats. Values are mean ± S.E.M of 5 rats.

3. 2. 4. The effects of verapamil and "verapamil + progesterone" on insulin sensitivity in female rats

Serum glucose level increased significantly in verapamil (100 mg/kg/day) treated, and non-significantly in "verapamil (100 mg/kg/day) + progesterone (20 mg/kg/day)" treated female rats compared with control group. In addition, increased serum glucose level was significantly more in verapamil treated group than "verapamil + progesterone" treated female rats ($p < 0.05$).

Serum insulin level significantly increased in verapamil treated ($p < 0.01$) but decreased in "verapamil + progesterone" treated ($p < 0.01$) female rats compared with control animals. Insulin sensitivity (glucose/insulin ratio) decreased non-significantly in verapamil treated but increased significantly in "verapamil + progesterone" treated group ($p < 0.001$) compared with control animals (Figure 14).

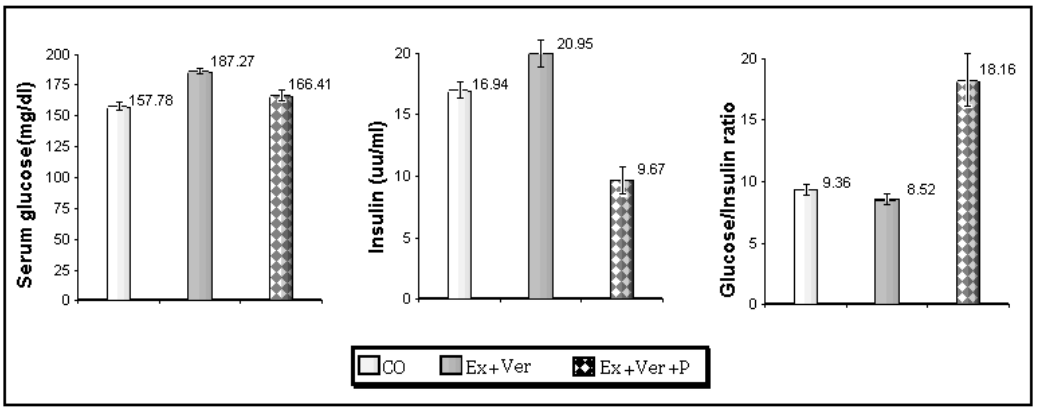

Figure 14: Serum glucose, insulin and glucose/ insulin ratio in control (CO), verapamil (100 mg/kg/day) treated (Ex + Ver) and "Verapamil (100 mg/kg/day) + progesterone (20 mg/kg/day) treated" (Ex + Ver + P) female rats. Values are mean ± S.E.M of 5 rats.

3. 2. 5. The effects of diazoxide and "diazoxide + progesterone" on insulin sensitivity in female rats

Serum glucose increased in diazoxide (30 mg/kg/day) treated and non-significantly in "diazoxide (30 mg/kg/day) + progesterone (20 mg/kg/day)" treated female rats compared with control animals. However, serum glucose increased non-significantly more in "diazoxide + progesterone" treated rats than diazoxide treated group. Serum insulin level decreased both in diazoxide and "diazoxide + progesterone" treated rats ($p < 0.001$) compared with control animals but there was no significant difference between serum insulin level of diazoxide and "diazoxide + progesterone" treated groups. Insulin sensitivity (glucose/insulin ratio) increased both in dazoxide and "diazoxide + progesterone" treated groups ($p < 0.01$) compared with control animals (Figure 15).

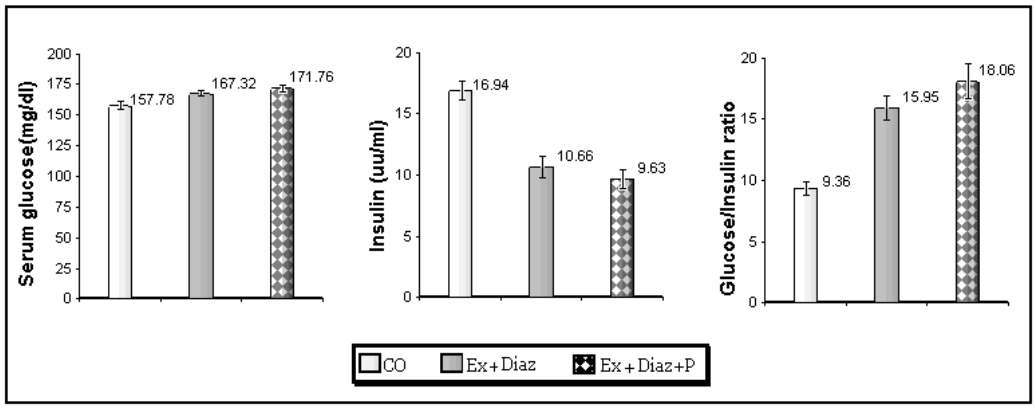

Figure 15: Serum glucose, insulin and glucose/ insulin ratio in control (CO), diazoxide (30 mg/kg/day) treated (Ex + Diaz) and "diazoxide (30 mg/kg/day) + progesterone (20 mg/kg/day) treated" (Ex + Diaz + P) female rats. Values are mean ± S.E.M of 5 rats.

3. 4. The effects of estradiol on testis and uterus

3. 4. 1. The effects of estradiol on testis weight, size and tissue structure

In estradiol treated (200 μg/kg/day) rats testis weight decreased compared with control group ($p < 0.001$) (Figure 16).

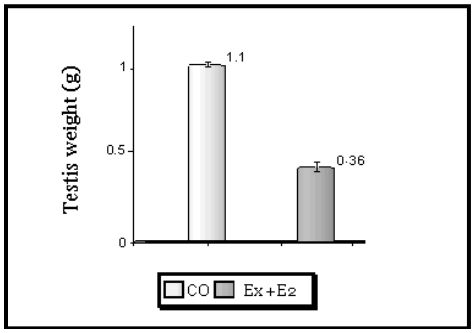

Figure 16: Testis weight in intact control (CO) and estradiol (200 μg/kg/day) treated (Ex + E_2) male rats. Values are mean ± S.E.M of 5 rats.

Qualitatively, testis size also reduced in estradiol treated rats compared with control animals (Figure 17).

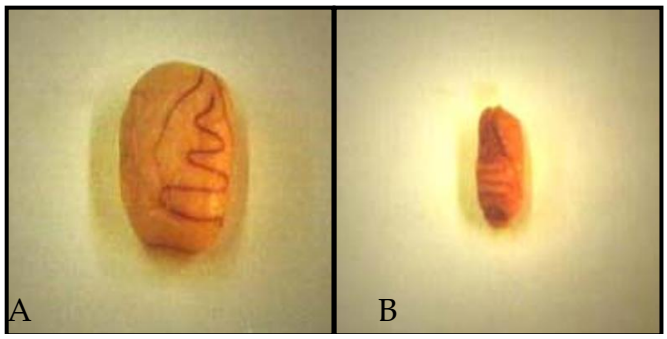

Figure 17: Testis size in control (A) and estradiol treated (B) rats.

Histological effects of estradiol on testis tissue indicated that the semeniferous tubules in estradiol treated rats were markedly different in appearance from those in control rats. They markedly reduced in size and showed a disruption of normal epithelial organization. The number of spermatozoa in testicular tunnel of estradiol treated rats was less than of control rats. Additionally, there was decreased number of germ cells in estradiol treated testis. As a whole, cellular concentration has been decreased in testes of estradiol treated rats compared with testes of control group (Figure 18).

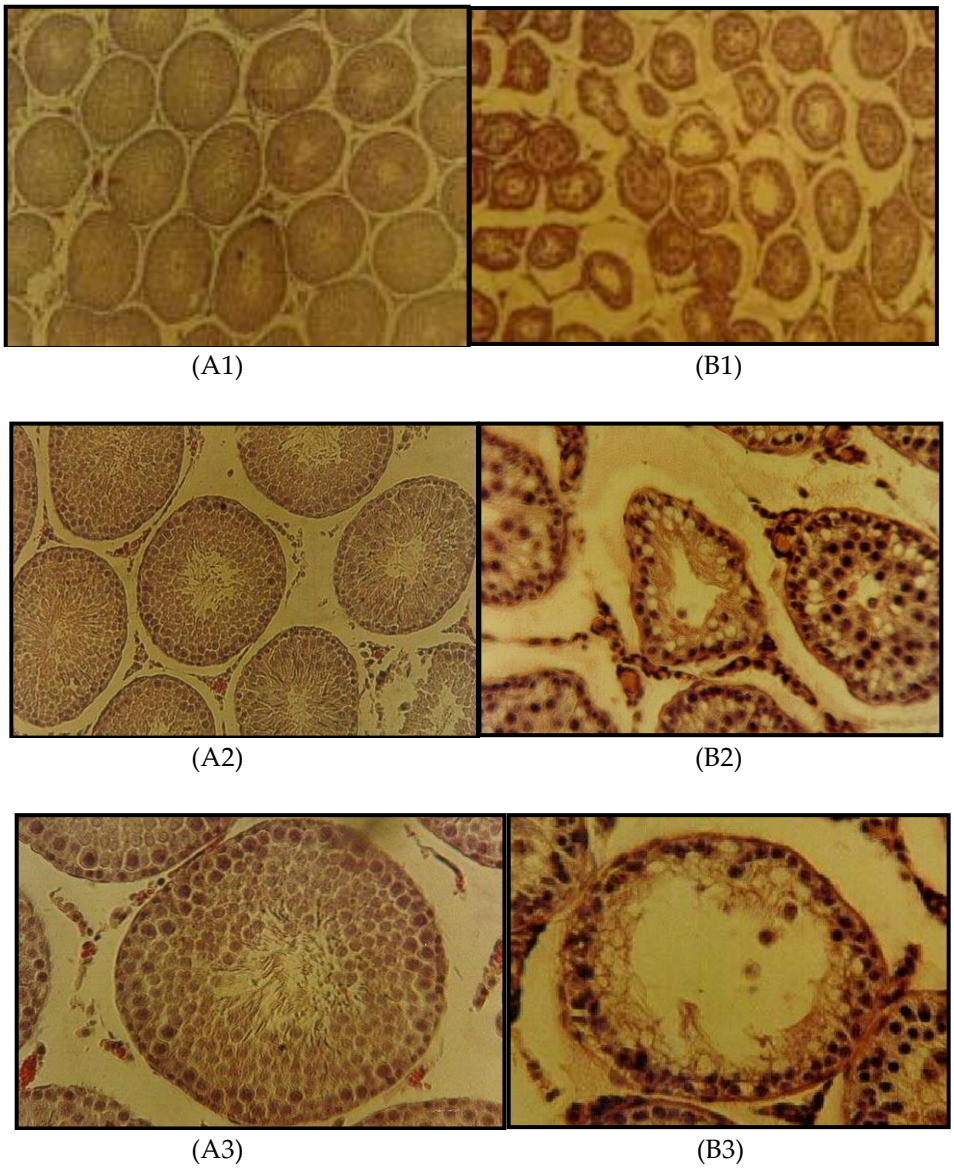

Figure 18: Hematoxylin - eosin stained sections of rat testis from intact controls (A1, A2 and A3) and estradiol treated (B1, B2 and B3) rats.

3. 4. 2. The effects of estradiol on uterus

Estradiol administration (200 μg/kg/day) for 4 weeks in female rats, indicated increased uterus size. Uterine epithelial tissue height was also larger in estradiol treated rats compared with intact control animals when were studied histologically. Therefore, the markedly

hypertrophic effect of estradiol on uterine epithelial tissue was observed in the uteri obtained from estradiol treated rats. (Figure 19).

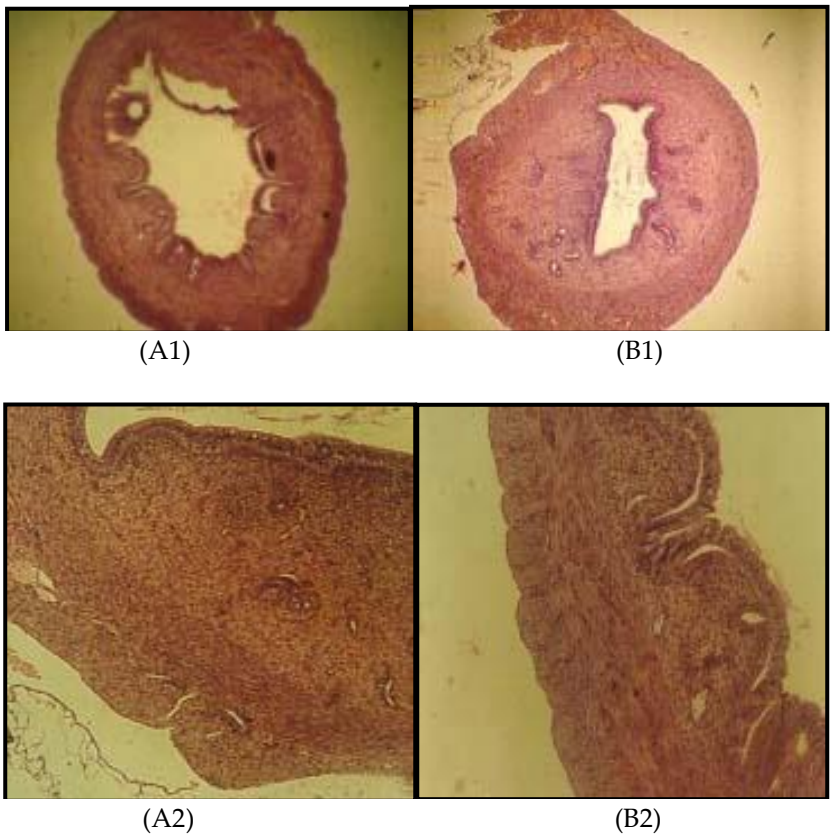

Figure 19: Hematoxylin - eosin stained sections of rat uteri , illustrating epithelial lining cells, obtained from intact control (A1 and A2) and estradiol treated (B1 and B2) rats.

Little is known about the interaction between testosterone levels and insulin sensitivity in men, in contrast to the abundant literature on this relationship in women. Cross-sectional studies demonstrate an inverse relationship between testosterone and fasting insulin levels in men independent of age, obesity, and body fat distribution.

Testosterone plays a key role in the regulation of glucose and lipid metabolism in men and women although in different manners. According to this study, testosterone has been shown to reduce insulin sensitivity. This result has been observed in previous and recent studies (Buffington et al., 1988; Holmäng and Björntorp, 1992; Morimoto et al., 2001). Contrary to our findings, however, it has been shown that testosterone improves cardiovascular risk factors such as insulin resistance and hypercholesterolemia (Jones and Kelly, 2018). In this study, bi-orchidectomy increased the sensitivity of cells to insulin. However, uni-orchidectomy reduced insulin sensitivity. Since testosterone administration compensates for the effect of bi-orchidectomy, it seems that the reason for the increase in insulin sensitivity in bi-orchidectomy is related to the decrease in testosterone levels after orchidectomy. Decreased insulin sensitivity due to testosterone is primarily dependent on increased insulin levels, which can be caused by: decreased metabolic clearance of insulin (McCarroll and Buchanan, 1973), impairment of hepatic capacity to extract insulin (Evans et al., 1984), increasing of insulin secretion from

pancreas, and increased insulin gene expression (Randle et al., 1964). Testosterone also can be converted to estrogens by aromatization and it might be considered that the effects of testosterone administration, in part, would be those of estrogens (Holmang et al., 1992). In orchidectomy, insulin gene expression decreases with decreasing testosterone levels, and eventually insulin concentration decreases. Thus, by replacing testosterone in bi-orchidectomy, mRNA insulin levels and serum insulin concentrations increase, ultimately reduce the cells' sensitivity to insulin (Morimoto et al., 2001; Nielsen, 1984). In our study, insulin sensitivity decreased in uni-orcx rats in part because of compensatory effects of remained testis to produce more insulin compared with control animals. This result requires more careful study. In addition to increasing insulin levels, testosterone also increases glucose levels. Therefore, testosterone may alter insulin sensitivity by influencing skeletal muscle fiber composition and shifting its morphology in to less insulin – sensitive fibers (Bergamini, 1975; Lefaucheur et al., 1986). In addition, testosterone administration reduces the incorporation of glucose in rat skeletal muscle and this effect could also be involved in decreased insulin sensitivity in testosterone treated groups of our study. However, administration of testosterone in bi-orchidectomy increases glucose concentration due to the increase in insulin levels, the glucose to insulin ratio (insulin sensitivity) in these groups not only does not increase, but also decreases.

In our study, verapamil 100 mg/kg/day was used as a blocker and diazoxide at a daily dose of 30 mg/kg was used as an ATP-sensitive K^+ channel opener in pancreatic beta cell membranes. Verapamil was used as an insulin-boosting drug and diazoxide was used as an insulin-lowering drug ((Dalponte et al., 1998; Shen et al., 2001; Dalponte et al., 1998; Shen et al., 2001; Quast and Cook, 1989). Co-administration of testosterone with verapamil decreased insulin sensitivity and administration of testosterone with diazoxide increased insulin sensitivity in male rats. However, insulin levels and insulin sensitivity were not significantly different between the verapamil and "testosterone + verapamil" groups and the diazoxide and "testosterone + diazoxide" groups. This probably indicates that testosterone has no effect on blocking or opening the ATP-sensitive K^+ channel in the beta cell membrane. Also, testosterone on the effects of verapamil or Diazoxide also has no effect on insulin secretion or insulin sensitivity.

Based on the findings of this study, uni-ovariectomy and bi-ovariectomydecreased serum insulin levels and increased insulin sensitivity. Also, insulin sensitivity in bi-ovariectomyrats showed a greater increase than uni-ovariectomy. For replacement, 20 mg/kg/day of progesterone and 200 μg/kg/day of estradiol were used in bi-ovariectomised rats. Research has shown that these doses are quite effective in insulin secretion and insulin sensitivity (Foster, 1998; Foster and Balfour, 1997; Lindheim et al., 1994). Estradiol replacement in bi-ovariectomyrats and administration of estradiol in non-surgical female rats reduced insulin sensitivity and increased insulin levels. Our research findings are consistent with previous studies. Decreased insulin sensitivity has also been observed in men receiving estrogen (Polderman et al., 1994). Estrogen appears to play an important role in improving and regulating glucose levels in insulin-sensitive tissues and organs (De Paoli et al., 2021). Another study also showed, fasting insulin levels were significantly lower in women taking estrogen than in women not taking estrogen (Brown et al., 2000). The stimulatory effects of estradiol on insulin secretion have also been reported in tissue culture studies (Etchegoyen et al., 1998). In

addition, a recent association between insulin secretion and serum estradiol concentrations has been established (Nagata et al., 2000). Progesterone administration increases in postmenopausal womenInsulin sensitivity (Foster and Balfour, 1997) Also, estradiol suppresses somatic gluconeogenesis with the ability of a transcription factor (Foxo1) (Yan et al., 2019). In addition, progesterone administration reduces insulin sensitivity in adipose tissue (Collison et al., 2000). New effects of estrogen signaling on pancreatic development and regeneration suggest that estrogen receptor α can be considered as a target for controlling glucose homeostasis and β cell formation in the treatment of diabetes (Yuchi et al., 2015). The place of specific estrogen and progesterone receptors in pancreatic cells indicates that the hormones estrogen and progesterone have a direct effect on pancreatic beta cells (Green et al., 1978; Tesone et al., 1979) Progesterone affects glucose homeostasis by increasing beta cells (Chang et al., 1983). Previous research has shown that single or combined administration of estrogen and progesterone can stop gluconeogenesis in female rats (Matute and Khalkhoff, 1973). Progesterone replacement in bilateral ovariectomized rats also reduces glucose uptake into brown adipose tissue (Nielsen, 1984).

In female rats, possible effects of progesterone on ATP-sensitive potassium channels through co-administration of progesterone (20 mg/kg/day), was evaluated with diazoxide (30 mg/kg/day) or verapamil (100 mg/kg/day). Co-administration of progesterone with diazoxide or verapamil decreased serum insulin levels and increased insulin sensitivity. Also, insulin sensitivity was not significantly different between the non-surgical groups receiving "progesterone + verapamil" and "progesterone + diazoxide". Verapamil administration alone reduced insulin sensitivity. But, "progesterone + verapamil" administration increased insulin sensitivity, indicating that progesterone counteracted the action of verapamil on pancreatic beta cells. By binding to its receptor on the surface of the pancreatic beta cell, verapamil blocks ATP-sensitive potassium channels, then opens voltage-sensitive calcium channels, and ultimately increases insulin secretion and decreases insulin sensitivity. Therefore, progesterone in response to verapamil may affect insulin secretion and ultimately insulin sensitivity by preventing the blockage of ATP-sensitive potassium channels or inhibiting voltage-gated calcium channels or at least interfering with verapamil receptors. In addition, there was no significant difference in insulin levels and insulin sensitivity between the non-surgical groups receiving diazoxide and "diazoxide + progesterone". According to the results of this study, diazoxide is a factor that increases insulin sensitivity. However, co-administration of progesterone with diazoxide did not significantly increase insulin sensitivity compared with diazoxide alone. Therefore, this result indicates that progesterone may not have a significant effect on the function of diazoxide on pancreatic beta cells.

4 Conclusion

The findings of this study show that testosterone has a decreasing effect on insulin sensitivity in male rats. However, it probably does not affect ATP-sensitive potassium channels in the pancreatic beta membrane. Estradiol has a decreasing effect and progesterone has an increasing effect on insulin sensitivity in female rats. Progesterone may reduce serum insulin and increase

insulin sensitivity by preventing the blockage of ATP-sensitive potassium channels in the pancreatic beta cell membrane or by inhibiting the effect of voltage-sensitive calcium channels.

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Conflict of interests

The authors declare no conflict of interests regarding the publication of this paper.

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