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Effect of Ghrelin on Some Sexual Hormones and Molecular Characterization of Luteinizing Hormone and Follicular Stimulating Hormone Genes in Hyperthyroidic Male Rats

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Abstract

Aim: The focus of this research was to see how the hormone ghrelin affected reproductive hormones and gene expression of LH and Fsh hormone in mature male rats.

Methods: Seventy male rats were used in the investigation, with twenty serving as controls and 50 being given a subcateaneous injection (s.c) of freshly manufactured thyroxine from (Sigma Aldrich Co.-USA) at a dose of 500 µg/kg dissolved in saline to induce hyperthyroidism. For thirty days, After inducing hyperthyroidism, The hyperthyroidic rats were divided into four groups at random. each with ten rats: a positive control group, a hyperthyroic group administrated with GRE at dose (0.5nmol/100µl saline), and a hyperthyroidic group administrated with administrated at dose (1nmol/100µl saline). The hyperthyroic group received GRE at a dose of 2nmol/100l saline. For thirty days,both the positive and negative control groups were given saline. (10µg/rat)

Results: when compared to the control group, the hyperthyroidism group had a significant ($P \leq 0.05$) increase in serum (T, FSH, and LH) concentration, whereas when compared to the hyperthyroidism group, all hyperthyroidism groups treated with ghrelin had a significant ($P \leq 0.05$) decrease in serum (T, FSH, and LH) concentration. In the hyperthyroidic group, LH mRNA expression was considerably higher ($p<0.0001$) than in the control group. When compared to the hyperthyroidic group, LH mRNA expression decreased considerably ($p<0.0001$) in all hyperthyroidic groups administrated with **GRE**. When comparing the hyperthyroidic group to the control group, FSH mRNA expression increased considerably ($p<0.0001$). FSH mRNA expression, on the other hand, decreased considerably ($p<0.0001$) in all hyperthyroidic groups administrated with **GRE**. **GRE** has been shown to have regulated effects on the reproductive hormones and expression of their related genes in adult male rats.

Conclusion: We discovered that GRE decreases the expression of the LH- and FSH hormones directly, which could be attributable to direct effects on the hypothalamusH-P-G. axis. ghrelin can regulator of the testicular functions and represents an important additional link between the male reproductive hormones homeostasis and the reproductive function.

Keywords: Hyperthyroidism, ghrelin, Levothyroxine, LH, FSH, T

Introduction

Thyroid hormone production and/or secretion are excessively high in hyperthyroidism. Thyrotoxicosis is a clinical disorder in which excessive thyroid hormone has an adverse effect on the tissues, resulting in systemic symptoms[1]. Changes in the endocrine, sexual, or reproductive systems are linked to thyrotoxicosis[2].The hypothalamic-pituitary-gonadal axis is the main operator of sex development and reproduction, which is triggered by the

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gonadotropin-releasing hormone (GnRH) [3]. The generation of SHBG and androgen binding protein (ABP), which transport testosterone, is affected by thyroid status in the liver and Sertoli cells. Due to a decrease in its metabolism, SHBG and testosterone levels rise in thyrotoxicosis[4]. Ghrelin (GRE) is a 28-amino-acid peptide hormone produced mostly by secretory granules in the stomach's submucosal layer's X/A-like endocrine cells[5],[6]. In the central nervous system, the hypothalamus has been identified as the primary source of ghrelin. Furthermore, the GHS-R1a receptor mRNA has been discovered in several locations of the brain, as previously mentioned. In rats, systemic ghrelin injection lowers the frequency of GnRH pulses in vivo. The full inhibition of the effects of ghrelin by the NPY-Y5 receptor antagonist suggests that NPY is involved in the modulation of ghrelin's effects on pulsatile GnRH production[7]. Ghrelin significantly inhibits GnRH release by hypothalamic lobes from ovariectomized females[8]. In rats, lambs, and monkeys, ghrelin reduces the frequency of LH pulses in the pituitary[9],[10], and humans [11]. Ghrelin controls gonadotropin release in mammalian and nonmammalian species via acting on the hypothalamus as well as the pituitary gland directly[12]. The action mechanism of ghrelin on the reproductive system is unknown; however, this peptide inhibits the hypothalamic–pituitary–gonadal axis, causing a decrease in luteinizing hormone and testosterone plasma levels[13]. In patients with thyroid dysfunction, ghrelin and hormones of the hypothalamic-pituitary-thyroid (HPT-) axis have a negative relation[14].

Materials and methods

Chemicals

L-Thyroxine (T4) take up from Merk-france , Ghrelin Lyphilized white powder taken up from Anaspec-UK Co. The testosterone, FSH and LH hormones were managed by using commercially available kits supplied by Elabscience Inc., China.

Gene Expresion Assay:

All of the male rats that treatment had their heads cut off. Pituitary glands were excised and kept at -80°C until the reverse transcriptase polymerase chain reaction was carried out (RTPCR).

Primers must be prepared before they can be used.

The primers (originally lyophilized) were dissolved in free ddH2O to reach a final concentration of 100 M/l, which functioned as a stock solution that was stored at -20 °C, according to the primer synthesiser company's instructions. The stock primers were concentrated to a concentration of 10 M/l to be utilized as a work primer.

Primers Used In This Study

Target gene	Sequence (5’-3’)		Tm (°C)	Product size
Rat GAPDH	F	5’- ATGACTCTACCCACGGCAAG-3’	60	89bp
	R	5’- CTGGAAGATGGTGATGGGTT -3’		

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LH-beta	F	5'-CTGTGGCTGCTGCTGAGCCCAAG-3'	60	115bp
	R	5'-TGCAGACTGGGCAGAACTCA-3'		
FSH-beta	F	5'-CACAGCCAGGCAATCTTATG-3'	60	70bp
	R	5'-AGACCAAACACCCAGAAAGT-3'		

Experimental animal design

Healthy adult male albino rats weighing 250-300 gm were procured from the animal house of the Faculty of Veterinary Medicine at Basrah University for this investigation. Rats were housed in hygienic steel wire cages (4 rats per cage) in the physiology animal house of the Faculty of Medicine, Basrah University. They had unlimited access to water, were kept at room temperature. Before the experiment began, the rats were allowed a week to acclimatize to their new place[15].

Seventy male rats were used in the investigation, with twenty serving as controls and 50 being given a subcateaneous injection (s.c) of freshly manufactured thyroxine from (Sigma Aldrich Co.-USA) at a dose of 500 µg/kg dissolved in saline to induce hyperthyroidism. For thirty days, After inducing hyperthyroidism, The hyperthyroidic rats were divided into four groups at random. each with ten rats: a positive control group, a hyperthyroic group administrated with GRE at dose (0.5nmol/100µl saline), and a hyperthyroidic group administrated with administrated at dose (1nmol/100µl saline). The hyperthyroic group received GRE at a dose of 2nmol/100l saline. For thirty days,both the positive and negative control groups were given saline. (10µg/rat)

Each group's animals were anesthetized with chloroform and murdered after the experiment period. For histological evaluation, the organ testis was taken and kept in 10% formalin. Blood was drawn from the heart through cardiac puncture with a 5cc sterile syringe and placed in plain tubes without anticoagulant. FSH, LH, and Testosterone hormones were measured in serum samples separated from blood by centrifugation at 3000rpm for 15 minutes.

Analytical Statistics

The single-direction covariance (ANOVA) test was used. Using the SPSS V. 21 (measurable bundles for sociologies) application.

Result

When compared to the control group, the hyperthyroidism group showed a significant ($P \leq 0.05$) increase in serum (T, FSH, and LH), whereas all hyperthyroid groups administrated with **GRE** had a significant ($P \leq 0.05$) drop in serum (T, FSH, and LH). While serum (T and LH) concentrations between the GRE (2 nmol) group and the control group were not significantly different. Furthermore, there were no significant differences in serum (LH) concentrations between the GRE (1 nmol) administrated and control groups. In comparison to the control group, the GRE (0.5 nmol) group demonstrated a significant ($P \leq 0.05$) increase in serum (T and LH) concentration. Furthermore, as compared to the control group, the group given **GRE** (1 nmol) demonstrated a significant ($P \leq 0.05$) increase in

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serum (T)concentration. On other hand, a significant (P≤0.05) decrease in serum **(Tes and LH)** concentration of group administrated with GRE (2nmol) as compared with the groups administrated with GHR (0.5 and 1nmol). Also a significant (P≤0.05) decrease in serum **(Tes and LH)** concentration of group administrated with GRE (1nmol) as compared with the groups administrated with GRE (0.5nmol). In contrast, serum FSH concentrations in groups administrated with GRE (1 and 2 nmol) were not significantly different from those in the control group. There were no significant differences in serum FSH concentrations in the GRE (2 nmol) group compared to the GRE (1nmol) group . Eventually, serum FSH concentrations in groups administrated with GRE (0.5nmol) increased significantly (P≤0.05) when compared to other groups administrated with GRE and the control group.

Table (1): Effects of Ghrelin Treatments on Serum Pituitary-Gonadal-Axis Hormones in hyperthyroidic Male Rats. (Mean±SD) (n=10)

Parameter / Groups	T (ng/ml)	LH (mlU/Ml)	FSH (mlU/Ml)
Control (Normal saline)	18.82±0.32d	7.23±0.27cd	5.91±0.36c
Hyperthyroidism	30.83±0.76a	19.44±0.49a	9.23±0.21a
Hyper+(0.5GHREL)	21.94±0.35b	8.91±0.35b	6.90±0.50b
Hyper+ (1 GHREL)	19.95±0.48c	7.65±0.47c	6.12±0.22c
Hyper+(2 GHREL)	18.67±0.37d	7.02±0.54d	5.98±0.62c
LSD	0.58	0.52	0.49

Values expressed in the small letters mean significant differences at the (P≤0.05) level.

Assay for Gene Expression:

Figure 1A shows the expression of LH mRNA in the pituitary of male rats with hyperthyriodism. LH mRNA expression in the hyperthyroidism group was considerably ($p<0.0001$) higher than in the control group. In all hyperthyroidism groups treated with ghrelin (0.5, 1 or 2 nmol) injection, LH mRNA expression decreased significantly ($p<0.0001$) as compared to the hyperthyroidism group.

There is no significant difference in LH mRNA expression between all hyperthyroidism groups treated with ghrelin and the control group ($p<0.0001$). Eventually, no significant differences in LH mRNA expression ($p<0.0001$) were found between the hyperthyroidism groups treated with ghrelin.

Figure 1B shows the expression of FSH mRNA in the pituitary of male rats with hyperthyriodism. FSH mRNA expression increased significantly ($p<0.0001$) in the hyperthyroidism group compared to the control group. In comparison to the hyperthyroidism group, FSH mRNA expression decreased significantly ($p<0.0001$) in all hyperthyroidism groups treated with ghrelin. While there is no significant difference in FSH mRNA expression between all hyperthyroidism groups treated with

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ghrelin and the control group . Consequently, no significant differences in FSH mRNA expression ($p<0.0001$) were found between all hyperthyroidism groups treated with ghrelin

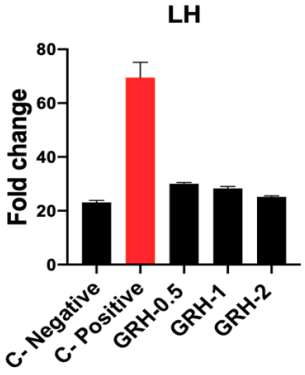

Fig.1A. Effects of ghrelin administration (0.5, 1 or 2 *nmol*) on the Expression of LHβ mRNA in the pituitary of male rats with hyperthyriodism.

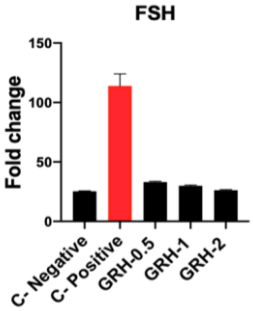

Fig.1B. Effects of ghrelin administration (0.5, 1 or 2 *nmol*) on the Expression of FSH mRNA in the pituitary of male rats with hyperthyriodism.

Histopathological Analysis

Testis

Control rats' testes had normal seminiferous tubule architecture, normal spermatogenesis, and normal spermatogenesis, as illustrated in Fig. 2 ,and leydig cells in the interstitial tissue. While, the testis in hyperthyroidism male rats treated with thyroxine shows histopathological changes including sever damage in the seminiferous tubules walls and leydig cells and the section shows sever blood vessels congestion in Fig.(3). The testis of hyperthyroid male rats treated with ghrelin (0.5nmol/100l saline) on the other hand. In several seminiferous tubules, there was no substantial occupied lesion (SOL) normal spermatogenesis, as demonstrated in Fig (4). Moreover, the testis in hyperthyroidism male rats treated with ghrelin (1nmol/100µl saline) showed an evidence of recovery of normal spermatogenesis with spermatozoa in the lumen of seminiferous tubules as shown in Fig.(5). Finally, the testis in hyperthyroidism male rats treated with ghrelin (2nmol/100µl saline) As illustrated in Fig(.6), the architecture of seminiferous tubules is normal, with normal spermatogenesis and normal interstitial tissue.

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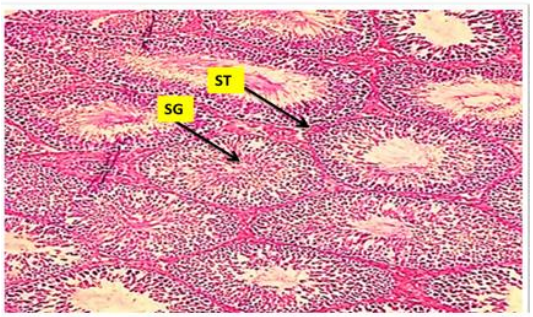

Fig.(2): Histological section for testis in control group showed no occupied lesion in the testis section, normal seminiferous tubules (ST), with normal spermatogenesis (SG). **(H&Estain) 10X.**

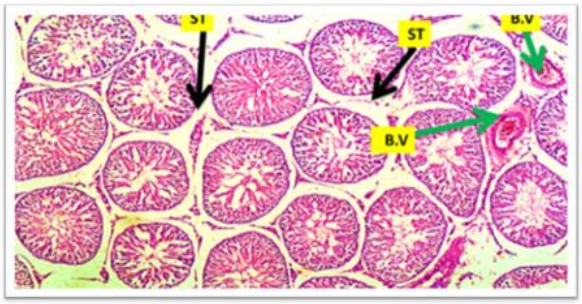

Fig.(3):The histopathological section of testis in hyperthyroidic rat group shows sever damage in the seminiferous tubules walls and leydig cells (Black arrow) and the section shows sever blood vessels congestion (Green arrow). **(H&Estain) 10X.**

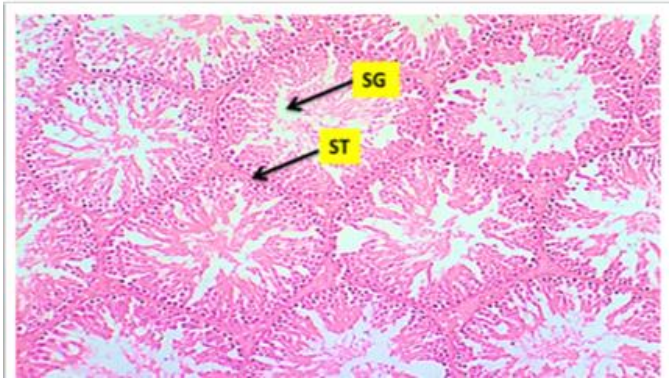

Fig.(4):Histopathological section for testis in hyperthyroidic male rats administrated with GRE(0.5nmol/100µl saline). Showed no occupied lesion (SOL) in the testis section. **(H&Estain) 10X.**

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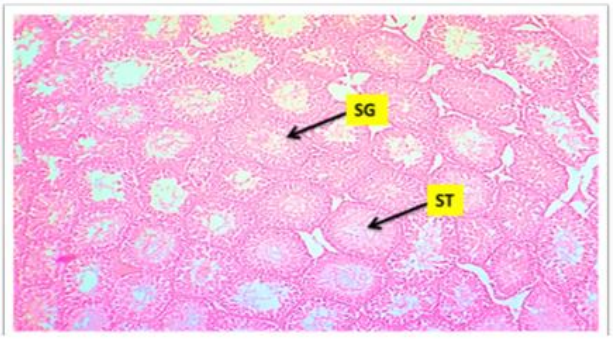

Fig.(5):Histopathological section for testis in hyperthyroidic male rat administrated with GRE(1nmol/100µl saline). Showed no occupied lesion (SOL) in the testis section. **(H&Estain) 10X.**

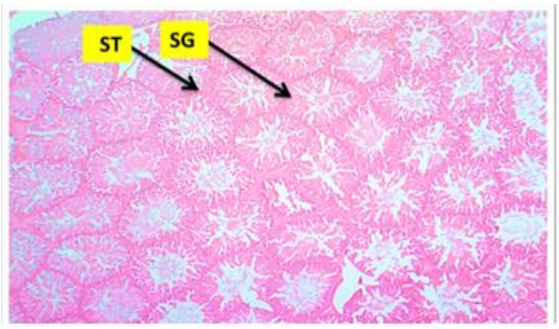

Fig.(6):Histopathological section for testis in hyperthyroidic male rats administrated with GRE(2nmol/100µl saline). Showed no occupied lesion (SOL) in the testis section. **(H&Estain) 10X.**

DISCUSSION

The results of this study's Table (1) show that thyroxine treatment resulted in a considerable elevation of serum T concentration when compared to the control group, These results are in agreement with Krassas et al., (2010)[16]; Singh et al.,(2011)[4], An increase in SHBG has been consistently linked to hyperthyroidism, resulting in higher total testosterone levels in the blood and a lower testosterone metabolic clearance rate. on the other hand, Free testosterone levels frequently remained normal, even if bioavailable testosterone levels were shown to be low in hyperthyroid individuals [17]

In hyperthyroid men, Kumar et al. (2012)[18] found an increase in serum SHBG. SHBG lowers the **T** metabolic clearance rate, which could explain why hyperthyroid males have higher serum total testosterone levels. Despite the elevated SHBG levels, there is enough circulating unbound testosterone accessible, as evidenced by an increase in estimated bio**T**.

According to Kumar et al., (2014)[19], hyperthyroid men's testosterone and estradiol levels were higher, but their progesterone levels were equal to euthyroid controls[19]. Others have found a comparable increase in serum testosterone and estradiol levels [20],[17]. The hyperthyroid group had a considerably higher testosterone to progesterone ratio, implying a higher progesterone to testosterone conversion rate. Normal progesterone levels, despite increased testosterone conversion, indicate that progesterone production has increased.

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Falling serum total cholesterol and LDL-C levels in hyperthyroid males suggest higher serum cholesterol utilization, which might be used to enhance progesterone production[18].

In the hyperthyroidism group, serum FSH and LH concentrations are significantly higher than in the control group, according to the same table. These findings coincide with Zähringer et al., (2000)[21], who note that no definitive pathophysiological mechanism has been identified, despite the fact that disruptions of the H-P-G axis are commonly implicated. The H-P-G axis involves episodic GnRH production in the hypothulmus, which causes pituitary release of LH and FSH, which stimulates the gonads. According to research conducted by Thomas and Reid (1987)[22],gonadotropin levels in hyperthyroidism patients are constantly increasing.

On other hand, the ghrelin adiminstration for 30 days this led to a reduction in the concentration of T, LH and FSH hormones in the treated groups compared to the hyperthyroid group, The endogenous ligand for the growth hormone secretagogue receptor is ghrelin, a peptide hormone produced mostly by the stomach[23]. In another study we conducted, ghrelin levels were reduced in rats with hyperthyroidism. Therefore, one objective of this study was designed to explore the potential effects of ghrelin administration in modulating the adverse effects of hyperthyroidism on testicular function. Ghrelin may regulate networks that control the gonadotrophic axis and gonadal function, among its many functions [24]. These findings correspond with those of Abou Heif et al., (2010)[25], who discovered a substantial inverse association between serum ghrelin and male hormones in rats. Changes in serum testosterone, FSH, and LH were also found to be dependent on changes in serum ghrelin.The inhibitory action of ghrelin on multiple critical enzymes in the steroidogenic pathway could explain the considerable drop in serum testosterone in rats. Human chorionic gonadotrophins and cyclic AMP-induced testosterone production were both reduced by GRE [26].

GRE reduced the frequency of GnRH pulses in rats, which is mediated through NPY and totally inhibited by the NPY-Y5 receptor antagonist. [27], and those who came to the conclusion that GRE inhibits both LH and FSH secretion in both male and female rats as well as humans [28] ,[9], [11].

Tena-Sempere (2007)[29],discovered that persistent GRE treatment resulted in overt reductions in plasma LH and testosterone levels. Yang et al., (2007)[30] and Sirotkin et al., 2008)[31], Massive hyper-ghrelinmia created by the simultaneous rise of endogenous GRE in calorie-restricted animals and exogenous administration of the peptide results in a condition of suppression. This finding is consistent with earlier research indicating that large doses of GRE have a strong inhibitory effect on LH and testosterone release in male rats[8].

In conclusion, an increase in serum ghrelin was found to be inversely related to each of the productive hormones, testosterone, FSH, and LH, suggesting that ghrelin may be one of the hormones responsible for the regulation of the male reproductive axis in hyperthyroidism male rats with negative energy balance. As demonstrated in the figures(1A-1B), the

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mechanism of gene expression also suggests that ghrerlin lowers the concentration of FSH and LH in the pituitary gland.

When all hyperthyroidism groups administered with GRE were compared to the hyperthyroidism group, LH and FSH mRNA expression decreased considerably. In male rats, GRE was also found to have a regulatory role on LH component gene expression. The expression of the LH component gene was reduced when GRE was administered centrally. Many ghrelin fibers have been projected to LH-containing neurons, according to previous neuroanatomical study[32],[33] and GRE secretion inhibited LH production by decreasing Fos induction in LH neurons [33],[34],[35]. GRE inhibitory effects on LH production are consistent with prior research that found ghrelin reduces LH release [36].few studies to date had addressed the suppressive role of ghrelin in LHβ subunit gene expression. in a study conducted by Babaei-Balderlou and Khazali., (2016)[36] antagonist, [D-Lys^3] DLS was employed to determine whether or not the influence of ghrelin on LH expression is mediated through the GHS-R1a. Ghrelin-induced effects on LH expression were inhibited by inhibiting the ghrelin receptor (GHS-R1a) with the antagonist DLS.

Numerous lines of research suggested that GRE has a direct and indirect stimulatory impact on brain areas involved in male sexual behavior regulation[37],[38] ,[35]. The fact that GRE reduces the frequency of LH pulses suggests that ghrelin has an effect on GnRH neurons.

GRE receptors are found in GnRH neurons [39]. GRE has an indirect inhibitory effect on GnRH neurons in addition to its direct action. Two findings back up the idea that ghrelin has an indirect influence on GnRH secreting neurons. Injections of ghrelin into the hypothalamus cause a considerable decrease in both LH secretion and Kiss1 expression[40]. Kisspeptin neurons in mice do not have ghrelin receptors (Smith et al., 2013)[41]. Kiss1 expression is inhibited by ghrelin. GRE inhibitory influence on kiss1R expression could be mediated by NPY neurons that express the proper ghrelin receptor[42]. Kisspeptin mRNA expression was also suppressed by ghrelin, according to Forbes et al., (2009)[43]. As a result, ghrelin injection and dietary restriction-induced hyperghrelinmia both result in a decrease in Kiss1 mRNA levels[43],[44]. Furthermore, an NPY Y5R antagonist bloked by ghrelin led to inhibited GnRH neurons[27]. These findings imply that Kiss1 and NPY-expressing neurons may play a role in GRE inhibitory effect on GnRH neurons[7]. The rate of FSH production is determined by GnRH pulse frequency, since several studies have demonstrated that a decrease in GnRH pulse frequency favors FSH production over LH production[45],[46],[47]. In mice lacking GnRH, serum FSH levels are lowered by 60–90% [48].GnRH injection elevated Fshb mRNA expression four - fold in rats[47], demonstrating that GnRH regulates FSH at the cellular regulation. nduction of immediate early genes (IEGs), such as Jun, Fos, Atf3, and Egr1, by GnRH affects FSH expression. Activator protein-1 (AP-1) is a GnRH-induced IEG product that comprises of several Fos and Jun dimeric isoforms (c-Fos, Fra-1, Fra-2, FosB, c-Jun, JunB, and JunD) [49],[50]. Calcium, calcineurin, and nuclear factor of activated T cells (NFAT), which gives responsiveness to different genes responsible for FSH production, are

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required for GnRH regulation of Jun and Atf3[51]. within the ovine Fshb promoter sequence, Strahl et al. discovered two AP-1 binding sites that are adequate to induce its production independently[52],[53]. In summary, GRE is one of the hormones that suppresses GnRH, which consequently suppresses FSH expression.

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References

[1]. Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia A, and Walter MA.. 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid, (2016) 26(10), 1343-1421.

[2]. AL-Saeed MH, and Al-Rufaei, IA. Ameliorative effect of selenium onreproductive efficiency in adult male rats with thyroid disturbance. Basrah jounral of veterinary reserch(2018)17(1), 21-35.

[3]. Terasawa E. Guerriero KA, and Plant TM.Kisspeptin and puberty in mammals. Kisspeptin signaling in reproductive biology, (2013) 253-273.

[4]. Singh R J, Hamada A, and Agarwal A. Thyroid hormones in male reproduction and fertility. The Open Reproductive Science Journal, (2011). 3(1), 98-10.

[5]. Kojima M, Hosoda H, Matsuo H, and Kangawa K. Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends in Endocrinology & Metabolism, (2001) 12(3), 118-122.

[6]. Holst B, and Schwartz TW. Constitutive ghrelin receptor activity as a signaling set-point in appetite regulation. Trends in pharmacological sciences, (2004). 25(3), 113-117.

[7]. Celik O, Celik N, Aydin S, Aygun BK, Haberal ET, Kuloglu T, Ulas M, Aktun LH, Acet M, and Celik S Ghrelin action on GnRH neurons and pituitary gonadotropes might be mediated by GnIH-GPR147 system. Hormone molecular biology and clinical investigation, (2016). 25(2), 121-128.

[8]. Fernández-Fernández R, Tena-Sempere M, Navarro VM, Barreiro M L, Castellano J M, Aguilar E, and Pinilla L. Effects of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretion in adult female rats: in vivo and in vitro studies. Neuroendocrinology, (2005). 82(5-6), 245-255.

[9]. Vulliemoz NR., Xiao E, Xia-Zhang L, Rivier J, and Ferin M. Astressin B, a nonselective corticotropin-releasing hormone receptor antagonist, prevents the inhibitory effect of ghrelin on luteinizing hormone pulse frequency in the ovariectomized rhesus monkey. Endocrinology, (2008). 149(3), 869-874.

[10]. Müller TD, Nogueiras R, Andermann ML, Andrews ZB, Anker SD, Argente J, and Tschöp MH. Ghrelin. Molecular metabolism, (2015). 4(6), 437-460.

[11]. Lanfranco F, Bonelli L, Baldi M, Me E, Broglio F, and Ghigo E. Acylated ghrelin inhibits spontaneous luteinizing hormone pulsatility and responsiveness to naloxone but not that to gonadotropin-releasing hormone in young men: evidence for a central

inhibitory action of ghrelin on the gonadal axis. The Journal of Clinical Endocrinology & Metabolism, (2008). 93(9), 3633-3639.

[12]. Dutta S, Biswas A, Sengupta P, and Nwagha U. Ghrelin and male reproduction. Asian Pacific Journal of Reproduction, (2019). 8(5), 227.

[13]. Poretti MB, Frautschi C, LuqueE, Bianconi S, Martini AC, Stutz G et al. Reproductive performance of male mice after hypothalamic ghrelin administration. Reproduction, (2018). 156(2), 121-132.

[14]. Wittekind DA, Kratzsch J, Mergl R, Baber R, Witte V, Villringer A, et al. Free triiodothyronine (T3) is negatively associated with fasting ghrelin serum levels in a population sample of euthyroid subjects. Journal of Endocrinological Investigation, (2021). 1-10.

[15]. Ghadhban RF, and Jawad AD. Effects of dexamethasone, estrogen administration on leptin, thyroid, reproductive hormone concentration and lipid profile of female rabbit's serum. Basrah jounral of veterinary reserch , (2013). 12(1) 41-53.

[16]. Krassas GE, Poppe K, and Glinoer D. Thyroid function and human reproductive health. Endocrine reviews, . (2010)31(5), 702-755.

[17]. Abalovich M, Levalle O, Hermes R, Scaglia H, Aranda C, Zylbersztein C, et al. Hypothalamic-pituitary-testicular axis and seminal parameters in hyperthyroid males. Thyroid, . (1999). 9(9), 857-863.

[18]. Kumar A, Dewan R, Suri J, Kohli S, Shekhar S, Dhole B, et al. Abolition of endocrine dimorphism in hyperthyroid males? An argument for the positive feedback effect of hyperoestrogenaemia on LH secretion. Andrologia, (2012) 44(4), 217-225.

[19]. Kumar A, Shekhar S, and Dhole B. Thyroid and male reproduction. Indian Journal of Endocrinology and Metabolism, . (2014) 18(1), 23.

[20]. Hudson RW, and Edwards AL. Testicular function in hyperthyroidism. Journal of andrology, . (1992) 13(2), 117-124.

[21]. Zähringer S, TomovaA, Von Werder K, Brabant G, Kumanov P, and Schopohl J. The influence of hyperthyroidism on the hypothalamic-pituitary-gonadal axis. Experimental and clinical endocrinology & diabetes, (2000). 108(04), 282-289.

[22]. Thomas R, and Reid R.L. Thyroid disease and reproductive dysfunction: a review. Obstetrics and gynecology, (1987). 70(5), 789-798.

[23]. Kojima M, Hosoda H, Nakazato M, and Matsuo H. Kangawa K. Ghrelin: structure and function. Physiol Rev, . (2005) 85, 495-522.

[24]. Gill, R. . (2022). Subalgebras over p-Adic Domains. International Journal on Recent Trends in Life Science and Mathematics, 9(1), 37–46. https://doi.org/10.17762/ijlsm.v9i1.140

[25]. Halem H A, Taylor JE, Dong J Z, Shen Y, Datta R, Abizaid A, et al. A novel growth hormone secretagogue-1a receptor antagonist that blocks ghrelin-induced growth hormone secretion but induces increased body weight gain. Neuroendocrinology, . (2005) 81(5), 339-349.

Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

[26]. Abou Heif H M, Deif MM, and Abdel Aziz HK. Effect of food restriction on ghrelin in adult male rats and its relation to male reproductive hormones. Andrologia, . (2010) 42(2), 97-105.

[27]. Tena-Sempere M, Barreiro ML, Gonzalez LC, Gaytan F, Zhang FP, Caminos JE, et al. Novel expression and functional role of ghrelin in rat testis. Endocrinology, (2002). 143(2), 717-725.

[28]. Lebrethon MC, Aganina A, Fournier M, Gerard A, Parent AS, and Bourguignon JP. Effects of in vivo and in vitro administration of ghrelin, leptin and neuropeptide mediators on pulsatile gonadotrophin-releasing hormone secretion from male rat hypothalamus before and after puberty. Journal of neuroendocrinology, . (2007)19(3), 181-188.

[29]. Fernández-Fernández R, Tena-Sempere M, Roa J, Castellano JM, Navarro VMAguilar E, and Pinilla L. Direct stimulatory effect of ghrelin on pituitary release of LH through a nitric oxide-dependent mechanism that is modulated by estrogen. Reproduction, (2007). 133(6), 1223-1232.

[30]. Tena-Sempere M. Roles of ghrelin and leptin in the control of reproductive function. Neuroendocrinology, (2007). 86(3), 229-241.

[31]. Pawan Kumar Tiwari, Mukesh Kumar Yadav, R. K. G. A. . (2022). Design Simulation and Review of Solar PV Power Forecasting Using Computing Techniques. International Journal on Recent Technologies in Mechanical and Electrical Engineering, 9(5), 18–27. https://doi.org/10.17762/ijrmee.v9i5.370

[32]. Yang H, Youm YH, Nakata C, and Dixit VD. Chronic caloric restriction induces forestomach hypertrophy with enhanced ghrelin levels during aging. Peptides, (2007). 28(10), 1931-1936.

[33]. Sirotkin AV, Chrenková M, Nitrayová S, Patraš P, Darlak K, Valenzuela F, et al. Effects of chronic food restriction and treatments with leptin or ghrelin on different reproductive parameters of male rats. Peptides, (2008) 29(8), 1362-1368.

[34]. Howard AD, Feighner SD, Cully DF, Arena J P, Liberator PA, Rosenblum CI,et al. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science, (1996)273(5277), 974-977.

[35]. Miller DW, Harrison JL, Brown YA, Doyle U, Lindsay A, Adam CL, and Lea RG. Immunohistochemical evidence for an endocrine/paracrine role for ghrelin in the reproductive tissues of sheep. Reproductive Biology and Endocrinology, (2005). 3(1), 1-14.

[36]. Gill, D. R. . (2022). A Study of Framework of Behavioural Driven Development: Methodologies, Advantages, and Challenges. International Journal on Future Revolution in Computer Science &Amp; Communication Engineering, 8(2), 09–12. https://doi.org/10.17762/ijfrcsce.v8i2.2068

[37]. Martini AC, Fernandez-Fernandez R, Tovar S, Navarro VM, Vigo E, Vazquez MJ, et al. Comparative analysis of the effects of ghrelin and unacylated ghrelin on luteinizing hormone secretion in male rats. Endocrinology, (2006)147(5), 2374-2382.

[38]. Dupont J, Maillard V, Coyral-Castel S, Ramé C, and Froment P. Ghrelin in female and male reproduction. International Journal of Peptides. (2010). Volume 2010, Article ID 158102, 8 pages doi:10.1155/2010/158102.

[39]. Babaei-Balderlou F, and Khazali H. Effects of ghrelin on sexual behavior and luteinizing hormone beta-subunit gene expression in male rats. Journal of reproduction & infertility, (2016). 17(2), 88.

[40]. Rissman EF. Behavioral regulation of gonadotropin- releasing hormone. Biol Reprod. (1996).54(2413-9).

[41]. Stoyanovitch AG, Johnson MA, Clifton DK, Steiner RA, and Fraley GS. Galanin-like peptide rescues reproductive function in the diabetic rat. Diabetes, (2005). 54(8), 2471-2476.

[42]. Odilia Gutiérrez, Cesar Padilla, Y Juan Cairo. (2022). Uso de la semilla de Moringa oleífera como clarificador de fluidos biológicos y calidad del agua potable. Nota técnica. Revista Electronica De Veterinaria, 20 - 24. Retrieved from https://www.veterinaria.org/index.php/REDVET/article/view/135

[43]. Farkas I, Vastagh C, Sárvári M, and Liposits Z. Ghrelin decreases firing activity of gonadotropin-releasing hormone (GnRH) neurons in an estrous cycle and endocannabinoid signaling dependent manner. PLoS One, (2013). 8(10), e78178.

[44]. Roa J, García-Galiano D, Castellano JM, Gaytan F, Pinilla L, and Tena-Sempere M. Metabolic control of puberty onset: new players, new mechanisms. Molecular and cellular endocrinology, (2010). 324(1-2), 87-94.

[45]. Smith JT, Reichenbach A, Lemus M, Mani BK, Zigman JM, and Andrews ZB. An eGFP-expressing subpopulation of growth hormone secretagogue receptor cells are distinct from kisspeptin, tyrosine hydroxylase, and RFamide-related peptide neurons in mice. Peptides, (2013). 47, 45-53.

[46]. Grove KL, and Cowley MA. Is ghrelin a signal for the development of metabolic systems?. The Journal of clinical investigation, (2005). 115(12), 3393-3397.

[47]. Forbes S, Li XF, Kinsey-Jones J, and O'Byrne K .Effects of ghrelin on Kisspeptin mRNA expression in the hypothalamic medial preoptic area and pulsatile luteinising hormone secretion in the female rat. Neuroscience letters, . (2009). 460(2), 143-147.

[48]. Frazao R, Lemko HM, da Silva RP, Ratra DV, Lee CE, Williams KW, et al. Estradiol modulates Kiss1 neuronal response to ghrelin. American Journal of Physiology-Endocrinology and Metabolism, (2014). 306(6), E606-E614.

[49]. Haisenleder DJ, Katt JA, Ortolano GA, El-Gewely MR, Duncan JA, Dee C, and Marshall JC. Influence of gonadotropin-releasing hormone pulse amplitude, frequency, and treatment duration on the regulation of luteinizing hormone (LH) subunit messenger ribonucleic acids and LH secretion. Molecular Endocrinology, (1988). 2(4), 338-343.

[50]. Dalkin AC, Haisenleder DJ, Ortolano GA, Ellis TR, and Marshall JC. The frequency of gonadotropin-releasing-hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression. Endocrinology, (1989). 125(2), 917-923.

[51]. Burger LL, Haisenleder DJ, Dalkin AC, and Marshall JC. Regulation of gonadotropin subunit gene transcription. Journal of molecular endocrinology, . (2004) 33(3), 559-584.

Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

[52]. Mason AJ, Hayflick JS, Zoeller RT, Young WS, Phillips HS, Nikolics K, and Seeburg PH. A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the hpg mouse. Science, (1986). 234(4782), 1366-1371.

[53]. Wurmbach E, Yuen T, Ebersole BJ, and Sealfon SC. Gonadotropin-releasing Hormone Receptor-coupled Gene Network Organization* 210. Journal of Biological Chemistry, (2001). 276(50), 47195-47201.

[54]. Kakar SS, Winters SJ, Zacharias W, Miller DM, and Flynn S. Identification of distinct gene expression profiles associated with treatment of LβT2 cells with gonadotropin-releasing hormone agonist using microarray analysis. Gene, (2003). 308, 67-77.

[55]. Binder AK, Grammer JC, Herndon MK, Stanton JD, and Nilson JH. GnRH regulation of Jun and Atf3 requires calcium, calcineurin, and NFAT. Molecular endocrinology, (2012). 26(5), 873-886.

[56]. Strahl BD, Huang HJ, Pedersen NR, Wu JC, Ghosh BR, and Miller WL. Two proximal activating protein-1-binding sites are sufficient to stimulate transcription of the ovine follicle-stimulating hormone-β gene. Endocrinology, (1997). 138(6), 2621-2631.

[57]. Strahl BD, Huang HJ, Sebastian J, Ghosh BR, and Miller WL. Transcriptional activation of the ovine follicle-stimulating hormone β-subunit gene by gonadotropin-releasing hormone: involvement of two activating protein-1-binding sites and protein kinase C. Endocrinology, (1998). 139(11), 4455-4465.