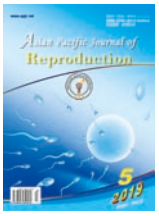

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Ghrelin and male reproduction

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ABSTRACT

Ghrelin is a multifunctional peptide hormone, conventionally known to be secreted by the stomach. The synthesis of ghrelin by the reproductive organs signifies its autocrine and/or paracrine actions upon the gonads. Expression of the functional ghrelin receptor is observed in different levels of the hypothalamic-pituitary-gonadal axis, suggesting its action on hypothalamic secretion of gonadotropin-releasing hormone and the pulsatile secretion of pituitary gonadotropins. It mainly acts to inhibit the secretion of the luteinizing hormone and thereby may also hinder proper testicular functions. This review article aims to provide a concise concept on (a) the characteristics, secretion and mode of actions of ghrelin, and (b) the role of ghrelin as a potential regulator of male reproductive functions. It may act upon the hypothalamic-pituitary-gonadal axis as well as directly regulate key testicular functions such as testosterone secretion, Leydig cell proliferation and expressions of prime functional proteins in the seminiferous tubule. These actions of ghrelin on testicular functions appear to be species-specific. Ghrelin and its versatile biological functions bring to a consensus that further research on ghrelin may establish one of the associations between body energy status with alterations in male reproductive functions.

1. Introduction

Ghrelin, following its discovery about 20 years ago, had been preliminarily designated only as a ligand for the growth hormone secretagogue receptor (GHS-R1a)[1], which has been covered in several potential pioneering reports[2,3]. The name of this multifunctional peptide hormone is derived from the Proto-Indo-European term, 'ghre' that refers to 'grow', and the term, 'relin' indicates that it acts as a releasing substance. Its prime secreting organ is the stomach, but its expressions are also evident in several other tissues, such as in the reproductive tissues where it acts as both endocrine and paracrine factor[4,5]. Besides its conventional role in inducing growth hormone secretion[1] *via* acting upon GHS-R, ghrelin has an array of other biological functions. The most documented functions of ghrelin include food intake[6], gastrointestinal motility[7], sleep[8], regulation of body weight[9], actions on cardiovascular system[10], fine-tuning of cell proliferation[11], anti-inflammatory cytokines secretion[12], as well as male and female reproductive functions[13].

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In the present global scenario, metabolic syndrome and infertility are among the most concerning threats to mankind. It is noteworthy that male fertility has been evidenced to follow a declining trend in the past few decades and most of the male infertility cases, the causatives remain unexplained[14–20]. Therefore, it is needed to emphasize potential endocrine molecules like ghrelin that may partly explain how energy balance associates with alterations in male reproductive functions. Thus, the aim of this article is to review the updated information on the role of ghrelin in the regulations of male reproductive functions.

2. Ghrelin and its receptors

Ghrelin is a peptide hormone which derives from a 28-amino acid peptide, preproghrelin[1]. As per its core origin, it appears in two different forms such as deacylated ghrelin and formacylated ghrelin. This metabolic hormone is indispensable for its post-translational acylation properties on several proteins[1,3]. In most of the vertebrates, the formacylated form of ghrelin is the most preserved one[1].

The major ghrelin receptor is the G-protein coupled receptor, which was first recognized in 1996 in human and pig stomach[21]. Ghrelin receptor, a rhodopsin-like seven transmembrane receptor, contains three more receptor types in it, the receptors for motilin peptides, neurotensin and neuromedin[22]. Molecular analysis of ghrelin in different species, like in rat, pig, dog and human, confirmed that it has 93% to 99% homogeneity in receptor sequence[23]. Prior to the discovery of ghrelin peptides, these receptors were implied to be responsible for growth hormone-related functions and was known as growth hormone secretagogues receptor (GHS-R1a)[21]. The role of ghrelin receptors in growth hormone production is still a matter of debate, owing to several contradictory reports[24]. Diverse other varieties of growth hormone receptor have also been identified, which also include GHS-R1b[21]. Like GHS-R1a receptor, GHS-R1b receptor is also functionally unproductive to stimulate the synthesis of ghrelin or growth hormone secretagogues. It has been observed that mRNA of GHS-R1 receptor is predominantly present in the pituitary[21] and brain cells of both mammals and non-mammals[25,26]. Yet, the same receptor is also present in several other organs of the human body, including thyroid gland, heart, pancreas, ovary, testes and adrenal gland[27].

3. Physiological actions of ghrelin

Till date, several studies have documented the multivariate functions of ghrelin[5,28]. As the primary source of ghrelin is stomach, it contributes about 75% of total circulating ghrelin[2,5]. Other major sources of this metabolic hormone include small intestine, placenta, kidney, and lungs[5,28]. In particular, ghrelin is quite ubiquitous in the gastrointestinal tract, and this local molecule has the ability to regulate the functions of the paracrine/autocrine glands[28,29]. Though ghrelin is a peripheral secretion of growth hormone secretagogues, it has been recognized as a potent endocrine molecule which is functionally active both *in vivo* and *in vitro*. The actions of growth hormone secretagogues have been established in pituitary and hypothalamus. On the other hand, ghrelin has been observed to regulate the functions of growth hormone-releasing hormone and somatostatin system[29]. Moreover, ghrelin is also responsible for neuroendocrine regulations, including the modulations of corticotropic, gonadotropic and lactotropic axes[30]. Subsequently, it has been recorded that the cloned ghrelin acts on the orexigenic pathway in the hypothalamus, which in turn controls numerous food-intake related neuropeptides, including orexins, neuropeptide-Y and agouti-related protein[5]. Ghrelin release from the gastrointestinal system increases following the food deprivation phase which is suggested as a molecular gesture for the maintenance of energy homeostasis[31]. The long term regulation of ghrelin secretion with alterations in body weight has already been established[32]. Along with the central functions of ghrelin, the growing evidences indicate that ghrelin is capable to control a wide range of metabolic functions in the human body, such as regulation of gastric movements, gastric acid secretion, several distinct effects on cardiovascular functions, regulation of glucose metabolism and insulin secretion from the pancreas, as well as the regulation of the cellular proliferation in numerous cancer types[5,28].

4. Ghrelin and male reproduction

Although conventionally ghrelin is known mostly for its role in regulation of feeding behavior and energy status *via* its action upon the central nervous system, several peripheral functions of this hormones are surfacing with recent advent in research which include its role in reproductive tissues. Since the last decades, ghrelin has been suggested as a potential modulator of reproductive functions. Ghrelin finds relevance in neuroendocrine integrators for concomitant control of energy balance and reproductive functions, along with other versatile metabolic and neuro-modulatory hormones such as leptin[33], adiponectin, obestatin, orexin, thyroid[34–36], melatonin[37] various hormones in the joint control of energy balance and reproduction[38,39]. The available data suggest that leptin and ghrelin have certain common relevant functional characteristics since both these hormones function as peripheral factors to primarily regulate the food intake behavior and the somatotropic axis. Leptin and ghrelin mediate opposite actions in regards to body weight homeostasis, such that leptin functions as satiety factor to signal for energy sufficiency while ghrelin being an orexigenic factor signal to convey energy insufficiency[40]. There are a substantial number of studies that confirm the potential role of leptin in modulating the reproductive functions, in contrast to which reports on ghrelin actions upon the reproductive functions are scanty. However, some studies, both *in vivo* and *in vitro* have demonstrated the actions of ghrelin upon the varying regulatory levels of hypothalamic-pituitary-gonadal axis as well as direct effects on gonadal functions *via* locally produced ghrelin.

4.1. Ghrelin and male reproductive axis

Emergent substantiation hints that ghrelin can act on a diverse level of reproductive functions along with the gonadal axis. Although the available information related to ghrelin is still inadequate. The categorization, its mode of action and its role in controlling the reproductive functions are largely uncharted. The relation with gonadotropin discharge by ghrelin is not explored yet. So far, the available information reveals that in ovariectomized female rats, central supervision of ghrelin leads to low production of luteinizing hormone (LH)[9]. Another study also confirms that ghrelin can be able to slow down the LH production in the prepubertal and gonadectomized male rats respectively. In contrast, in the female rats, the follicle-stimulating hormone (FSH) level was not affected[9]. Fascinatingly, ghrelin has direct effects on both the gonadotropic hormones at the pituitary level and differs in the response to LH-releasing hormone. The LH production was reduced while the FSH secretion was improved which is noticed in various experimental and physiological studies. Along with these, ghrelin also linked in regulating the prolactin production which has been documented in humans[30]. In contrast, studies with the rat model ghrelin show an inhibitory effect on the prolactin production in the early pubertal male and females, which acts mainly through the hypothalamic axis[41]. Actual cause of these different actions of ghrelin in rat and human models are unexplained. The most probable reasons of ghrelin's inhibitory effect on prolactin secretion lie on the maturation period. As reported by Pinilla and Tena-Sempere (unpublished data), chronic utilization of ghrelin to matured cyclic rats improves blood prolactin levels. Practically, the ghrelin-dependent prolactin production in early pubertal rat was reduced and similar trend was also observed in humans. This relationship waits for the further confirmation.

4.2. Ghrelin system in testis

The proven functions of leptin upon testis[41] made it convenient to speculate the actions of ghrelin upon testicular functions. It is evident that ghrelin has inhibitory effects upon the pulsatile LH secretion for anterior pituitary[42]. Furthermore, a testis-specific ghrelin gene-derived transcript has also been identified in murine model[43], along with evidence of ghrelin gene expressions in human testis[27,41]. Studies in rats showed expressions of ghrelin in Leydig cells at advanced maturational stages, irrespective of the origin[41]. Ghrelin expressions have also been demonstrated in the human testicular cells which have been found to be higher in the Leydig cells and at lower levels in the Sertoli cells[44]. It has been suggested that testicular ghrelin expressions may partially be regulated by pituitary LH, as may also be hinted by the presence of testicular LH/chorionic gonadotropin receptors in the Leydig cells as well as testicular expression of ghrelin receptor (GHS-R1a) in both rodents and human[41,44]. Studies have revealed that ghrelin along with the pituitary FSH is responsible for the regulation of the testicular *GHS-R* gene expressions. Thus, homologous and heterologous hormonal signals regulate the testicular sensitivity towards ghrelin actions, suggesting that ghrelin has well-coordinated direct actions upon testicular functions.

Inhibitory effects of ghrelin upon testosterone secretion have been put forth by some studies. Since ghrelin downregulates both human choriogonadotropin (hCG) and cyclic adenosine monophosphate-induced secretion of testosterone, it may be speculated that these actions occur *via* intracellular signaling that is beyond cyclic adenosine monophosphate production. Ghrelin inhibition over testosterone secretion has been linked to decline in hCG-induced mRNAs expressions of some key steroidogenic factors. These include 3β-hydroxyl steroid dehydrogenase and testis-specific17β-hydroxyl steroid dehydrogenase type ⅢⅠ, steroid acute regulatory protein, and the enzymes P450[41]. Thus, there is a conflicting hypothesis where studies show inhibitory effects of ghrelin on hCG induced testosterone secretion and also some studies revealed that pituitary LH, which is a conventional stimulant hormone for testosterone secretion, actually stimulates testicular ghrelin expressions[41]. It may be interpreted in a way that ghrelin may act as a local testicular regulator in the fine-tuning of the LH mediated steroidogenic functions, by auto-limiting the responses of testicular cells towards pituitary gonadotropin in secreting testosterone. *In vivo* experiments in rats have shown that impact of ghrelin upon plasma levels of testosterone is dependent upon the nutritional status, such that in fed rats, administration of ghrelin reduced testicular mass without affecting plasma levels of testosterone, while food-restricted rats were characterized by high endogenous ghrelin levels, and further chronic ghrelin administration had reduced plasma testosterone[45]. Thus, high ghrelin levels may alter the male reproductive axis in conditions of energy deficit.

Besides the steroidogenic regulations of ghrelin, its receptor (GHS-R1a) expressions in the testicular tubular compartments may indicate its direct actions on the functions of seminiferous tubule functions. Ghrelin has been suggested to have inhibitory effects on the gene expression for stem cell factor, which is secreted by the Sertoli cells as a prime paracrine inducer of germ cell development, aiding survival of germ cells at different stages of spermatogenic cycle in the seminiferous epithelium, as well as playing vital roles in development and survival of the Leydig cells[46]. Thus, inhibition of ghrelin upon tubular stem cell factor expressions may suggest the role of ghrelin in regulation of Leydig cell proliferation and spermatogenesis.

4.3. Ghrelin on testicular cell proliferation

Ghrelin administration has shown to be associated with inhibited immature Leydig cells proliferations both at the time of pubertal development and following ethylene dimethanesulfonate mediated specific ablation of already existing mature Leydig cells[47]. Expressions of ghrelin and its receptors have been found in testicular tumors. Ghrelin expressions in Leydig tumor cells and not the expressions of its receptors in this site may be associated with the degree of Leydig cell differentiation[44].

4.4. Ghrelin and semen quality

The study by Kheradmand *et al*[48] claimed that chronic administration of ghrelin in rats could enhance sperm membrane integrity with no alterations in sperm motility and concentration. However, when ghrelin was administered in chronic doses with the rat spermatozoa being incubated at 37 ℃, significantly higher sperm motility, progressive forward movement of the sperm and improved sperm membrane integrity were reported. It had been suggested that these ameliorating effects of ghrelin may be attributed to its antioxidative effects upon the spermatozoa, particularly on the sperm plasma membrane[49]. In 2012, Lukaszyk *et al*[50] had shown ghrelin receptor (GHSR-1a) expressions in rat spermatids and epididymal spermatozoa. *In vitro* studies by the same group had also demonstrated actions of ghrelin on the spermatozoa. These studies showed ghrelin receptor expressions in spermatozoa, along with its influence upon intracellular concentration of calcium ion and sperm motility, suggesting participation of ghrelin in the intracellular signaling of spermatozoa needed for its motility.

4.5. Ghrelin and sexual behavior

Ghrelin has been shown to play major roles in mediating normal sexual behavior *via* dopaminergic actions in male murine model. In 2016, Babaei-Balderlou and Khazali[51] had studied the copulatory actions of ghrelin and a specific ghrelin antagonist, [D-Lys3]-GHRP-6 (DLS). They had observed the effects of different doses of ghrelin as well as its antagonist (through the central nervous system on sexual behavior and *LHβ* subunit gene expression in male rats. They revealed that ghrelin in a dose-dependent manner reduced the sexual potency and desire as well as delayed the onset of intercourse. Administration of [D-Lys3]-GHRP-6 (DLS) had antagonized the supposed inhibitory actions of ghrelin upon sexual behavior, which included indirect mode of action *via* inhibiting LH synthesis. Yet another study by Egecioglu *et al* in 2016[52] had reported that administration of ghrelin could increase the sexual motivation in male mice and sexual behavior with female mice in oestrus. In separate experiments, this group had reported that ghrelin and its receptor (GHS-R1A) antagonism had no impact upon the duration in female bedding as determined through the androgen-dependent bedding test[53]. Prieto-Garcia *et al*[54] had shown that administration of JMV2959, a ghrelin receptor (GHSR-1A) antagonist, locally in the laterodorsal tegmental area or in the ventral tegmental area decreased the preference of male mice for female mice with reduced mounting frequency as well as the duration of mounting, and in sexually naïve male mice, the administration prolonged the latency period to mount. Whereas, administration of ghrelin into the ventral tegmental area or laterodorsal tegmental area significantly enhanced the mounting frequency and duration of mounts along with reduced latency to mount. Moreover, when ghrelin was administered into the laterodorsal tegmental area, increase in the preference in sexually naïve male mice for female mice was evident. It had been reported that systemic ghrelin administration in males exposed to sexual interaction increases the turnover of dopamine in the ventral tegmental area, while the opposite was observed with JMV2959 administration. Thus, it may be suggested that ghrelin and its receptor in ventral tegmental area and laterodorsal tegmental area are needed for normal male sexual behavior and that ghrelin may mediate its action upon male sexual behavior *via* pathways involving the dopamine neurotransmission. However, there are very less studies on the impacts of ghrelin upon male reproductive behavior and further research interventions are required to postulate possible direct ghrelin effects on specific components of central nervous system in regulation of male sexual behavior.

5. Ghrelin and male hypogonadism

Endocrine glands mostly operate following a sexually dimorphic phenomenon, which even includes the secretion of metabolic hormones such as leptin[55]. Ghrelin secretion seems to follow the same pattern being reported higher in women than in men[56]. In men, it has been reported that ghrelin levels positively correlate with that of testosterone (total or bioavailable). In fact, decreased ghrelin levels were associated with hypogonadism in male as compared with normal-weight control men or weight-matched eugonadal men[57,58]. Ghrelin also showed negative correlation with insulin resistance[59]. In addition, testosterone administration in hypogonadal men could restore the ghrelin levels to normal[60]. Thus, androgens may have direct effects on ghrelin expression and synthesis, or may have indirect effects over its secretion *via* regulation of free fatty acid metabolism[61], while the latter mechanism is still controversial[62].

Since there are reports suggesting detectable ghrelin concentration in the semen of normospermic and dyspermic men, it may be hypothesized that ghrelin may have regulatory roles in spermatogenesis[63]. However, the exact role of ghrelin and its mechanism of actions in the regulation of spermatogenesis and maintenance of sperm vitality are not completely known. Given the possible potential effects of ghrelin upon male fertility, further studies are encouraged to unveil the specific actions of ghrelin in male reproductive functions.

6. Conclusions

The present review article suggests that ghrelin has a significant role in the regulation of key testicular functions such as testosterone secretion, Leydig cell proliferation as well as gene expressions of prime functional proteins in the seminiferous tubule. Its testicular expressions are stimulated by pituitary LH, and it may act as a local regulator of the testicular functions mediated by LH[64]. Ghrelin expressions in testicular Leydig cells rely upon the degree of cell differentiation and have been found in the highest levels in the maturation advanced stages, irrespective of the fetal or adult origin[4]. It is shown that mature Leydig cells express both ghrelin and its functional receptor, but these cells have almost no proliferative

activities[41]. Whereas, proliferating progenitors of Leydig cell and Leydig tumors in human which are poorly differentiated are devoid of ghrelin immunoreactivity[44,64]. Finally, ghrelin receptor expressions in the seminiferous tubules are suggestive of its potential actions upon seminiferous epithelium. This is also evident that ghrelin regulates spermatogenesis by its inhibitory effects on tubular stem cell factor gene expressions[46]. The role of ghrelin the integration of energy balance and reproduction may involve multifactorial mechanisms which need thorough investigations. There are several open questions regarding the possible reproductive functions of ghrelin.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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