# The Effect of Kisspeptin on Male Rat Reproductive System

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## Abstract

**Background**: Kisspeptin (KP), a peptide secreted by the hypothalamic neurons, is a critical regulator of reproduction and puberty but its role in the regulation of gonadal maturation in sexually immature males is elusive.
**Objectives:** to investigate the effects of single dose of administration of KP on gonadotropins and testosterone release and maturation of immature rat male gonads.
**Materials & methods**: Kisspeptin-10 was administered intraperitoneally at different dosage concentrations (50 and 12.5 nmol) to 45 days old prepubertal male rats, as single dose. Plasma LH, FSH and testosterone concentrations were measured. Spermatogenesis was histologically studied.
**Results**: At the end of the treatments plasma LH increased significantly after 15 minutes, 6 hours and 24 hours. Testosterone concentration increased significantly after 15 minutes but decreased after 6 and 24 hours.
Histologically, there was an evident degeneration of seminiferous tubules showing tubular necrosis, multinucleated giant cell formation, intratubular vacuolization, widened lumen and deshaped germ cells. Marked microscopic changes characterized by enlarged intratubular spaces, degenerative tissues filling tubular lumen and disrupted germ cells were noticeable.
**Conclusion**: Pharmacological doses of kisspeptin-10 can cause induction of LH, FSH and testosterone and cause development of the spermatogonia but at high doses it causes testicular degeneration (dose dependent) in the prepubertal male rat.

**Keywords**: Kisspeptin, Hypothalamic-pituitary-gonadal axis, Spermatogenesis.

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## Introduction

Maturation and initiation of reproductive function in animal is a complex process. It requires the interaction of forebrain, pituitary and gonads. The onset of puberty is triggered by impaired sensitivity of certain hypothalamic neurons to the small quantity of the sex hormones that are secreted by the supra-renal cortex. This will lead to the activation of neurons in the hypothalamus that produce gonadotropin releasing hormone (GnRH) [1].

The amplified secretion of GnRH evokes the release of luteinizing hormone (LH) and follicular stimulating hormone (FSH), which then awakens the gonads [2, 3].

Although this cascade has been well characterized for many mammalian species; the molecular and cellular events at the central level that actually initiate this process, remain unclear. Recently, it was shown that dysfunctional or deletional mutations

in the gene encoding the G protein-coupled receptor, GPR54, cause hypogonadotropic hypogonadism [4, 5].

Very soon, kisspeptin (KP), a product of the Kiss1 gene was found to be the endogenous ligand of GPR54[5, 6]. The gene for kisspeptin encodes a 145-amino-acid precursor peptide that is proteolytically cleaved into a family of peptides collectively referred to as kisspeptins, the most abundant of which is an amidated 54-amino-acid protein, kisspeptin-54. Further cleaving gives rise to shorter products namely kisspeptin-14, kisspeptin-13 and kisspeptin-10[7,8,9]. All of these kisspeptins are reported to have a similar affinity and efficacy in vitro at GPR54[7].

Both kisspeptin and GPR54 (now called Kiss1r[10]) transcripts are expressed in a variety of tissues including the placenta, several brain regions, spinal cord, testes, ovaries, liver, small intestine, pancreas, heart, kidney and uterus[11]. Expression of both the Kiss1 and Kiss1r mRNA is regulated developmentally as well as hormonally in the hypothalamus, with a sharp increase at prepubertal age in both male and female rats, changes throughout the estrous cycle in adult females, and increases after gonadectomy; an effect that is prevented by sex steroid replacement[12,13].

Kisspeptins are likely to be the most potent elicitors of GnRH/gonadotropin secretion[11,14] however, little is known about the effects of kisspeptin on testicular tissue and the process of spermatogenesis. Thompson[15,16] showed that chronic administration of kisspeptin to adult rats causes testicular degeneration, an effect that can be prevented by pretreatment with cetrorelix, a GnRH antagonist. This feature, together with its pivotal role in the control of the gonadotropic axis, makes this peptide a suitable target for pharmacological intervention of the reproductive system dysfunction.

## Material and methods

*Animals*

Prepubertal male rats of Albino strain, aged (45 days) postnatal. The animals were obtained from the Animal House at the National Center for Drug Control and Research / Ministry of Health. All the rats were housed in the animal house of The High Institute of Infertility Diagnosis and Assistant Reproductive Technology/Al-Nahrain University in box cages of opaque plastic measuring (length: 42, width: 25, height: 20) cm. Floors of cages were covered with soft crushed wood shaving. Temperature was maintained between (21–23°C) with a photoperiod (13+2) hours. They received free diet (Billet) with ad libitum access to food and water. To minimize stress due to crowding, five rats were housed per cage.

Animals of albino rats are divided into two experimental sets of treatment groups.

The first set is divided in to two groups:

Group I are pre-pubertal (45-50 days) animals (n=20 in each) treated with 50 nmol/kg (64.75 µg/kg) kisspeptin-10 and Group II (control) are pre-pubertal animals (45-50 days) (n=20 in each) treated with the solvent that is used in preparation of kisspeptin stock solution (5% DMSO).

The second set is divided in to two groups:

Group III are pre-pubertal (45-50 days) animals (n=30 in each) treated with 12.5 nmol/kg (16.18 µg / kg) kisspeptin-10 and Group IV (control) are pre-pubertal animals (45-50 days) (n=30 in each) treated with the solvent that is used in preparation

of kisspeptin stock solution (5% DMSO).

*Drug used*

Kisspeptin-10, metastin (45-54), was purchased from SIGMA. Kisspeptin is a hygroscopic white powder. Kisspeptin-10 is soluble in dimethylesulphoxide (DMSO) and stable under recommended storage conditions (-20ºc).

**Procedure**

*Thawing*

Kisspeptin-10 was dissolved and stored according to the manufacturer's recommendations in 5% (DMSO). To start with, it was firstly brought to room temperature by thawing and then diluted to a working concentration.

## Drug administration

Male rats were given a single intraperitonial (i.p.) injection of kisspeptin-10 at a concentration of (50 nmol/kg body weight: 64.75 µg / kg body weight and 12.5 nmol/kg body weight: 16.18 µg / kg body weight) for the tested group animals and 5% DMSO in saline (saline-DMSO) for the control group animals. The animals were left freely moving in the animal cages.

*Tests performed*

Blood samples (1 ml each) were drawn from the heart (under halothane inhalation anesthesia) at zero time (basal line), 15 minutes, 6 hours and 24 hours intervals after the administration of the kisspeptin-10 or saline injection.

The blood samples were transferred into centrifuge tubes and were immediately placed in an incubator at 37ºC for 15 minutes then serum samples were obtained after centrifugation at 2500 rpm for 10 minutes and stored at -20ºC until assayed for: LH, FSH and testosterone. The animals then were killed by anesthesia (under deep halothane inhalation anesthesia. The testes were quickly removed, immersed and fixed in buffered 10% formalin and subsequently stored in 70% ethanol until processing and embedding in paraffin. Then the testes were serially sectioned and stained with hematoxylin and eosin (H&E) for histology.

## Result

*Effect on plasma gonadotropins and testosterone*

Plasma LH concentrations increased significantly at both 50 and 12.5 nmol after 15 minutes, 6 hours and 24 hours (table 1) and (Fig 1). Plasma FSH levels increased significantly in all treatment groups (table 2) and (Fig 2). Plasma testosterone concentration was significantly increased at both 50 nmol and 12.5 nmol doses after 15 minutes as compared to base line and an increased was evident after 6 hours and 24 hours at both kisspeptin-10 doses (insignificant at 12.5 nmol; (table 3) and ( Fig 3).

Table 1: The effect of kisspeptin-10 on plasma LH:

| | Agents | n | Base line | 15 min | 6 hrs | 24 hrs |
|---|---|---|---|---|---|---|
| Set 1 | KP 50 nmol (Group I ) | 20 | 0.29(0.06) | 0.87(0.16)** | 1.19(0.26)** | 0.95(0.20)** |
| | DMSO (Group II) | | 0.3(0.063) | 0.33(0.06)# | 0.32(0.05)# | 0.35(0.05)# |
| Set 2 | KP 12.5 nmol (Group III ) | 30 | 0.39(0.05) | 1.06(0.12)** | 1.32(0.14)** | 2.05(0.16)** |
| | DMSO (Group IV) | | 0.32(0.03) | 0.33(0.039)# | 0.32(0.037)# | 0.36(0.14)# |

Data presented as mean ± S.E.M, n represents the number of animals; # P > 0.05, ** P < 0.001.
Comparisons of values were made with their respective base line values using paired t –test.

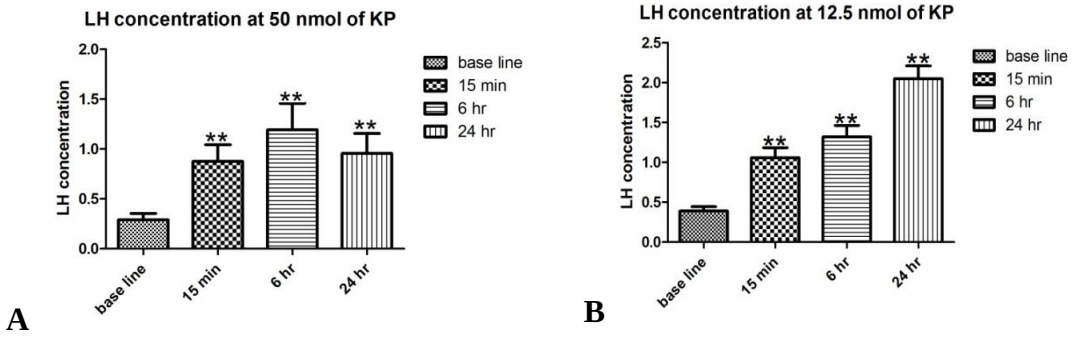


**Figure 1**: The effect of single, i.p. administration of 50 nmol (A) and 12.5 nmol (B) KP-10 in prepubertal male Albino rats on plasma levels of LH at 15 minutes, 6 hours , and 24 hours post injection *vs.* base line. All data are expressed as mean ± SEM. Significance is indicated by ** P<0.001.

Table 2: The effect of kisspeptin-10 on plasma FSH:

| | Agents | n | Base line | 15 min | 6 hrs | 24 hrs |
|---|---|---|---|---|---|---|
| Set 1 | KP 50 nmol (Group I ) | 20 | 7.05(0.42) | 20.7(2.68)** | 18.2(1.95)** | 20.3(3.07)** |
| | DMSO (Group II) | | 6.87(0.4) | 6.92(0.3)# | 6.8(0.4)# | 6.96(0.38)# |
| Set 2 | KP 12.5 nmol (Group III ) | 30 | 6.86(0.41) | 15.36(1.22)** | 15.66(1.02)** | 16.66(2.54)** |
| | DMSO (Group IV) | | 6.87(0.38) | 6.91(0.39)# | 6.89(0.38)# | 6.96(0.38)# |

Data presented as mean ± S.E.M, n represent the number of animals; # P > 0.05, ** P < 0.001.

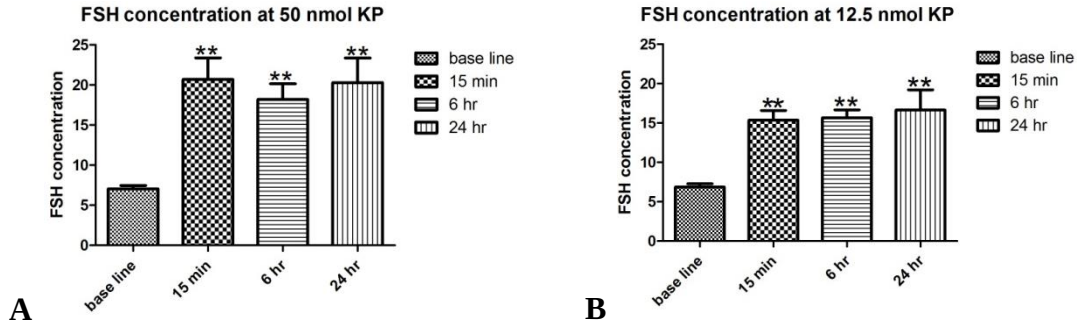


**Figure 2**: The effect of single i.p. administration of 50 nmol (A) and 12.5 nmol (B) KP-10 in prepubertal male Albino rats on plasma levels of FSH at 15 minutes, 6 hours and 24 hours post injection *vs.* base line. All data are expressed as mean ± SEM. Significance is indicated by ** P<0.001.

Table 3: The effect of kisspeptin-10 on plasma testosterone:

| | Agents | n | Base line | 15 min | 6 hrs | 24 hrs |
|---|---|---|---|---|---|---|
| Set 1 | KP 50 nmol (Group I ) | 20 | 0.5(0.05) | 2.2(0.53) ** | 1.1(0.18) ** | 1.1(0.43)* |
| | DMSO (Group II) | | 0.5(0.053) | 0.56(0.05)# | 0.54(0.05)# | 0.49(0.05)# |
| Set 2 | KP 12.5 nmol (Group III ) | 30 | 0.43(0.04) | 0.93(0.15) ** | 0.82(0.11) ** | 0.6(0.16)# |
| | DMSO (Group IV) | | 0.51(0.05) | 0.53(0.048)# | 0.55(0.04)# | 0.54(0.04)# |

Data presented as mean ± S.E.M, n represent the number of animals; # P > 0.05, * P < 0.05 ** P < 0.001.

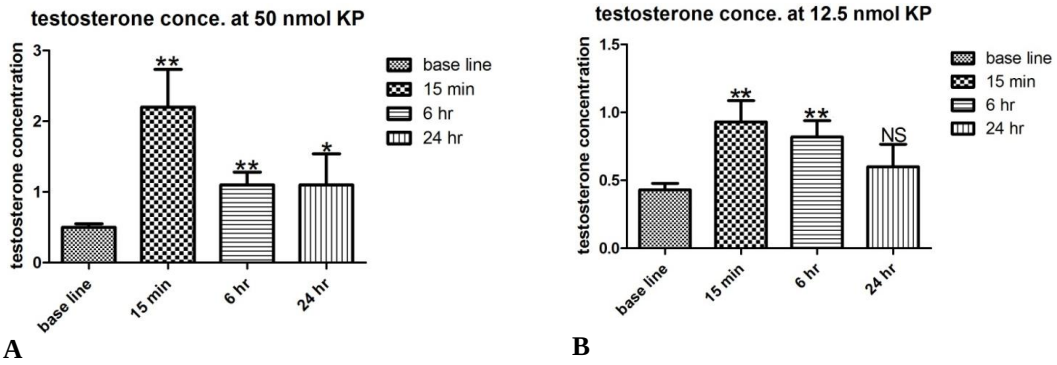


**Figure 3**: The effect of single, i.p. administration of 50 nmol (A) and 12.5 nmol (B) KP-10 in prepubertal male Albino rats on plasma levels of testosterone at 15 minutes, 6 hours, and 24 hours post injection *vs*. base line. All data are expressed as mean ± SEM. Significance is indicated by * P<0.05, ** P<0.001.

*Histomorphology of seminiferous tubules*

Light microscopic examination of testicular sections from saline treated control rats showed normal seminiferous tubules with intact germinal epithelium, spermatogonia were evenly spaced (Fig 4) (A).

At 12.5 nmol kisspeptin dose, degeneration of seminiferous tubules was evident with intraepithelial vacuolizations found in some tubules. Germ cells were regressed, atrophied and showed necrosis. Few necrotic tissues were found in tubular lumen (fig 4) (B). At 50 nmol/kg kisspeptin dose, degeneration of seminiferous tubules was increased further (show marked cellular degeneration). Abnormal cell associations and germ cell maturation arrest were evident. Intraepithelial spaces were increased and multinucleated giant cells were frequent occurrences. Germ cells were regressed, atrophied and showed necrosis. Spermatogonial cells were highly regressed and atrophied. Spermatogonia were regressed and hyperchromatic. Seminiferous tubular lumen was filled with necrotic tissue. The germinal epithelium was irregular shaped. Intratubular spaces were evident. Development of spermatogonia is evident but the cells are atrophied and hyperchromatic (Fig-4)(C).

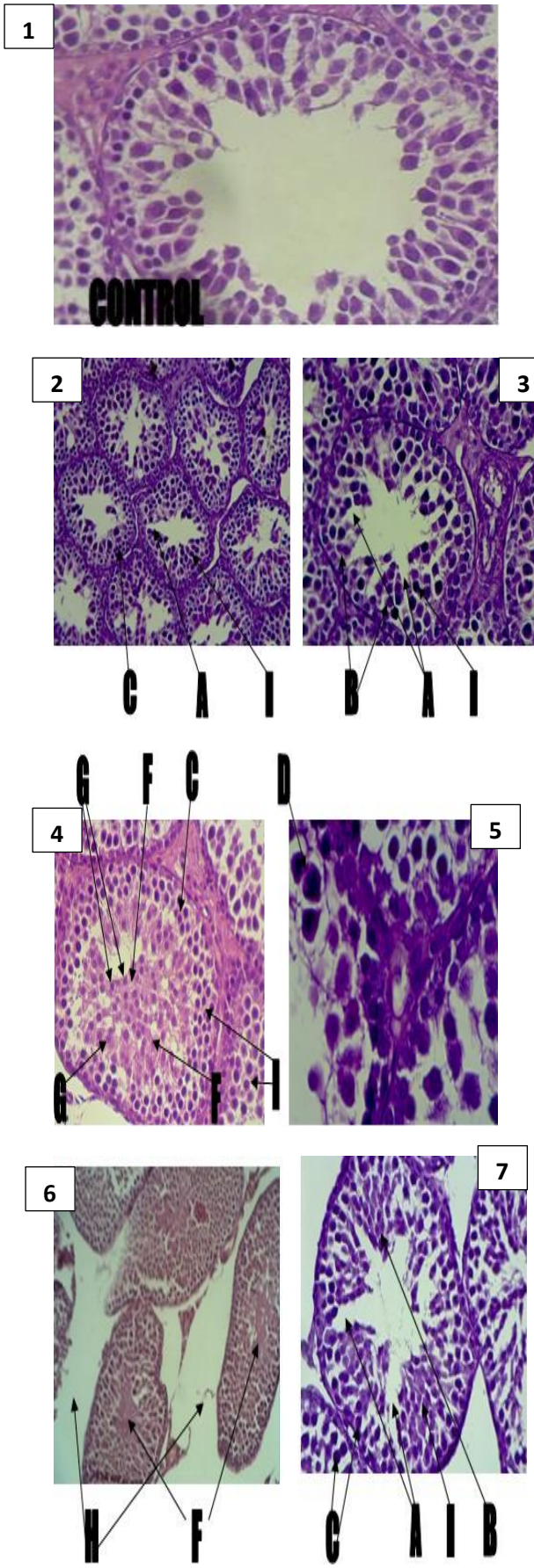


Figure 4: Photomicrographs of seminiferous tubules of prepubertal rats treated with kisspeptin showing cellular alterations. (1): Light microscopic examination of testicular sections from saline treated control rats showed normal seminiferous tubules with intact germinal epithelium. spermatogonia were evenly spaced. (2, 3): At 12.5 nmol/kg kisspeptin dose, seminiferous tubules were deshaped and appeared degenerated. Tubular lumen contained little necrotic tissue. Degeneration of seminiferous tubules was evident with intraepithelial spaces found in the tubules (A). Germ cells were regressed, atrophied and showed necrosis (B). Spermatogonia were regressed and hyperchromatic (C). (4, 5, 6, and 7): At 50 nmol/kg kisspeptin dose, degeneration of seminiferous tubules was increased further (show marked cellular degeneration). Abnormal cell associations and germ cell maturation arrest were evident. Intraepithelial spaces (A) were increased and multinucleated giant cells (D) were frequent occurrences. Germ cells were regressed, atrophied and showed necrosis (B). Spermatogonial cells were highly regressed and atrophied. Spermatogonia were regressed and hyperchromatic (C). Seminiferous tubular lumen was filled with necrotic tissue (F). The germinal epithelium was irregular shaped (G). Intratubular spaces were evident (H). Development of spermatogonia is evident but the cells are atrophied and hyperchromatic (I).

## Discussion

Over the last few years, a lot of attention has been made for the role of kisspeptin in mammalian reproduction[4,5,17,18,19]. It regulates the secretion of pituitary gonadotrophines, which control the level of sex steroid hormones [20,21,22].

In this study we concentrate our work on the first kisspeptin effect (initiation of puberty and maturation of the gonads) in Albino rats at pre-pubertal stage.

In the first set of experiments of the present study, our findings are in agreement with those reported by Thompson[15], who found that the levels of LH, FSH and testosterone were increased, but that of the latter was decreased after 12 days. Also, he found that testicular degeneration was apparent after 13 days. Although, Thompson used kisspeptin-54 and he administered kisspeptin subcutaneously for 13 days continuously, whereas, in the present study we used single dose of kisspeptin-10 intraperitonially. It is suggested that kisspeptin at high dose and not only the prolonged administration produces testicular degeneration as a result of changes in LH.

Our data suggests that the use of kisspeptin at 12.5 nmol concentration cause an early increase in LH and FSH after 15 minutes from time of kisspeptin injection, and this continues up to 24 hours. These findings are in agreement with Tovar and Thomson results[16, 23], who found that the levels of LH and FSH were increased after 15 minutes from the intravenous (i.v.) administration of KISSPEPTIN-10 and they suggested that kisspeptin has a stimulatory effect on the release of LH and FSH.

This LH surge which was induced by kisspeptin injection was associated by an increase in the testosterone level after 15 minutes followed by a decrease in testosterone after 6 and 24 hours from the time of kisspeptin injection; but during the whole period (15 minutes to 24 hours) the level of testosterone was always above the initial value. These findings support the idea that kisspeptin is a stimulatory factor for the process of reproduction through HPG axis and it may give a clue that the maturation process is induced initially by an increase in the basal secretion of kisspeptin[24]. This conclusion may need support by a further study to measure kisspeptin level around pubertal period.

The development and maturation of testes has been evaluated by our histological data. There are signs of maturation of spermatogonia, but in the same histological figure there are pieces of evidence of degeneration of seminiferous tubules (increased intraepithelial spaces, atrophied and necrosis of some epithelial cells and hyperchromatic spermatogonia) which may suggest an early evidence of degenerative process in the seminiferous tubules. This finding has been suggested by previous studies[16,24].

In the present study single i.p. administration of 50 or 12.5 nmol kisspeptin-10 led to a dramatic increase in plasma LH, FSH and testosterone at 15 minutes, 6 hours, and 24 hours in comparison to the base line level but the increase at 6 hours and 24 hours was not as high as seen in 15 minutes. These results may suggest that, there is a positive feedback mechanism to increase plasma LH as a result (secondary) to testicular degeneration which in turn decreases plasma testosterone. A decrease in the testosterone after 24 hours appears to be due to the down regulation of LH

secretion implicating active suppression of testosterone secretion by the testes. Alternatively, as indicated by the histomorphological damage, this could be due to the degenerating testes rather than per se the decreased LH secretion. The reduction in LH and changes in FSH may be explained by the burnout of hypothalamic GnRH neurons or due to the feedback mechanism of testosterone.

In the present study, the route of kisspeptin administration was i.p., the testicular degeneration was most likely mediated via the HPG axis, this is in agreement with previous pharmacological studies that shown kisspeptin is capable of eliciting LH secretion via different routes of administration including the intracerebral , intracerebroventricular, intrahypothalamic, intravenous (i.v.), intraperitoneal (i.p.), and subcutaneous (s.c.) [12,23,25,26,27,28].

The testicular degeneration following a single i.p. injection of kisspeptin-10 was dose-dependent. An i.p. injection of 12.5 nmol kisspeptin-10 has been shown to produce submaximal degenerative changes (milder than that produced by 50 nmol). The high-dose i.p. kisspeptin-10-induced testicular degeneration was completely preventable by pretreatment with a GnRH-R antagonist and this strongly supports the hypothesis that the testicular degeneration seen in the present study following high doses of kisspeptin-10 is a result of hyper-stimulation of the HPG axis and is driven by the marked, and presumably supraphysiological increase in plasma LH. Kisspeptin-induced increase in LH is blocked by GnRH antagonist[29] who suggest a suprapituitary action of kisspeptin.

Since testosterone concentration declined at 6 hours and 24 hours while it increased at 15 minutes compared with the base line value, this may be due to testicular degeneration resulting in a positive feedback effect on either the GnRH secreting neurons or directly on the pituitary, to potentiate the LH secretion.

There is a strong relationship between kisspeptin and the reproduction (spermatogonial development), but kisspeptin is very potent and the dose should be determined carefully to improve testicular function without damage. Alteration in kisspeptin production may lead to abnormal gonadal function and impaired reproduction. Hence, it is concluded that KISSPEPTIN may have important applications in the treatment of some male reproductive disorders.

## Conclusions

1. Kisspeptin has a utility in initiating puberty (initiate puberty).
2. Kisspeptin use leads to increase in plasma testosterone level and development of testes through hypothalamic gonadal axes.
3. Pharmacological doses of kisspeptin-10 can cause testicular degeneration in the pre-pubertal male rats, which is likely to be a result of central hyper-stimulation of the HPG axis. The dose must be determined carefully.

The kisspeptins therefore represent a potential tool for HPG axis manipulation in humans, but our results suggest that the doses used must be chosen with considerable care.

Our results have important implications for targeting the kisspeptin system to treat reproductive disease.

The best results were achieved at 12.5 nmol (not at 50 nmol); this is because of the high testicular damage at 50 nmol resulted from high LH surge which cause (burnout) of the seminiferous tubules.

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