



# The Kisspeptin System in Male Reproduction

Rosaria Meccariello

Department of Movement Sciences and Wellness, University of Naples Parthenope, 80133 Naples, Italy; rosaria.meccariello@uniparthenope.it; Tel.: +39-081-5474668

**Abstract:** The kisspeptin system includes the cleavage products Kiss1 precursor and kisspeptin receptor (Kiss1R). It was originally discovered and studied in cancer metastasis, but the identification of *KISS1/KISS1R* gene mutations causing hypogonadotropic hypogonadism (HH) revealed unexpected effects in reproduction. Nowadays, the kisspeptin system is the main central gatekeeper of the reproductive axis at puberty and adulthood, but it also has a widespread functional role in the control of endocrine functions. At the periphery, Kiss1 and Kiss1R are expressed in the testes, but the need for kisspeptin signaling for spermatogenesis and sperm quality is still unclear and debated. This brief manuscript summarizes the main findings on kisspeptin and male reproduction; upcoming data on sperm maturation are also discussed.

**Keywords:** Kiss1; Kiss1 receptor; reproduction; spermatogenesis; epididymis; spermatozoa

## 1. Introduction

In the last 25 years, the discovery of kisspeptins and their receptor Kiss1R strongly contributed to the discovery of new signaling mechanisms in biological systems [1]. The *Kiss1* gene was first discovered in 1996 as an anti-metastasis gene in melanoma cell lines [2]. This gene encodes a precursor that is then cleaved into shorter peptides (Kp-54 or metastin, Kp-14, Kp-13, Kp-10), all sharing the carboxy-terminal region and capable of activating the G coupled membrane receptor Kiss1R; the 10-aminoacid-long Kp-10 represents the shorter peptide for Kiss1R activation [3,4].

Since the *Kiss1* discovery, the knowledge of the kisspeptin system in cancer significantly enlarged, nowadays revealing anti-metastasis, cancer-promoting or pro-metastasis activity, depending on cancer types, cancer microenvironment and steroid receptor status in the cancer cells [5,6]. However, the discovery in 2003 that inactivating mutations in the human *KISS1R* gene [7,8] caused hypogonadotropic hypogonadism (HH) with deficiencies in gonadotropin-releasing hormone (GnRH) secretion, gonadotropin discharge and infertility opened new perspectives in the biological action of the kisspeptin system, revealing unexpected roles in the central control of reproduction.

Furthermore, the characterization of the system in non-mammalian vertebrates [9] and the expression of Kiss1 and Kiss1R (at both mRNA and protein levels) in peripheral tissues, such as gonads, placenta, pancreas, adipose tissues, liver or vasa, additionally enlarged the biological role of the system from cancer progression to reproduction, fertility and energy homeostasis, suggesting that the system has more general roles in the control of endocrine functions in vertebrates [1].

In this Communication, the main findings on kisspeptin activity in the control of male reproduction are briefly summarized, with a focus on studies published during the last 5 years on the effects of kisspeptin at the periphery. Upcoming data from epididymal spermatozoa (SPZ) and future perspectives are also discussed.

## 2. Kiss1 and Kiss1R in the Central Control of Reproduction

The production of gametes is under the control of the hypothalamus–pituitary–gonadal (HPG) axis and requires endocrine, paracrine and autocrine communications



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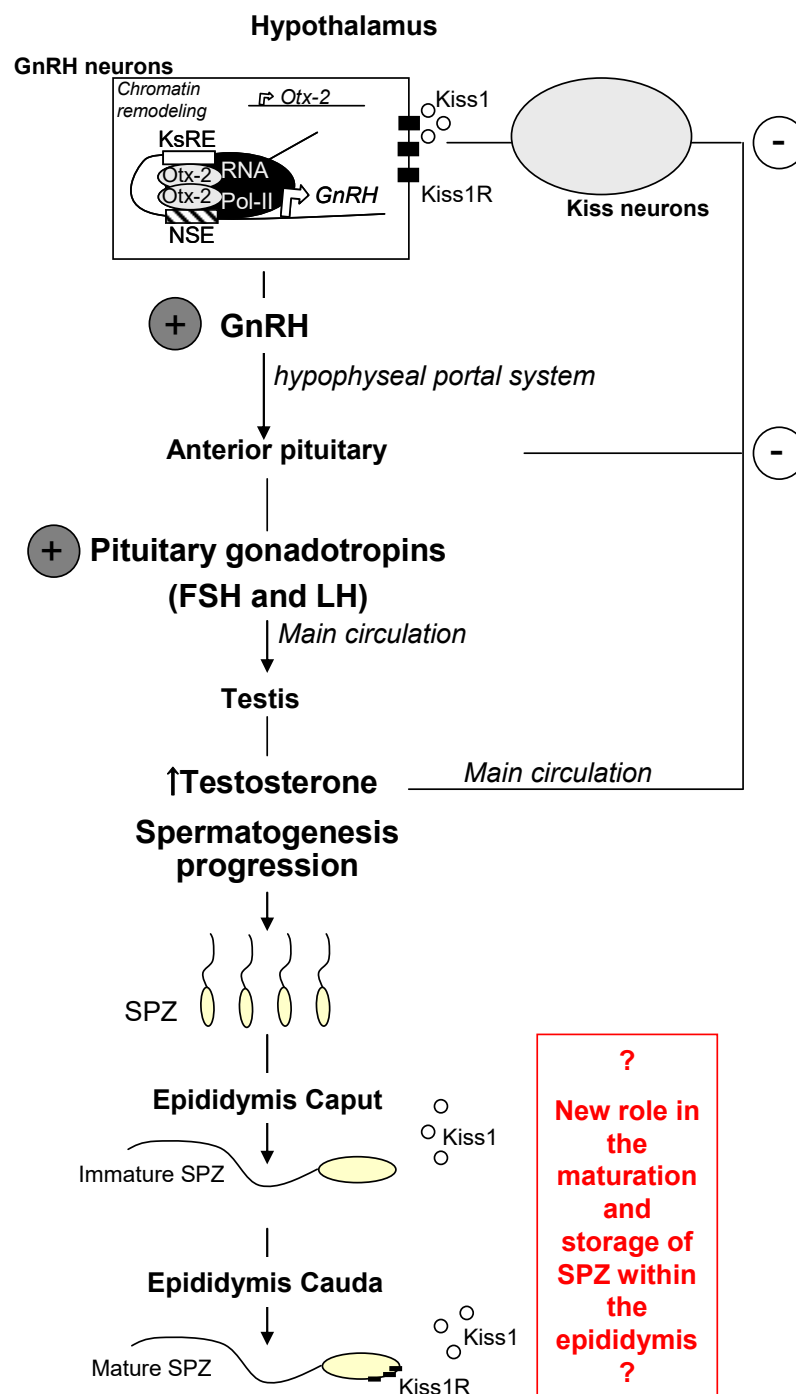
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among the hypothalamus, which drives the pulsatile secretion of the GnRH; the pituitary, which is responsible for the discharge of gonadotropins (Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH)); and gonads, which, in turn, produce sex steroids [10].

Notably, the gain of function and the loss of function mutations in *KISS1/KISS1R* genes cause precocious puberty and HH, respectively, in both human and animal models [7,8,11–13], as a consequence of the precocious stimulation or impairment of the HPG axis at the hypothalamic level. In fact, hypothalamic GnRH-secreting neurons possess on their membranes Kiss1R [14], and express and secrete the neurohormone GnRH under the control of kisspeptin, which is produced in the brain by neuronal populations located within the arcuate nucleus and the anteroventral periventricular nucleus [14].

The molecular mechanism that mediates kisspeptin action on *GnRH* gene expression involves kisspeptin-induced dynamic chromatin modifications [15,16]. A kisspeptin-response element (KsRE) between –3446 and –2806 bp of the mouse *GnRH* gene enhancer has been detected in GnRH-neuronal cell lines and in transgenic mice. KsRE represents a binding site for the transcription factor Orthodenticle Homeobox 2 (*Otx-2*). Kisspeptin treatment causes nucleosome-depleted DNA in KsRE, induces the transcription of the *Otx-2* gene and the synthesis of the *Otx-2* protein, promotes *Otx-2* protein binding to KsRE, and consequently activates *GnRH* gene transcription [15]. Specifically, kisspeptin significantly increased histone 3 (H3) acetylation at lysine (K) 14 and K27 and the trimethylation of H3K4 within the KsRE, but did not affect the dimethylation of H3K9, thus resulting in being effective on well-known markers of active chromatin, but devoid of action on gene repression markers. Furthermore, a chromosome conformation capture assay revealed that kisspeptin promoted the functional formation of a chromatin loop between KsRE and the downstream located neuron-specific element to gain the transcriptional activation of the *GnRH* gene [16] (Figure 1).

Kisspeptin neurons are also intermediate in the sex-steroid mediated feedback mechanisms on reproduction [14] and convey on the HPG axis several environmental cues, thus modulating reproduction with epigenetic mechanisms [17,18]. In this respect, diet or exposure to environmental toxicants centrally affect reproduction, modulating the activity of Kiss-secreting neurons. The best example in the field is the epigenetic modulation of puberty, a process that requires specific methylation changes in the *Kiss1* and *Kiss1R* gene promoters [19] and the activity of the NAD<sup>+</sup> dependent deacetylase Sirtuin 1 (Sirt1) that epigenetically modulates *Kiss1* expression within the hypothalamus [20]. In fact, reproduction strongly depends on energy availability and Sirt1 acts as metabolic sensors capable of activating or repressing gene expression through mechanisms involving the deacetylation of histone proteins, transcriptional factors or cofactors [21].



**Figure 1.** Schematic representation of kisspeptin activity along the hypothalamus–pituitary–testis axis and the shift in Kiss1R localization recently observed in rat and dog SPZ collected in caput and cauda epididymis. See the main text for details. GnRH, Gonadotropin-ReleasingHormone; Kiss1R, kisspeptin receptor; KsRE, kisspeptin-response element; NSE, neuron-specific element; Otx-2, Orthodenticle Homeobox 2; RNA PolII, RNA Polymerase II; SPZ, spermatozoa.

### 3. Kiss1 and Kiss1R in Testis and Spermatozoa

Spermatogenesis is a complex process modulated step-by step by the autocrine, paracrine and endocrine route. It needs the coordination between germ cell proliferation and death, meiotic division and differentiation events, and the key contribution of Leydig cells in the interstitium to produce sex-steroids, and Sertoli cells in the seminiferous tubules to provide structural and nourishment support to developing germ cells [10]. The

kisspeptin system has been characterized in the testis of mammalian and non-mammalian vertebrates, revealing possible roles in the autocrine and paracrine intra-testicular communications, steroid biosynthesis, spermatogenesis progression and sperm functions, but also species-specific differences in localization and possible functions in turns [22–24].

Kisspeptin, but not GnRH, has been detected in human plasma and measured in different health conditions [12]. In males, circulating kisspeptin levels change in relation to fertility status, being significantly higher in fertile than in infertile men [25]. Some HH patients have high levels of kisspeptin in the plasma, but, after GnRH replacement therapy, circulating kisspeptin levels decrease as a consequence of the restored sex-steroid feedback mechanisms at hypothalamic levels [26]. However, gonadotropin stimulation is not always able to rescue testosterone biosynthesis and spermatogenesis in clinical cases of *KISS1R* inactivating mutations [27,28], suggesting the need for testicular Kiss1R signaling for steroidogenesis. Similarly, the specific reactivation of the *Kiss1R* gene in the GnRH secreting neuron of *KISS1R*<sup>-/-</sup> knockout mice does not restore spermatogenesis, further confirming the need of the intratesticular kisspeptin signal for successful spermatogenesis [29]. By contrast, testosterone replacement in *KISS*<sup>-/-</sup> mice that exhibit HH restores plasma and intratesticular testosterone levels and sustains spermatogenesis until the production of spermatozoa capable of fertilizing eggs in vitro, but treated mice failed to impregnate females [30].

The administration of kisspeptin usually promotes spermatogenesis in intact animal models [31–33]. Nevertheless, the kisspeptin-dependent over-stimulation of the HPG axis causes testis damage if prolonged [34] and the switch-off of the HPG axis through Kiss1R desensitization [35].

Post-natal testis development and Leydig cells maturation require kisspeptin signaling, and synergistic effects involving both the hypothalamic and LH-dependent intratesticular production of kisspeptin have been suggested in rodents [36]. In fact, centrally produced kisspeptin activates the HPG axis, leading to hypothalamic GnRH secretion and gonadotropin LH production by the anterior pituitary; in turn, LH signaling in Leydig cells induces *Kiss1* expression through cyclic adenosine monophosphate and the activation of the protein kinase A pathway [37]. In vitro effects on *GnRH* expression in Leydig cells [38] and the modulation of intratesticular GnRH system, estradiol and testosterone levels in non-mammalian vertebrates [32,39] have also been reported. Recently, the co-culture of germ and somatic cells revealed kisspeptin-dependent effects on spermatogenesis progression [40], confirming observations in more physiological conditions, such as ex vivo testes explants [31,32,39].

Hence, in spite of in vivo, ex vivo and in vitro observation revealing a possible role in Leydig cells physiology and steroid-secreting activity, or spermatogenesis progression as well [24], the intratesticular role of the kisspeptin system is far from being elucidated and still debated [23].

Additionally, humans, bulls, rodents and frogs express Kiss1R [24] and the system has been characterized in goat, hamster, mouse and rat epididymis [24,41]. Nevertheless, the need for kisspeptin to gain SPZ competence for fertilization has been poorly investigated and remains unclear. The localization of Kiss1R differs in humans and mice, being mainly localized in the post-equatorial segment in humans [42] and acrosomic region in mice [43]. Currently, the modulation of the kisspeptin system in SPZ by specific agonists/antagonists in physiological conditions has been reported in human [42] and mouse spermatozoa [43] only. These studies revealed Kp-13-dependent effects on human sperm hypermotility [42] and effects of Kiss1R antagonism by Kp-234 on the fertilizing ability of mouse SPZ collected in the epididymal tail [43]. Recently, we characterized the kisspeptin system in dog and rat SPZ [41,44], providing evidence of Kiss1R trafficking in SPZ head during the transit from caput to cauda epididymis (Figure 1). In the dog, a model close to humans, the combined use of flow cytometry, epifluorescence microscopy and Western blot on specific membrane protein fractions revealed Kiss1R on membrane-intact SPZ collected from the epididymal tail. In particular, the presence of Kiss1R on SPZ surface parallels the acquisition

of the typical features for successful epididymal maturation (i.e. protamination rate and motility) [44]. In the rat, immunofluorescence analysis carried out on permeabilized SPZ collected in caput and tail epididymis revealed that Kiss1R shifts from the posterior region of the SPZ head in the epididymal caput to the perforatorium in the epididymal tail [41]. Interestingly, by dot blot assay, Kiss1 was detected in the epididymal fluid of both rats and dog [41,44] and, in rat, a specific ELISA assay was used to measure significant Kiss1 levels in the epididymal fluid and in the plasma, used as a positive control [41]. Thus, Kiss1R trafficking seems to be a marker of appropriate sperm maturation, and kisspeptin signaling may represent a signal for SPZ storage within the epididymis. The main limitations of these studies are the lack of functional data on the activity of Kiss1R in SPZ collected at different tracts of the epididymis in both physiological and pathological conditions. Thus, further studies are required in the field. Interestingly, kisspeptin levels have been measured in seminal plasma and blood plasma in a large cohort of healthy men, revealing higher kisspeptin levels in the seminal plasma than blood and a positive association between sperm quality and kisspeptin within the seminal plasma [45]. Hence, it could be interesting to analyze the kisspeptin system in SPZ-defective animal models, but also in the seminal plasma of normospermic, subfertile and infertile men as an additional sperm quality parameter.

#### 4. Conclusions

In conclusion, kisspeptin signaling has a fundamental role in the central control of reproduction at puberty and adulthood, but additional studies are required to elucidate the need for peripheral kisspeptin signaling in spermatogenesis and sperm functions. Nevertheless, upcoming data revealed the differential localization of Kiss1R in the SPZ head during epididymal maturation. In parallel, the detection of significant Kiss1 amounts in seminal plasma and epididymal fluid strongly encourages additional studies in the field and candidates for the kisspeptin system to become a promising marker and target in evaluating and addressing male fertility troubles in the near future.

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#### References

1. Chianese, R.; Colledge, W.H.; Fasano, S.; Meccariello, R. Editorial: The multiple facets of Kisspeptin activity in biological systems. *Front. Endocrinol.* **2018**, *9*, 727. [[CrossRef](#)] [[PubMed](#)]
2. Lee, J.H.; Miele, M.E.; Hicks, D.J.; Phillips, K.K.; Trent, J.M.; Weissman, B.E.; Welch, D.R. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *J. Natl. Cancer Inst.* **1996**, *88*, 1731–1737. [[CrossRef](#)]
3. Kotani, M.; Dethoux, M.; Vandenbogaerde, A.; Communi, D.; Vanderwinden, J.-M.; LePoul, E.; Brezillon, S.; Tyldesley, R.; Suarez-Huerta, N.; Vandeput, F.; et al. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J. Biol. Chem.* **2001**, *276*, 34631–34636. [[CrossRef](#)] [[PubMed](#)]
4. Muir, A.I.; Chamberlain, L.; Elshourbagy, N.A.; Michalovich, D.; Moore, D.J.; Calamari, A.; Szekeres, P.G.; Sarau, H.M.; Chambers, J.K.; Murdock, P.; et al. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J. Biol. Chem.* **2001**, *276*, 28969–28975. [[CrossRef](#)] [[PubMed](#)]
5. Ciaramella, V.; Della Corte, C.M.; Ciardiello, F.; Morgillo, F. Kisspeptin and Cancer: Molecular Interaction, Biological Functions, and Future Perspectives. *Front. Endocrinol.* **2018**, *9*, 115. [[CrossRef](#)] [[PubMed](#)]
6. Guzman, S.; Brackstone, M.; Radovick, S.; Babwah, A.V.; Bhattacharya, M.M. KISS1/KISS1R in Cancer: Friend or Foe? *Front. Endocrinol.* **2018**, *9*, 437. [[CrossRef](#)] [[PubMed](#)]
7. Seminara, S.B.; Messenger, S.; Chatzidaki, E.E.; Thresher, R.R.; Acierno, J.S., Jr.; Shagoury, J.K.; Bo-Abbas, Y.; Kuohung, W.; Schwino, K.M.; Hendrick, A.G.; et al. The GPR54 gene as a regulator of puberty. *N. Engl. J. Med.* **2003**, *349*, 1614–1627. [[CrossRef](#)] [[PubMed](#)]



8. de Roux, N.; Genin, E.; Carel, J.C.; Matsuda, F.; Chaussain, J.L.; Milgrom, E. Hypogonadotropic Hypogonadism due to loss of function of the Kiss1-derived peptide receptor GPR54. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10972–10976. [[CrossRef](#)]
9. Oakley, A.E.; Clifton, D.K.; Steiner, R.A. Kisspeptin signaling in the brain. *Endocr. Rev.* **2009**, *30*, 713–743. [[CrossRef](#)]
10. Pierantoni, R.; Cobellis, G.; Meccariello, R.; Fasano, S. Evolutionary aspects of cellular communication in the vertebrate hypothalamo-hypophysio-gonadal axis. *Int. Rev. Cytol.* **2002**, *218*, 69–141.
11. d'Anglemont de Tassigny, X.; Fagg, L.A.; Dixon, J.P.; Day, K.; Leitch, H.G.; Hendrick, A.G.; Zahn, D.; Franceschini, I.; Caraty, A.; Carlton, M.B.; et al. Hypogonadotropic Hypogonadism in mice lacking a functional Kiss1. *Gene. Proc. Natl. Acad. Sci. USA* **2007**, *104*, 10714–10719. [[CrossRef](#)] [[PubMed](#)]
12. Trevisan, C.M.; Montagna, E.; de Oliveira, R.; Christofolini, D.M.; Barbosa, C.P.; Crandall, K.A.; Bianco, B. Kisspeptin/GPR54 System: What Do We Know About Its Role in Human Reproduction? *Cell Physiol. Biochem.* **2018**, *49*, 1259–1276. [[CrossRef](#)] [[PubMed](#)]
13. Lapatto, R.; Pallais, J.C.; Zhang, D.; Chan, Y.M.; Mahan, A.; Cerrato, F.; Le, W.W.; Hoffman, G.E.; Seminara, S.B. Kiss1<sup>-/-</sup> mice exhibit more variable hypogonadism than Gpr54<sup>-/-</sup> mice. *Endocrinology* **2007**, *148*, 4927–4936. [[CrossRef](#)] [[PubMed](#)]
14. Pinilla, L.; Aguilar, E.; Dieguez, C.; Millar, R.P.; Tena-Sempere, M. Kisspeptins and Reproduction: Physiological Roles and Regulatory Mechanisms. *Physiol. Rev.* **2012**, *92*, 1235–1316. [[CrossRef](#)] [[PubMed](#)]
15. Novaira, H.J.; Fadoju, D.; Diaczok, D.; Radovick, S. Genetic mechanisms mediating kisspeptin regulation of GnRH gene expression. *J. Neurosci.* **2012**, *32*, 17391–17400. [[CrossRef](#)]
16. Novaira, H.J.; Sonko, M.L.; Radovick, S. Kisspeptin Induces Dynamic Chromatin Modifications to Control GnRH Gene Expression. *Mol. Neurobiol.* **2016**, *53*, 3315–3325. [[CrossRef](#)]
17. Motti, M.L.; Meccariello, R. Minireview: The epigenetic modulation of KISS1 in reproduction and cancer. *Int. J. Environ. Res. Public Health* **2019**, *16*, 2607. [[CrossRef](#)]
18. Santoro, A.; Chianese, R.; Troisi, J.; Richards, S.; Nori, S.L.; Fasano, S.; Guida, M.; Plunk, E.; Viggiano, A.; Pierantoni, R.; et al. Neuro-toxic and reproductive effects of BPA. *Curr. Neuropharmacol.* **2019**, *17*, 1109–1132. [[CrossRef](#)]
19. Wyatt, A.K.; Zavodna, M.; Viljoen, J.L.; Stanton, J.A.; Gemmell, N.J.; Jasoni, C.L. Changes in methylation patterns of kiss1 and kiss1r gene promoters across puberty. *Genet. Epigenet.* **2013**, *5*, 51–62. [[CrossRef](#)]
20. Vazquez, M.J.; Toro, C.A.; Castellano, J.M.; Ruiz-Pino, F.; Roa, J.; Beiroa, D.; Heras, V.; Velasco, I.; Dieguez, C.; Pinilla, L.; et al. SIRT1 mediates obesity- and nutrient-dependent perturbation of pubertal timing by epigenetically controlling Kiss1 expression. *Nat. Commun.* **2018**, *9*, 4194. [[CrossRef](#)]
21. D'Angelo, S.; Mele, E.; Di Filippo, F.; Viggiano, A.; Meccariello, R. Sirt1 activity in the brain: Simultaneous effects on energy homeostasis and reproduction. *Int. J. Environ. Res. Public Health* **2021**, *18*, 1243. [[CrossRef](#)] [[PubMed](#)]
22. Chianese, R.; Cobellis, G.; Chioccarelli, T.; Ciaramella, V.; Migliaccio, M.; Fasano, S.; Pierantoni, R.; Meccariello, R. Kisspeptins, estrogens and male fertility. *Curr. Med. Chem.* **2016**, *23*, 4070–4091. [[CrossRef](#)] [[PubMed](#)]
23. Sharma, A.; Thaventhiran, T.; Minhas, S.; Dhillon, W.S.; Jayasena, C.N. Kisspeptin and testicular function-is it necessary? *Int. J. Mol. Sci.* **2020**, *21*, 2958. [[CrossRef](#)] [[PubMed](#)]
24. Meccariello, R.; Fasano, S.; Pierantoni, R. Kisspeptins, new local modulators of male reproduction: A comparative overview. *Gen. Comp. Endocrinol.* **2020**, *299*, 113618. [[CrossRef](#)] [[PubMed](#)]
25. Ramzan, M.H.; Ramzan, M.; Ramzan, F.; Wahab, F.; Jelani, M.; Khan, M.A.; Shah, M. Insight into the serum kisspeptin levels in infertile males. *Arch. Iran Med.* **2015**, *18*, 12–17. [[PubMed](#)]
26. Kotani, M.; Katagiri, F.; Hirai, T.; Kagawa, J. Plasma kisspeptin levels in male cases with hypogonadism. *Endocr. J.* **2014**, *61*, 1137–1140. [[CrossRef](#)]
27. Semple, R.K.; Achermann, J.C.; Ellery, J.; Farooqi, I.S.; Karet, F.E.; Stanhope, R.G.; O'Rahilly, S.; Aparicio, S.A. Two novel missense mutations in G protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 1849–1855. [[CrossRef](#)]
28. Nimri, R.; Lebenthal, Y.; Lazar, L.; Chevrier, L.; Phillip, M.; Bar, M.; Hernandez-Mora, E.; De Roux, N.; Gat-Yablonski, G. A novel loss-of-function mutation in GPR54/KISS1R leads to hypogonadotropic hypogonadism in a highly consanguineous family. *J. Clin. Endocrinol. Metab.* **2011**, *96*, E536–E545. [[CrossRef](#)]
29. León, S.; Barroso, A.; Vazquez, M.J.; Garcia-Galiano, D.; Manfredi-Lozano, M.; Ruiz-Pino, F.; Heras, V.; Romero-Ruiz, A.; Roa, J.; Schutz, G.; et al. Direct actions of kisspeptins on GnRH neurons permit attainment of fertility but are insufficient to fully preserve gonadotropic axis activity. *Sci. Rep.* **2016**, *6*, 19206. [[CrossRef](#)]
30. Goto, T.; Hirabayashi, M.; Watanabe, Y.; Sanbo, M.; Tomita, K.; Inoue, N.; Tsukamura, H.; Uenoyama, Y. Testosterone Supplementation Rescues Spermatogenesis and In Vitro Fertilizing Ability of Sperm in Kiss1 Knockout Mice. *Endocrinology* **2020**, *161*, bqaa092. [[CrossRef](#)]
31. Chianese, R.; Ciaramella, V.; Fasano, S.; Pierantoni, R.; Meccariello, R. Kisspeptinreceptor, GPR54, as a candidate for the regulation of testicular activity in the frog, *Rana esculenta*. *Biol. Reprod.* **2013**, *88*, 1–11. [[CrossRef](#)] [[PubMed](#)]
32. Chianese, R.; Ciaramella, V.; Fasano, S.; Pierantoni, R.; Meccariello, R. Kisspeptin drives germ cell progression in the anuran amphibian *Pelophylax esculentus*: A study carried out in ex vivo testes. *Gen. Comp. Endocrinol.* **2015**, *211*, 81–91. [[CrossRef](#)] [[PubMed](#)]
33. Ayturk, N.; Firat, T.; Kukner, A.; Ozoğul, C.; Tore, F.; Kandirali, İ.E.; Yilmaz, B. Theeffect of kisspeptin on spermatogenesis and apoptosis in rats. *Turk. J. Med. Sci.* **2017**, *47*, 334–342. [[CrossRef](#)] [[PubMed](#)]

34. Ramzan, F.; Qureshi, I.Z. Intraperitoneal kisspeptin-10 administration induces dose-dependent degenerative changes in maturing rat testes. *Life Sci.* **2011**, *88*, 246–256. [[CrossRef](#)] [[PubMed](#)]
35. Seminara, S.; Di Pietro, M.J.; Ramaswami, S.; Crowley, W.F.; Plant, T.M. Continuous human metastatin 45–54 infusion desensitizes G protein-coupled receptor 54-induced gonadotropin-releasing hormone release monitored indirectly in the juvenile male Rhesus monkey (*Macaca mulatta*): A finding with therapeutic implications. *Endocrinology* **2006**, *147*, 2122–2126. [[CrossRef](#)]
36. Wang, J.Y.; Hsu, M.C.; Tseng, T.H.; Wu, L.S.; Yang, K.T.; Chiu, C.H. Kisspeptin expression in mouse Leydig cells correlates with age. *J. Chin. Med. Assoc.* **2015**, *78*, 249–257. [[CrossRef](#)]
37. Chiang, C.M.; Chiu, H.Y.; Jong, D.S.; Wu, L.S.; Lee, Y.J.; Chiu, C.H.J. Role of the Kisspeptin/KISS1 Receptor System in the Testicular Development of Mice. *J. Chin. Med. Assoc.* **2021**, *84*, 203–211. [[CrossRef](#)]
38. Petrucci, L.; Maranesi, M.; VeriniSupplizi, A.; Dall’Aglio, C.; Mandara, M.T.; Quassinti, L.; Bramucci, M.; Miano, A.; Gobbetti, A.; Catone, G.; et al. Kisspeptin/GnRH1 system in Leydig cells of horse (*Equus caballus*): Presence and function. *Theriogenology* **2020**, *152*, 1–7. [[CrossRef](#)]
39. Chianese, R.; Ciaramella, V.; Fasano, S.; Pierantoni, R.; Meccariello, R. Kisspeptin regulates steroidogenesis and spermiation in the anuran amphibian. *Reproduction* **2017**, *154*, 1–12. [[CrossRef](#)]
40. Toolee, H.; Rastegar, T.; Solhjoo, S.; Mortezaee, K.; Mohammadipour, M.; Kashani, I.R.; Akbari, M. Roles for Kisspeptin in proliferation and differentiation of spermatogonial cells isolated from mice offspring when the cells are cocultured with somatic cells. *J. Cell. Biochem.* **2019**, *120*, 5042–5054. [[CrossRef](#)]
41. Mele, E.; D’Auria, R.; Scafuro, M.; Marino, M.; Fasano, S.; Viggiano, A.; Pierantoni, R.; Santoro, A.; Meccariello, R. Differential Expression of Kisspeptin System and Kisspeptin Receptor Trafficking During Spermatozoa Transit in the Epididymis. *Genes* **2022**, *13*, 295. [[CrossRef](#)] [[PubMed](#)]
42. Pinto, F.M.; Cejudo-Román, A.; Ravina, C.G.; Fernández-Sánchez, M.; Martín-Lozano, D.; Illanes, M.; Tena-Sempere, M.; Candenas, M.L. Characterization of the kisspeptin system in human spermatozoa. *Int. J. Androl.* **2012**, *35*, 63–73. [[CrossRef](#)] [[PubMed](#)]
43. Hsu, M.C.; Wang, J.Y.; Lee, Y.J.; Jong, D.S.; Tsui, K.H.; Chiu, C.H. Kisspeptin modulates fertilization capacity of mouse spermatozoa. *Reproduction* **2014**, *147*, 835–845. [[CrossRef](#)] [[PubMed](#)]
44. Gloria, A.; Contri, A.; Mele, E.; Fasano, S.; Pierantoni, R.; Meccariello, R. Kisspeptin Receptor on the Sperm Surface Reflects Epididymal Maturation in the Dog. *Int. J. Mol. Sci.* **2021**, *22*, 10120. [[CrossRef](#)] [[PubMed](#)]
45. Zou, P.; Wang, X.; Chen, Q.; Yang, H.; Zhou, N.; Sun, L.; Chen, H.; Liu, J.; Ao, L.; Cui, Z. Kisspeptin Protein in Seminal Plasma Is Positively Associated with Semen Quality: Results from the MARHCS Study in Chongqing, China. *Biomed. Res. Int.* **2019**, *2019*, 5129263. [[CrossRef](#)] [[PubMed](#)]