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Kisspeptin and attributes of infertile males and females: A cross-sectional study in a subset of Pakistani population

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Title:

**Kisspeptin and attributes of infertile males and females: a cross sectional
study in a subset of Pakistani Population**

Running Title:

Infertility and Kisspeptin

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Abstract

Kisspeptin and attributes in infertile males and females: a cross sectional study in a subset of Pakistani Population.

Kisspeptin; a peptide hormone, plays a pivotal role in fertility and neuroendocrine regulation of hypothalamo-pituitary gonadal axis. Increased kisspeptin and reproductive hormones are responsible for fertility in male and females. This study aimed to explore the role of kisspeptin on hypothalamo-pituitary-gonadal axis by comparing the levels of kisspeptin in fertile and infertile subjects and identifying single-nucleotide polymorphisms(SNP) of *KISS1* gene in exon 2 and 3 of infertile male and female cohorts. A cross-sectional study was carried out on 80 males (44 infertile and 36 fertile) and 88 females (44 in each group). Significantly high levels of kisspeptin(KP), follicle stimulating hormone(FSH), luteinizing hormone and testosterone were observed in fertile male and female subjects except low FSH levels in comparison to infertile female subjects. One polymorphism in exon 2 [E1225K (G/A 3673)] and three in exon 3 [P1945A (C/G 5833); Insertion of T at 6075; G2026G (C/G 6078)] in infertile group were detected; with low KP and hormonal levels. Male subjects had abnormal sperm parameters and unsuccessful attempt of Intracytoplasmic sperm injection in females. Expression of SNP in exon 2 and 3 of *KISS1* could be responsible for alteration in release of reproductive hormones and gonadal functions, hence causing infertility.

Key words: Kisspeptin, Infertility, *KISS1*, polymorphism

1. Introduction:

51 Kisspeptin (KP) encoded by *KISS1* belongs to a family of peptide hormones
52 which play a principal role in fertility and neuroendocrine regulation of
53 hypothalamo-pituitary gonadal axis (Vaziri, Rafeie et al. 2017). KP secreting
54 neurons are present in the different nuclei of hypothalamus; arcuate nucleus
55 (ARC) also known as infundibular nucleus, the anteroventral peri-ventricular
56 nucleus (AVPV), anterodorsal preoptic nucleus (APN), stria terminalis and
57 Amygdale (Funes, Hedrick et al. 2003). The pulsatile secretion of Gonadotropin
58 Releasing Hormone (GnRH) in central regulation of the Hypothalamo-pituitary
59 gonadal (HPG) axis is played by hypothalamic *KISS1/KISS1R* (receptor of KISS
60 1) system (Skorupskaite, George et al. 2014). Consequently, the cross-talk
61 between Kisspeptin and the receptor (*KISS1R*) stands crucial in regulating the
62 commencement of puberty and release of hormones from the involved
63 reproductive axis (Luan, Zhou et al. 2007)

64 *KISS1* translates for a 145 amino acid long protein identified as kisspeptin-145
65 which produces a peptide containing 54 residues after cleavage, called
66 Kisspeptin 54 or metastin that can further be sliced into much smaller amino acid
67 sequences, recognized as kisspeptin-14, kisspeptin-13 and kisspeptin-10,
68 however they represent a common structural motif (ArgPhe-NH₂) in their C-
69 terminal (de Tassigny, Fagg et al. 2007). Along the length of gene *KISS1*, more
70 than approximately 294 single nucleotide polymorphism (SNPs) are already
71 identified; among which the untranslated region (UTR) contributes to have 42
72 mutations, exon for 30 and the rest by intronic regions. [“A database of human
73 single nucleotide polymorphisms” 2014, <http://www.ncbi.nlm.nih.gov/SNP/>].

74 *KISS1R* gene mutations result in loss of function of the *KISS1R*, leading to
75 down-regulation of GnRH pulsatile secretion as well as infertility. On the other

76 hand, activating mutations cause prevention of desensitization of the
77 *KISS/KISSIR* pathway and ultimately lead to precocious puberty. Two *KISSI*
78 mutations, p.P74S and p.H90D, have been recognized as genetic causes of
79 Central Precocious Puberty (Silveira, Noel et al. 2010). Furthermore,
80 polymorphism of the *KISSI* gene with amino acid substitution (P110T)
81 documented to have significant association with central precocious puberty
82 (CPP) in Korean girls (Luan, Zhou et al. 2007) (Ko, Lee et al. 2010)

83 The role of KP in feedback regulation of GnRH secretion and hence release of
84 gonadal hormones required for normal reproductive functions has been
85 elucidated (Irwig, Fraley et al. 2004). Evidence advocates the loss of gene
86 functionality or presence of SNPs in *KISSI* and *KISSIR* to be a risk factor for
87 sexual immaturity and infertility axis in humans (Ko, Lee et al. 2010).

88 A number of studies have verified role of Kisspeptin on reproductive axis,
89 unexplained infertility and as a therapeutic agent to trigger oocyte maturation
90 and ovulation (Abbara, Jayasena et al. 2014), (Mumtaz, Khalid et al. 2017).
91 Literature has proved that mutations which inhibit the action of *KISSI* and
92 *KISSIR* in idiopathic hypo-gonadotropic-hypogonadism (IHH) subjects resulted
93 in delayed puberty and subfertility (Semple, Achermann et al. 2005) .

94 Although mutations inhibiting *KISSI* gene and its receptor activity can instigate
95 infertility yet, the information about *KISSI* gene mutations and its
96 polymorphisms are scarce. A research done on Q36R (rs35431622) *KISSI* gene
97 in infertile female subjects in northern Iran documented that it has no association
98 with female infertility and suggested that variation of results might be possible
99 due to genetic variations on account of different geographic situations (Vaziri,

Rafeie et al. 2017). In addition to that, low levels of KP have also been identified in infertile male subjects of our region (Haris Ramzan, Ramzan et al. 2015).

We thus aimed to explore role of KP on HPG axis by comparing KP levels in fertile and infertile male and female subjects and identify the sequence variations, including mutations and single-nucleotide polymorphisms (SNPs) of *KISS1* gene in exon 2 and 3 of infertile males and female cohorts.

Subjects and Methods:

This cross-sectional study was conducted in the Department of Biological & Biomedical Sciences, Aga Khan University, Karachi, from April 2016 till March 2018 after acquiring ethical approval (3331-BBS-ERC-14). The estimated sample size by observing 94% power, with prevalence of 21.9% infertility and 22% +/-6 confidence limit, was 79. To avoid drop out of subjects we recruited 88 infertile (male and female) subjects and matched 80 fertile subjects, who fulfilled our inclusion criteria and consented to be part of our study.

Phenotypic Characterization:

Inclusion Criteria for Male Subjects;

All males between the ages of 25 to 55 years during the study phase who concurred to take part in the study were selected. A comprehensive history related to the diagnosis of infertility was obtained for excluding secondarily infertile males. A semen analysis report was obtained on request (6 to 9 months old). In case of failure to do so, male subjects were demanded a fresh semen samples by masturbation following a 3 - 5 days of asceticism. Samples were then processed and stored in sterilized containers, later were analyzed as per World Health Organization guidelines.

124 Fertile Group:

125 The “fertility status” of the recruited subjects was established according to the
126 semen parameters observing the World Health Organization criteria “2010”
127 which states “had total sperm number (TC) >39 million per ejaculate, total sperm
128 motility (Progressive and Non-progressive) measured within 60 minutes of
129 collection of more than 40%, and normal morphology of $\geq 4\%$.” (Cooper,
130 Noonan et al.)

131 Infertile group:

132 Men with history of primary infertility; sperm number (TC) less than 39
133 million/ejaculate, decreased sperm motility of all sperms (less than 40%) and
134 having normal sperm morphology of less than 4% (Cooper, Noonan et al. 2010)
135 were included in the study.

136 Exclusion Criteria for Male Subjects:

137 Subjects having diabetes, hypertension, arthritis, malignancy, epilepsy,
138 tuberculosis, endocrinal disorders, liver/renal disease, cryptorchidism, testicular
139 trauma, orchitis, testicular hypotrophy along with those who had general health
140 issues were excluded. Additionally, those who suffered from secondary
141 infertility were discounted. Moreover, subjects who were receiving any
142 hormonal or steroids therapy were also excluded.

143 Inclusion Criteria for Female Subjects:

144 Fertile females:

145 All healthy females between the ages of 18 - 35 years, with a child less than 2
146 years of age from all ethnic groups were recruited as controls.

147 Infertile females:

148 All females who fulfill the criteria of primary infertility (never conceived in last
149 more than one years) between the ages of 18 - 35 years, from all ethnic
150 backgrounds, were enrolled in the study as cases.

151

152 Exclusion criteria for Female subjects:

153 The females who were diagnosed with secondary infertility, being treated with
154 oral contraceptive pills, having thyroid disorders, preexisting diabetes and
155 hypertension were excluded from the study.

156 Clinical Data Collection: The clinical data including age, height, weight, blood
157 pressure, menstrual/obstetric and gynecological history with general physical
158 examination was recorded in all study subjects. The height in centimeters
159 (converted to meters) and weight in kilograms of all the recruited subjects were
160 noted to calculate the body mass index (BMI), and categorized consulting
161 cutoffs for Asians, where 18 - 22.9 kg/m² was normal weight, overweight 23 -
162 24.9 kg/m² was overweight and BMI $\geq$ 25 kg/m² was considered obese (WHO
163 2004). Gender, age and height of every participant was entered manually into
164 the BIA machine by a digital keyboard, and it immediately revealed the
165 percentage fat mass (% FM) of the individual (Lazzer, Boirie et al. 2003)

166 **Biochemical Measurement**

167 **Serum samples** were used to detect the hormones levels using commercially
168 available Enzyme Linked Immuno Sorbent Assay (ELISA) kits, **following the**
169 **manufacturer's protocol**. Follicle stimulating hormone (FSH) by Human FSH
170 Enzyme Immunoassay (Kit Cat. No DKO010; DiaMetra), LH by Human LH
171 Enzyme Immunoassay (Kit Cat. No DKO010; Dia Metra). Immunoassay for

172 FSH, the inter assay **coefficient of variation**, <8% and intra assay coefficient of
173 variation, <9.7% and similarly LH immunoassay, the inter assay **coefficient of**
174 **variation** was <7.91%; intra assay **coefficient of variation** was <9.21%. **Serum**
175 KP was measured by ELISA kit (Cat. No: 95611, Glory BioScience, USA). The
176 analytical sensitivity was 10.16 ng/L and intra and inter assay **coefficients of**
177 **variation** was less than 10% and 12%, respectively. For assessing total
178 testosterone levels commercially available Human Total Testosterone (TT)
179 immune-enzymatic kit for serum analysis was utilized (Cat. No DKO002 by
180 Diametra).

181 **Genotype Characterization:**

182 *Blood Sampling and genotyping:*

183 Ten ml of venous blood was collected from all study subjects. DNA was isolated
184 from the leukocytes in the peripheral blood of the study subjects using a DNA
185 isolation kit (Genomic DNA Purification Kit Cat. No A1125 by Promega, USA).
186 The isolated DNA was quantified by measuring the ultraviolet (UV) absorbance
187 and determining the absorbance ratio (A280/A260) for 2 μ L samples, employing a
188 Nanodrop-ND1000 (Thermo Fisher Scientific, Waltham, MA). Extracted DNA was
189 considered pure at an absorbance ratio of ~1.8. Furthermore, gel electrophoresis
190 was run to visualize the PCR products on 5 μ L of sample in 2% agarose gel against
191 a 100bp ladder on approximately 15% of all samples for confirmation. Gel was
192 observed in Gel Doc Imaging system (Biorad, **United Kingdom**) (Figure 1a & b).

193 For exon 2: Polymerase chain reaction (PCR) was executed using the 2X PCR
194 Hotstart Master Mix (Cat# G906, ABM (Applied Biological Materials Inc, **Canada**)
195 according to the instructions mentioned on the provided manual. The cycle
196 conditions during PCR were: 1 cycle for 5 min at 95 °C for initial denaturation

197 followed by 40 cycles at 95 °C for 20 seconds, 65°C for 15 seconds s, 72 °C for 15
198 seconds, followed by a final extension of 1 min at 72 °C.

199 For exon 3: PCR was executed employing the Go Taq (R) Hotstart Green Master
200 mix (Cat #M5122, Promega Corporation, USA) according to the instructions
201 mentioned on the provided manual. The cycle conditions during PCR were: 1 cycle
202 for 5 min at 95 °C for initial denaturation followed by 35 cycles at 95 °C for 30 s,
203 58°C for 45 s, 72 °C for 45 s followed by a final extension of 10 min at 72 °C.

204 Purification of the PCR products was done using PCR Clean Up for **DNA**
205 **Sequencing (Cat. No BT5100, Bio Basic Inc, Canada)** following the manufacturer
206 protocol. Genotypic analysis was performed to detect mutations responsible for
207 infertility by PCR amplification of the fertile and infertile male (n=80) and female
208 subjects (n=88) within the region of exon 2 (214bp) and exon 3 (606bp) of *KISS1*
209 gene. The obtained sequences were directly compared to previously published
210 *KISS1* gene sequence using the MEGABLAST search tool in the National Center
211 for Biotechnology Information (NCBI) database. Sequence files were imported into
212 Chromas Lite, and then assembled using Molecular Evolutionary Genetic Analysis
213 version 6.0.

214 All coding exons (exons 2 and 3) of *KISS1* gene were PCR amplified with specific
215 primers as follows:

	Forward primer (5' to 3')	Reverse primer (3' to 5')
exon 2	CAGATCCTGTGCCTGACCT	CCCACTCCTTTCCCCAGAG
	A	

216

exon 3	ATGGGATGACAGGAGGTGT TG	ACCATCCATTGAGGATGGA AG
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217 **Statistical Analysis:** Statistical analyses were performed using IBM Statistical
218 Package for the Social Sciences (IBM SPSS version 21; IBM Corp Inc, Armonk,
219 NY). Continuous variables (such as age, LH, FSH etc.) were represented as Mean
220 $\pm$ standard deviation and the evaluation of the categorical variables (BMI) was
221 expressed in terms of frequencies and percentages. To compare continuous and
222 categorical variables, Independent sample t-test and Pearson's chi square test was
223 applied. Correlations were adjusted for age and BMI for hormonal associations
224 (logistic regression). SNP data was calculated by chi-squared statistics, Odds ratio
225 with 95% confidence interval was calculated for genotype and allele frequency
226 analysis.

227 **Results:**

228 The results of the study showed that infertile males were obese and were at a
229 lower age bracket as compared to fertile group ($p < 0.001$). Furthermore, the
230 infertile females also demonstrated a higher BMI as compared to the fertile
231 females ($p < 0.001$). The sperm count and motility was decreased along with
232 increased abnormal morphology in the infertile males as compared to the fertile
233 males ($p < 0.01$) (Table 1). A similar trend in terms of hormonal profile was
234 observed in the infertile female subjects; however, no difference for age was
235 recorded (Table 1). Comparison of hormones in Table 2 show significantly high
236 levels of KP, FSH, LH and Testosterone in fertile male, however KP, LH and
237 Estradiol levels were significantly higher in fertile female subjects with low FSH
238 levels. When tested for the correlations of Kisspeptin levels on the male
239 hormones of hypothalamo-hypophyseal-gonotrophic axis; FSH showed

240 moderate positive **relationship** with KP levels ($r=0.67$; $p<0.001$), while
241 Testosterone ($r=0.38$; $p<0.01$) showed **weak correlation with** fertility in all
242 subjects. Similarly, KP showed weak positive **correlation** with Estradiol
243 ($r=0.466$; $p=0.001$) while no **relationship** was observed with LH and FSH. All
244 correlations were lost when adjusted for age and BMI ($p>0.05$).

245 **Table 3 shows** the genotype distribution of the polymorphic EXON 2 and 3
246 sequences. This study was able to identify one polymorphism in exon2 [E1225K
247 (G/A 3673)] and 3 unique polymorphisms in exon3 [P1945A (C/G 5833);
248 Insertion of T at 6075; G2026G (C/G 6078)] in the study population.
249 Interestingly, these polymorphisms were observed in higher frequency in
250 infertile group (both genders) versus the fertile; yet the presence or absence of
251 polymorphic site in both exons of interest failed to reveal any significant
252 difference in this study population ($p>0.05$).

253 This study documented one novel result in the study cohort. In a sample of $n=80$
254 males and $n=88$ females; 03 subjects in each sex group tested positive for all 03
255 of the polymorphisms in exon-3 region. When their data was linked with the
256 polymorphic status; it was observed that 04 out of 06 individuals (male female
257 combined) were smokers with low KP and its related hormonal levels at various
258 points during the study. Furthermore, their sperm parameters fell in the abnormal
259 category (males) or attempt at **Intracytoplasmic Sperm injection** (ICSI) was
260 unsuccessful (females) (**Table 4 and 5**). **This result shows a probable effect of**
261 **environmental changes on genetic alterations in the KP gene and its secondary**
262 **effect on the reproductive axis.**

263 **Discussion:**

264 The relationship of KP with the interruption of hypothalamic-pituitary-gonadal
265 axis can be demonstrated by high concordance of the phenotypes between
266 comparable genetic variants present in GnRH receptor, FSH and its receptor, LH
267 and its receptor in mice and humans (Rehman, Jamil et al. 2015). In male
268 subjects, we have observed low levels of KP with concomitant decrease in
269 gonadotropin and sex steroid hormone levels, which is comparable to studies in
270 male infertile subjects(Haris Ramzan, Ramzan et al. 2015). This can probably
271 be an explanation of KP role in preservation of spermatogenesis and hence
272 fertility. Low FSH levels was observed in normozoospermic and azospermic
273 infertile male subjects by Ramzan et al which was not significantly different in
274 fertile and infertile males (Haris Ramzan, Ramzan et al. 2015). The significant
275 low FSH levels in infertile male subjects of our study may be explained by lack
276 of stratification of subjects into infertile categories on the basis of sperm
277 parameters. In the female infertile subjects a high FSH explains the negative
278 feedback interplay on HPG axis due to decrease in Estradiol secretion. Literature
279 also supports a raised FSH in infertile females (Prasad, Parmar et al. 2015).

280 Kisspeptin is now safely and successfully used in both healthy and infertile
281 human subjects after trials in United Kingdom, and it is possible that in the future
282 the Kisspeptin signaling may be used as a target in the treatment of reproductive
283 disorders (Hameed, Jayasena et al. 2011).

284 The role of Kisspeptin injections to stimulate the secretion of LH and FSH in
285 numerous mammalian species including rats, mice, sheep, cows and monkeys is
286 supportive of our statement (Navarro, Castellano et al. 2005). Furthermore, a
287 study done by Dhillon et al 2015, documents that KP infusion significantly
288 increased plasma LH, FSH, and testosterone levels (Clarke, Dhillon et al. 2015)

289 is supportive of our study in which infertile study subjects had low KP, LH,
290 FSH and testosterone in male subjects. The correlation of KP with estradiol in
291 both genders explains that KP requires estradiol secretion to stimulate GnRH
292 secretion as suggested by the study in which ovariectomy abolished the KP-
293 induced GnRH release in pubertal monkeys, and estradiol replacement resulted
294 in partial recovery of KP- induced GnRH release (Guerriero, Keen et al. 2012).
295 Kisspeptin represents and should be investigated in the treatment of fertility
296 disorders characterized by low gonadotropins or anovulation. (Clarke, Dhillon et
297 al. 2015)

298 Higher KP in non-obese males and non-obese young females were observed in
299 our study, which might be due to the fact that KP effects negatively on body
300 weight and calorie consumption (Walewski, Ge et al. 2014, Stengel 2011 #214,
301 (Lin, 2015 #216). BMI and body fat % age were noted to be high in infertile
302 groups while KP was low which is perhaps due to certain changes in the sex
303 hormones which regulates obesity by increasing the serum triglycerides levels
304 (Shamai, Lurix et al. 2011, Kołodziejewski, Pruszyńska-Oszmałek et al. 2018). It
305 is a well-established fact that there are various mechanisms how obesity causes
306 infertility (Talmor and Dunphy 2015). There is an ample evidence that KP
307 signaling tends to decrease the metabolic rate and initiates glucose intolerance
308 and increased body fat (Holmes 2014).

309 In this study, no clear-cut difference was observed in the genetic mutations
310 amongst fertile and infertile males and female subjects. Three unique *KISS1*
311 mutations were identified all together in unrelated subject in each gender
312 category. The absence of this variant in the fertile female group suggests that
313 this is a rare mutation which has a major qualitative effect on the *KISS* gene.

314 However only 1 fertile male tested positive for this mutation; when we checked
315 the paternal age and status of fertility in the last 5 years, it was identified that
316 this gentleman was 33-year-old and had a baby 4 years ago. Perhaps this
317 substitution mutation is related to the age group and environmental or stress
318 related changes. There are examples in literature where same SNPs resulted in
319 diverse expressions. As an example in Chinese population a SNP of amino acid
320 substitution (P110T) in KISS1 in females with CPP was found to be statistically
321 interconnected to infertility whereas in Korean girls the same mutation had a
322 protective effect on infertility (Luan, Zhou et al. 2007) (Ko, Lee et al. 2010).
323 Yet, no data is available from the current literature to support this claim.
324 Therefore, more work is required to assess the functionality or causality of this
325 mutation with infertility.

326 In terms of mutations in exon 3, we found no link of the SNP's with infertility.
327 These mutations i.e. insertion of Thiamine at position 6075 and substitution of
328 Proline to Alanine at position 1945 may be explained as due to faulty gene
329 regulation process related with age, since all these individuals were in the age range
330 of 34 to 48 year. Interestingly, in the fertile group with these mutations; one female
331 had delivered a baby within last 7 months. This finding suggests that silent
332 mutations at these positions, does not affect the functional role of Kisspeptin
333 protein. The study is limited in terms of being a uni-centric study with a small
334 sample size in which the impact of polymorphism in infertile females on the basis
335 of cause of infertility has not been taken into consideration. Furthermore, the
336 association of gene variation of KISS1 could have been further validated in terms
337 of impact on different altered sperm parameters.

338 However, this is the first study in this region that has attempted to explore a cause
339 effect relationship of *KissI* gene variation with hormones of reproduction and
340 impact on fertility status in both male and female infertile subjects.

341 **Conclusion:** Role of KP in regulation of normal reproductive functions can be
342 explained on the basis of its effect on secretion of gonadotropins and sex steroids.
343 Polymorphism in exon2 [E1225K (G/A 3673)] and 3 unique polymorphisms in
344 exon3 [P1945A (C/G 5833); Insertion of T at 6075; G2026G (C/G 6078)] can be
345 further explored as plausible cause of decreased KP production in infertile male and
346 female subjects. Further detailed studies are warranted for understanding of the
347 mechanistic role of genetic variations of KP in infertility.

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353

- Abbara, A., C. Jayasena, A. Comninou, M. Nijher, G. Christopoulos, C. Izzi-Engbeaya, M. Sridharan, S. Narayanaswamy, D. Ashby and M. Ghatei (2014). "Kisspeptin: a novel physiological trigger for oocyte maturation in in-vitro fertilisation treatment." The Lancet **383**: S17.
- Clarke, H., W. S. Dhillon and C. N. Jayasena (2015). "Comprehensive review on kisspeptin and its role in reproductive disorders." Endocrinology and Metabolism **30**(2): 124-141.
- Cooper, T. G., E. Noonan, S. Von Eckardstein, J. Auger, H. Baker, H. M. Behre, T. B. Haugen, T. Kruger, C. Wang and M. T. Mbizvo (2010). "World Health Organization reference values for human semen characteristics." Human reproduction update **16**(3): 231-245.
- de Tassigny, X. d. A., L. A. Fagg, J. P. Dixon, K. Day, H. G. Leitch, A. G. Hendrick, D. Zahn, I. Franceschini, A. Caraty and M. B. Carlton (2007). "Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene." Proceedings of the National Academy of Sciences **104**(25): 10714-10719.
- Fatima, S. S., R. Rehman and B. Chaudhry (2014). "Body mass index or body fat! which is a better obesity scale for Pakistani population?" JPM: Journal of the Pakistan Medical Association **64**(11): 1225.
- Funes, S., J. A. Hedrick, G. Vassileva, L. Markowitz, S. Abbondanzo, A. Golovko, S. Yang, F. J. Monsma and E. L. Gustafson (2003). "The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system." Biochemical and biophysical research communications **312**(4): 1357-1363.
- Guerriero, K. A., K. L. Keen, R. P. Millar and E. Terasawa (2012). "Developmental changes in GnRH release in response to kisspeptin agonist and antagonist in female rhesus monkeys (*Macaca mulatta*): implication for the mechanism of puberty." Endocrinology **153**(2): 825-836.
- Hameed, S., C. N. Jayasena and W. S. Dhillon (2011). "Kisspeptin and fertility." Journal of Endocrinology **208**(2): 97-105.
- Haris Ramzan, M., M. Ramzan, F. Ramzan, F. Wahab, M. Jelani, M. Aslam Khan and M. Shah (2015). "Insight into the Serum Kisspeptin Levels in Infertile Males." Archives of Iranian Medicine (AIM) **18**(1).
- Holmes, D. (2014). "Metabolism: kisspeptin signalling linked to obesity." Nat Rev Endocrinol **10**(9): 511.
- Irwig, M. S., G. S. Fraley, J. T. Smith, B. V. Acohido, S. M. Popa, M. J. Cunningham, M. L. Gottsch, D. K. Clifton and R. A. J. N. Steiner (2004). "Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat." **80**(4): 264-272.
- Ko, J. M., H. S. Lee and J. S. Hwang (2010). "KiSS1 gene analysis in Korean girls with central precocious puberty: a polymorphism, p. P110T, suggested to exert a protective effect." Endocrine journal **57**(8): 701-709.
- Kołodziejewski, P. A., E. Pruszyńska-Oszmałek, E. Korek, M. Sassek, D. Szczepankiewicz, P. Kaczmarek, L. Nogowski, P. Maćkowiak, K. W. Nowak and H. Krauss (2018). "Serum levels of spexin and kisspeptin negatively correlate with obesity and insulin resistance in women." Physiological research **67**(1): 45-56.
- Lizzer, S., Y. Boirie, M. Meyer and M. Vermorel (2003). "Evaluation of two foot-to-foot bioelectrical impedance analysers to assess body composition in overweight and obese adolescents." British Journal of Nutrition **90**(05): 987-992.
- Luan, X., Y. Zhou, W. Wang, H. Yu, P. Li, X. Gan, D. Wei and J. Xiao (2007). "Association study of the polymorphisms in the KiSS1 gene with central precocious puberty in Chinese girls." European Journal of Endocrinology **157**(1): 113-118.

403 Mumtaz, A., A. Khalid, Z. Jamil, S. S. Fatima, S. Arif and R. Rehman (2017). "Kisspeptin: a
 404 potential factor for unexplained infertility and impaired embryo implantation."
 405 International journal of fertility & sterility **11**(2): 99.

406 Navarro, V., J. Castellano, R. Fernandez-Fernandez, S. Tovar, J. Roa, A. Mayen, R.
 407 Nogueiras, M. Vazquez, M. Barreiro and P. Magni (2005). "Characterization of the potent
 408 luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54."
 409 Endocrinology **146**(1): 156-163.

410 Prasad, B., D. Parmar, N. J. I. J. o. M. R. Sharma and H. Sciences (2015). "A study on serum
 411 FSH, LH and prolactin levels among infertile women." **4**(4): 876-878.

412 Rehman, R., Z. Jamil, S. S. Fatima and F. Alam (2015). "Silent Mutation in KISS1and KISS1R
 413 and Unexplained Infertility."

414 Semple, R., J. Achermann, J. Ellery, I. Farooqi, F. Karet, R. Stanhope, S. O'rahilly and S.
 415 Aparicio (2005). "Two novel missense mutations in g protein-coupled receptor 54 in a
 416 patient with hypogonadotropic hypogonadism." The Journal of Clinical Endocrinology &
 417 Metabolism **90**(3): 1849-1855.

418 Shamai, L., E. Lurix, M. Shen, G. M. Novaro, S. Szomstein, R. Rosenthal, A. V. Hernandez
 419 and C. R. Asher (2011). "Association of body mass index and lipid profiles: evaluation of a
 420 broad spectrum of body mass index patients including the morbidly obese." Obesity
 421 surgery **21**(1): 42-47.

422 Silveira, L. G., S. D. Noel, A. P. Silveira-Neto, A. P. Abreu, V. N. Brito, M. G. Santos, S. D. C.
 423 Bianco, W. Kuohung, S. Xu and M. Gryngarten (2010). "Mutations of the KISS1 gene in
 424 disorders of puberty." The Journal of Clinical Endocrinology & Metabolism **95**(5): 2276-
 425 2280.

426 Skorupskaite, K., J. T. George and R. A. Anderson (2014). "The kisspeptin-GnRH pathway
 427 in human reproductive health and disease." Human reproduction update **20**(4): 485-500.

428 Talmor, A. and B. Dunphy (2015). "Female obesity and infertility." Best Practice &
 429 Research Clinical Obstetrics & Gynaecology **29**(4): 498-506.

430 Vaziri, H., A. Rafeie and Z. Siapoosh (2017). "Q36R (rs 35431622) Polymorphism in KISS1
 431 Gene and Idiopathic Female Infertility in a Northern Iranian Population." Gene, Cell and
 432 Tissue **4**(3).

433 Walewski, J. L., F. Ge, H. Lobdell Iv, N. Levin, G. J. Schwartz, J. R. Vasselli, A. Pomp, G. Dakin
 434 and P. D. Berk (2014). "Spexin is a novel human peptide that reduces adipocyte uptake of
 435 long chain fatty acids and causes weight loss in rodents with diet-induced obesity."
 436 Obesity **22**(7): 1643-1652.

437 WHO, E. C. (2004). "Appropriate body-mass index for Asian populations and its
 438 implications for policy and intervention strategies." Lancet **363**: 157-163.

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