Circulating kisspeptin and anti-müllerian hormone levels, and insulin resistance in women with polycystic ovary syndrome: A systematic review, meta-analysis, and meta-regression

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Appendix A: The online version of this article contains the following Supplementary Information: (a) one eMethod (Pubmed search strategy); (b) two eTables; and (c) seven eFigures.

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ABSTRACT

Objective: This systematic review and meta-analysis aimed to summarize the available evidence regarding circulating kisspeptin and anti-müllerian hormone (AMH) and the homeostasis model assessment of insulin resistance (HOMA-IR) index in adolescents and women with and without polycystic ovary syndrome (PCOS).

Method: We performed a comprehensive literature search in Medline, Embase, Cochrane, Scopus, and Web of Science for studies evaluating circulating kisspeptin levels in women with and without PCOS published until September 24th, 2020. Coprimary outcomes were the HOMA-IR index and AMH. The quality of included studies was assessed using the Newcastle-Ottawa Scale. Random-effects models were used to estimate outcomes, and effects reported as mean difference (MD) or standardized MD (SMD) and their 95% confidence interval (CI). The systematic review and meta-analysis was registered in the International Prospective Register of Systematic Reviews (PROSPERO) as number CRD42020205030.

Results: We evaluated 18 studies including, 1,282 PCOS cases and 977 controls. Participants with PCOS were younger (MD=-2.38 years, 95%CI -4.32 to -0.44), with higher BMI (MD=1.16, 95% CI 0.54 to 1.78), waist-to-hip ratio (MD=0.04, 95%CI 0.02 to 0.05), circulating kisspeptin (SMD=1.15, 95%CI 0.68 to 1.62), luteinizing hormone (SMD=1.29, 95%CI 0.76 to 1.83), AMH (SMD=0.97, 95%CI 0.60 to 1,34), total testosterone (SMD=2.48, 95%CI 1.73 to 3.23), free testosterone (SMD=1.37, 95%CI 0.56 to 2.17), and dehydroepiandrosterone sulfate (SMD=0.72, 95%CI 0.32 to 1.13) levels, and Ferriman-Galweg score (SMD = 5.08, 95%CI 2.76 to 7.39), and lower sex hormone-binding globulin level (SMD=-1.34, 95%CI -2.15 to -0.52). Besides, participants with PCOS had higher HOMA-IR index (SMD=0.76, 95%CI 0.35 to 1.17), and circulating insulin (SMD=0.75, 95%CI 0.30 to 1.19), leptin (SMD=2.82, 95%CI 1.35 to 4.29), and triglycerides (SMD=2.15, 95%CI 1.08 to 3.23) levels than participants without the syndrome. The meta-regression did not identify significant factors influencing circulating kisspeptin.

Conclusion: Patients with PCOS showed higher kisspeptin, LH, insulin, AMH, and androgen levels and HOMA-IR index, and lower sex hormone-binding globulin levels than those without the syndrome.

KEYWORDS: Polycystic ovary syndrome; Kisspeptin; Metastin; HOMA-IR; Insulin; Antimüllerian hormone

Abbreviations

AMH: Anti-müllerian hormone; BMI: Body mass index; CI: Confidence interval; DHEA-S: dehydroepiandrosterone sulfate; FSH: Follicle-stimulating hormone; GnRH: gonadotrophinreleasing hormone; HDL: High density lipoprotein; HOMA-IR: Homeostatic model assessment of insulin resistance; IQR: Interquartile range; LDL: Low density lipoprotein; LH: Luteinizing hormone; MD: Mean difference; NOS: Newcastle–Ottawa Scale; PCOS: polycystic ovary syndrome (PCOS); SD: Standard deviation; SHBG: Sex hormone-binding globulin; SMD: Standardized mean difference; VLDL: Very low density lipoprotein cholesterol

Introduction

Kisspeptins are a group of brain neuropeptides initially described as metastasis suppressors [1]. The kisspeptin precursor has 145 amino acids that, by proteolysis, produce kisspeptin-54 (also know as metastin) that is considered the active product. Kisspeptin 13 and kisspeptin-14 are products from the degradation of kisspeptin-54 [2,3]. Kisspeptin (encoded by *KISS1*) influences gonadotrophin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion and might contribute to the development of some features of the PCOS [4,5]. Although kisspeptin is expressed in discrete brain regions, it is also present in peripheral tissues like fat, liver, and pancreas [4]. Women with PCOS have an increased expression of kisspeptin receptors in granulosa lutein cells [6]. Clinical studies reported variable and heterogeneous results concerning circulating kisspeptin levels in women with PCOS [7,8].

PCOS is associated with different degrees of hypothalamic-pituitary-ovarian axis function disorders, hyperandrogenemia, excessive body weight, insulin resistance, and genetic factors [9-12]. The prevalence of insulin resistance is higher in women with PCOS than in age- and weight-matched women without it [13,14], being about 30% and 70%, respectively, in lean and obese women with PCOS [13]. On the other hand, women with PCOS display increased circulating anti-müllerian hormone (AMH) that correlates with the number of antral follicles, and the AMH measurement diagnostic efficacy is superior to follicular count [15]. Besides, it seems to be a specific PCOS and reproducible marker from one menstrual cycle to another [16]. Metabolic syndrome components and indexes of insulin resistance, including homeostasis model assessment IR index (HOMA-IR), are frequently altered in patients with PCOS [10,11]. This systematic review and meta-analysis aim to study kisspeptin levels in patients with PCOS, to assess possible associations between kisspeptin and insulin resistance and AMH levels, and to perform meta-regression analyses of factors influencing kisspeptin levels.

Methods

This study was undertaken following the Meta-Analysis Of Observational Studies in Epidemiology (MOOSE) Group Recommendations [17]. The protocol was registered with the international Prospective Register of Systematic Reviews (PROSPERO: CRD42020205030). A formal institutional review board approval was not required, since this analysis consisted of the pooling of published studies.

Search strategy

A literature search was performed on PubMed/Medline, Scopus, Cochrane, Web of Science, and Embase databases, using a combination of the following terms and their synonyms: "polycystic ovary syndrome", "Stein-Leventhal syndrome", "kisspeptin", and "metastin". The full PubMed search strategy using Boolean operators AND or OR is shown in Appendix 1, eMethod. The search included articles in any language from initial publications of human kisspeptin measurements in 2005 until September 24th, 2020. Found abstracts were pooled into an EndNote X7.2 (EndNote, Clarivate Analytics, Philadelphia, Pennsylvania, United States) to identify and remove duplicate records. Besides, references from selected articles and Google Scholar were screened for additional potential publications.

Eligility criteria and outcomes of interest

This systematic review and meta-analysis include prospective and retrospective observational studies assessing circulating kisspeptin levels and endocrine, metabolic, and biochemical outcomes in non-pregnant adolescents and women, with and without PCOS, irrespective of age, parity, ethnicity, country of origin, publication date, and language. Studies reporting women with other clinical or biochemical forms of hyperandrogenism, diabetes or chronic diseases, metabolic alterations, or receiving hormone treatments were excluded. The PI(E)COS (Population, Intervention or Exposure, Comparators, Outcomes, Study Design) criteria were developed a priori to guide the scope of the review, along with the procedures, selection, and synthesis of the literature search. Studies were eligible if they met the following inclusion criteria: *Population:* non-pregnant adolescents and women not receiving any treatment. Intervention/Exposure: PCOS diagnoses reached by the revised Rotterdam ESHRE/American Society of Reproductive Medicine Criteria or other internationally recognized scientific organizations [18-20]. Comparator: participants without PCOS. *Outcomes*: The primary outcome was circulating kisspeptin level, and co-primary outcomes were HOMA-IR index and AMH levels. Secondary outcomes: reproductive

hormones, androgen-related endpoints, and glucose and lipid metabolites. *Study design*: observational studies including patients with all types and stages of PCOS.

Study selection and data extraction

We included prospective and retrospective observational studies reporting circulating kisspeptin in non-pregnant participants with and without PCOS. Studies reporting clinical, endocrine, metabolic, or biochemical outcomes of interest, such as body mass index (BMI), waist-to-hip ratio, HOMA-IR, insulin, leptin, reproductive hormones, androgens, glucose, and lipid metabolites were eligible for inclusion. Exclusion criteria were: (a) Circulating kisspeptin concentration was not available or could not be extracted from the study groups; (b) no appropriate control group; (c) participants receiving any treatment that might modify endocrine or metabolic outcomes; and (d) presence of another endocrine, metabolic or chronic disorders different from PCOS. Authors were contacted if supplementary information or clarification was required to analyze study eligibility.

Three of the authors (LO, MTLB, FRPL) independently evaluated full-text articles for compliance with inclusion and exclusion criteria. Disagreements were managed through discussion with all authors to reach a consensus. Extracted data included the year of publication, country of study conduction, the sample size for PCOS and control groups, participants characteristics, and outcomes per group. Data extraction was independently performed by 2 authors (LO, RSC) and disagreements were solved by a discussion with all authors.

Risk of bias assessment

The risk of bias of selected studies was assessed independently by two authors (GRPR, MTLB) using the Newcastle–Ottawa scale (NOS) [21]. The NOS consists of three parameters of quality: selection, comparability, and outcome assessment. The NOS assigns a maximum of four points for selection, two points for comparability, and three points for exposure or outcome. NOS scores of \geq 7 were considered as high-quality studies and NOS scores of 5–6 were considered moderate quality. Any discrepancies were addressed by a re-evaluation of the original article to reach a consensus.

Statistical analyses

Effects of the PCOS on outcomes were described as mean differences (MDs) or standardized mean differences (SMDs) and their 95% confidence interval (CI). MDs were used for age and BMI whereas all other outcomes were pooled as SMDs since the heterogeneity of the PCOS, studied population, lifestyle differences (including nutrition and physical activity), and laboratory differences. Studies reporting medians (m), and interquartile ranges (IQR), means were estimated by x=(a+2m+b)/4, where m is median and a and b are P25 and P75, respectively [22]. Standard deviations (SDs) were estimated using SD=IQR/1.35. When median and ranges were provided, the mean was estimated by x=(a+2m+b)/4 using the values of the median (m), the smallest and largest value (a and b, respectively); SD was estimated by SD=range/4 if the sample size was <70 and SD=range/6 if the sample size was >70 [23] (GRPR, FRPL). The fixedeffect model was initially planned if moderate or lower heterogeneity ($I^2 \leq 65\%$) was found. If $I^2 > 65\%$, the random-effect model was adopted. Assessment for among-study heterogeneity was performed by calculating I²: An I² value of 0-30% define low heterogeneity, 30-65 % moderate heterogeneity, and >65 % substantial heterogeneity [24]. A p < 0.1 for the Chi² test was defined as an indicator of heterogeneity; a Tau² >1 was defined as the presence of substantial statistical heterogeneity. The effects of clinical and statistical heterogeneity from meta-analyses were tested by the exclusion of one or several studies sufficed to decrease heterogen eity [25,26].

Calculated PCOS mean age, mean BMI, and mean HOMA-IR were pre-specified for subgroups analyses. We predefined subgroup for exploring potential sources of heterogeneity by (i) mean age groups, (ii) mean BMI groups, and (iii) mean HOMA-IR index (GRPR, FRPL, JS). Meta-regression analyses was used to explore kisspeptin heterogeneity (JS). Potential publication bias was estimated by the Begg's funnel plot [27] and the Egger's linear regression test [28].

Statistical analyses were conducted using Review Manager (RevMan 5.3; Cochrane Collaboration, Oxford, UK) and STATA software (version 10.0; College Station, TX, USA).

Results

Selection of studies

After the removal of duplicates, a total of 150 abstracts were identified through search engines, and one additional doctoral thesis was identified by manual search. Twenty-eight full-text items were evaluated for eligibility. Five papers did not report separated information for control groups, 4 included duplicate information, and one did not report the primary outcome (Figure 1). Finally, a total of 18 studies were evaluated for qualitative and quantitative assessment [29-47].

We make an effort to include all available studies or complementary information by contacting some authors. Dr. Zahraa H Al-Jelawy kindly provided a full complementary document (a doctoral thesis [30] of the article [29]. Dr. Huiying Zang provided the mean ages ± SD results not included in the full-publication [47]. Dr. Xiaoli Chen informed us that a publication in the Chinese language [48] was a preliminary article of the final publication in English [34]. We also contacted the correspondent author of 4 papers published over years [35, 49-51] without getting any response. Therefore, we excluded earlier publications to ensure the use of only the largest and most recent participants and to prevent data set inflation [35].

Characteristics of included studies

The 18 selected studies included 1,282 participants who suffer PCOS and 997 controls, published between 2006 [44] and 2020 [35, 39, 42] (Table 1). PCOS sample sizes across studies ranged from 20 [33] to 250 [37]. The PCOS diagnosis followed (i) the Rotterdam criteria in 17 studies [29-43, 45-47], and (ii) one study was based on the association of chronic anovulation (< 6 cycles in 12 months) with hyperandrogenemia [44]. Six studies were carried out in Turkey [33, 37, 38, 41,42,47}, two in China [34, 46], two in Iraq [29, 39], and one in each of the following countries: Bulgaria [43], Egypt [45], Ghana [31], Greece [44], Korea [40], Kuwait [36], Saudi Arabia [35] and Sri Lanka [32]. Table 1 displays information on the location and period of study, number of participants, mean age and BMI of participants with and without PCOS, as well as the main findings of studies meta-analyzed. Women with PCOS were younger than those without the syndrome (Table 2; Fig. 2A), had higher BMI (Table 2; Fig. 2B), and higher waist-to-hip ratio (Table 2; Fig. 2C).

Risk of bias assessment

Using the NOS scale, 13 studies were identified as high-quality [35-47], and the other five of moderate quality [29, 31-34] (Appendix A, eSupplementary Table 1). All publications identified the study population, patients were representative of average PCOS cases, and controls were derived from the same population as cases. In all studies, secure patient records were used for the ascertainment of PCOS and assessment of outcomes.

Meta-analyses of outcomes

Kisspeptin and reproductive homones

In 18 studies (n=2,259), circulating kisspeptin was significantly increased in women with PCOS as compared to control women (Table 2; Fig. 3A). In 16 studies (n=2,017), circulating LH was significantly increased in women with a previous diagnosis of PCOS (Table 2; Fig. 3B). In 15 studies (n=1,880), circulating FSH was not different in women with and without PCOS (Table 2; Fig. 3C). In 5 studies (n=743), circulating AMH was significantly higher in women with PCOS (Table 2; Fig. 3D). In 5 studies, there were no differences for both prolactin (Table 2; Fig. 3E), and in 8 studies estradiol between women with and without PCOS (Table 2; and Figure 3F). There were high heterogeneity of effects on outcomes across studies (I² 76%-98%; Table 2).

Androgen-related outcomes

In 14 studies (n= 1802), total testosterone was significantly increased in participants with PCOS (Table 2, Figure 4A). In 7 studies (n = 999), free testosterone was increased in participants with PCOS (Table 2, Figure 4B). In 9 studies (n = 1297), DHEA-S was significantly increased in participants with PCOS (Table 2, Figure 4C). In 7 studies (n = 999) SHBG, was significantly reduced in participants with PCOS (Table 2, Figure 4D). Finally, in 5 studies (n = 1037,) the modified Ferriman-Gallweg score was significantly increased in participants with PCOS (Table 2, Figure 4e). There were high heterogeneity of effects on these outcomes across studies (I² 91%-99%; Table 2).

Glucose and insulin-related outcomes

In 11 studies (n = 1593), glycemia was not significantly different in participants with and without PCOS (Table 2, Figure 4A). In 10 studies (n = 1398), the HOMA-IR index was significantly increased in participants with PCOS (Table 2, Figure 4B). In 8 studies (n = 1104), mean circulating insulin was significantly increased in participants with PCOS (Table 2, Fig. 4C). In four studies (n = 832), circulating leptin was significantly increased in women with PCOS (Table 2; Fig. 3D). There was a high heterogeneity of effect on these outcomes across studies (I² 92%-98%; Table 2).

Lipid-related outcomes

In 4 studies (n = 957) there were no significant differences in total cholesterol, HDL-cholesterol and LDL-cholesterol (Table 2; Figures 6A, 6B, 6C) between participants with and without PCOS. In four studies (n = 1045) triglycerides were significantly increased in participants with PCOS (Table 2; Figure 6D). There was a high heterogenity of effects on lipid outcomes across studies (I² 95%-100%; Table 2).

Subgroup analyses and meta-regression

Evaluation of subgroup effects on circulating kisspeptin matched studies and not matched studies by the mean age of studied groups ($I^2 = 97\%$) displayed a similar trend with higher levels in patients with PCOS (eSuplementary figure 1). Subgroup analysis of circulating kisspeptin studies matched by mean BMI ($I^2 = 36\%$) and not matched by mean BMI of studied groups showed higher levels in participants with PCOS ($I^2 = 97\%$; eSuplementary figure 2). There was not a significant difference in subgroup analysis of circulating kisspeptin by matched mean HOMA-IR index with a difference < 0.60 ($I^2 = 87\%$) whereas non-matched mean HOMA-IR (difference > 0,70) displayed a significant difference ($I^2 = 19\%$; eSuplementary figure 3). The results of subgroup of kisspeptin meta-analysis showed moderate heterogeinity for studies matched by mean age, mean BMI and HOMA-IR index <0.60.

Meta-regression analyses showed that are no influences of age, HOMA-IR index, circulating insulin, LH, and anti-müllerian hormone on circulating kisspeptin in women with PCOS (eSuplementary Figures 4A, 4B, 4C, 4D and 4E, respectively).

Sensitivity analysis

Sensitivity analyses were performed, including the removal of studies one by one, for kisspeptin, HOMA-IR index and AMH (Table 3). The I² values remained high for circulating kisspeptin (94% to 96%, n = 18 studies) and HOMA-IR index (80% to 92%), whereas for AMH was 61% by deleting the Kaya et al. study [41]. A sensitivity analysis for kisspeptin was also performed deleting 4 studies [29,31,32,44] and heterogenity was reduced to 67% (eSuplementary figure 4).

Publication bias

Funnel plots of outcomes available in more than 10 studies (kisspeptin and HOMA-IR) showed that there was asymmetry of points for kisspeptin (Egger test =7.535, p<0.001) and symetry for HOMA-IR index (Egger test = 0.136, p=0.902). When 3 studies [29,31,32] with suspected publication bias for kisspeptin, the remaining 15 studies did not display publication bias (Egger test = -0.172, p=0.863; eSupplementary figure 5, eSuplementary table 2). Funnel plots and Egger's tests were also calculated for HOMA-IR index (eSuplementary figure 6, eSupplementary table 2). There were publication bias for circulating LH, FSH, and total testosterone levels (eSuplementary table 2).

Discussion

Meta-analyzed PCOS patients were younger with higher BMI and waist-to-hip ratio in comparison to participants without the syndrome. They had increased circulating kisspeptin, LH, AMH, total and free testosterone, DHEA-S, insulin, and leptin whereas the SHBG was lower than in the control group. They also had increased HOMA-IR index, and circulating insulin, leptin, and triglyceride levels. There were no significant differences for circulating FSH, estradiol, prolactin, glucose, total cholesterol, HDL-cholesterol, and LDL-cholesterol. Meta-regression analysis indicates that kisspeptin is not influenced by age, HOMA-IR index, circulating insulin, LH, and AMH.

In this meta-analysis of 18 studies, circulating kisspeptin levels were significantly higher in 1282 PCOS cases than in 977 participants without the syndrome. Kisspeptin was initially described as a suppressor of metastasis in human melanoma and named metastin [52]. It acts on an orphan G-protein coupled receptor (GPR-54) and is present in the central nervous system and the pituitary gland [53]. New research has now shown that the novel kisspeptin receptor agonist MVT-602 mediates the increase of LH levels in healthy women and those with PCOS or hypothalamic amenorrhea [54]. Hypothalamic kisspeptin has a regulatory effect on GnRH neurons to control the reproductive axis, and its release is under the influence of nutrients and energy reserve [55]. In both rodents and humans, kisspeptin is also present in limbic brain regions [56]. Kisspeptin neurons may integrate information from other hormones, environmental factors, stress, and metabolic variables. Experimental and clinical evidence suggests that kisspeptin may switch the onset of puberty [57,58]. Both the neurokinin B receptor and the kisspeptin receptor are also present in normal human granulosa cells, while their lower expression alters normal follicle development [59]. Therefore, the increased kisspeptin levels detected in PCOS patients may act on both the pituitary and the ovaries. Preliminary studies suggest that kisspeptin administration to women with PCOS partially stimulates gonadotropins and ovulation [60]. However, kisspeptin does not neutralize menstrual dysfunction and long cycles [61].

In the present study, PCOS patients showed significantly increased AMH levels when compared to non-PCOS subjects. AMH is s a dimeric glycoprotein secreted by the granulose cells that regulates folliculogenesis and is a marker of the number of antral follicles [62, 63]. In women with normal ovaries, high AMH has an inhibitory effect on antral follicle development while low serum AMH is associated with ovulation, pregnancy rate and greater live birth rates [64]. AMH levels are related to increased fasting glucose and insulin levels , testosterone, and BMI in PCOS patients [65]. AMH values higher than 5.1 ng/mL are considered a surrogate marker of hyperandrogenism since positively correlates with total testosterone, 17-hydroxyprogesterone, and LH [66]. AMH levels are highest when patients have all three major characteristics of PCOS (menstrual disorders, hyperandrogenism, and ultrasound evidence of polycystic ovaries) [65,67].

PCOS menstrual disfunction and amenorrhea are associated with increases in circulating LH, androgens, and insulin, and reduced SHBG [68]. The increased LH levels in PCOS patients may be a consequence of the sustained increase of kisspeptin release

that might promote alterations on the hormone feedback mechanism and persist permanently disrupted. Besides, there is a correlation between AMH and kisspeptin in PCOS patients that does not exist in normal women [42]. We found higher levels of circulating glucose, insulin and leptin, as well as the HOMA-IR index, BMI, and waistto-hip ratio in patients with PCOS as compared to the control group. The full-length kisspeptin has 54 aminoacid may regulate glucose metabolism, although several smaller peptides (with 10, 13, and 14 aminoacids) are also produced in vivo with a similar biologic effect to the principal kisspeptin-54 [1]. In non-diabetic subjects, higher kisspeptin levels are associated with hyperinsulinism independently of sex, age, adiposity post-load glucose, and insulin sensitivity, and is inversely correlated with BMI and waist circumference [69]. In normal subjects, kisspeptin administration does not alter gut hormone, appetite, and food consumption [70].

Abdominal fat tissue accumulation during puberty has been postulated that is central in the progression of PCOS and alterations of kisspeptin, hyperandrogenism, and insulin resistance which at the same time increases the abdominal fat deposition [71,72]. Previous studies demonstrates that PCOS women have significantly higher levels of triglycerides closely related to waist circumference and insulin resitance [73]. The alteration of lipid metabolism in PCOS patients are related with the risk of insulin resistance and higher HOMA-IR [74]. In non-diabetic subjects, these endocrine and metabolic responses have been associated with circulating kisspeptin levels [56]. Animal experiments and human studies have demostrated associations between circulating kisspeptin levels and glucose metabolism and insulin secretion by altering adiposity [70]. A positive correlation between kisspeptin levels and HOMA-IR index has been reported in women with PCOS [43]. However, our meta-regression of metaanalyzed women showed that circulating kisspeptin is not influenced by age, HOMA-IR index, and AMH.

Our meta-analyses also showed that PCOS patients had increased circulating total and free testosterone, and DHEA-S levels, and reduced levels of DHEA-S. Circulating androgen levels are bound to proteins that determine distribution, metabolism, and biological effects. The unbound fraction of androgens are responsible for the caption on target organs and functions. SHBG is the principal plasma transport protein for sex steroids, regulating the androgen bioavailability to target tissues [75]. The reduction of SHBG in patients with PCOS has been related with SHBG gene polymorphisms [76,77].

Strengths

This meta-analysis has the strength that PCOS patients were diagnosed according to standardized international scientific recommendations, including 1282 cases, and without possible duplicated populations. Since published studies were from 2006 to 2020 measuring hormones and metabolic outcomes with different methods, we calculated all those outcomes as SMD to neutralize the risk of bias related to methodological laboratory evolution. Our meta-analysis fills some gaps and controversies from individual studies concerning endocrine and metabolic PCOS knowledge concerning pituitary and ovarian hormones, androgens, and insulin secretion.

Limitations

The high statistical heterogeneity is a limitation of our study that may be due to (i) the small sample size, varying from only 20 to 250 women with PCOS, which may cause unexpected sampling error, (ii) to variable age of participants and PCOS characteristics; (iii) difference in nutrition and physical activity. Different PCOS phenotypic variants may likely have different levels of kisspeptin since its short half degrees of insulin resistance and hyperandrogenism. Physical activity is also a determinant of the endocrine and metabolic status of patients with PCOS that was not reported in studies meta-analyzed. Exercise training and physical activity in PCOS may have benefits, improving the anthropometric measurements such as BMI, waist circumference, and free androgen index, whereas metabolic parameters are not improved [62,79,80].

New studies are needed to answers questions and doubts generated by the ongoing meta-analysis, and to clarify relationships among kisspeptin, AMH, insulin resistance, and androgens. Future clinical studies should clearly define PCOS women according to the admitted phenotypes.

Conclusions

In comparison to controls, adolescents and women with PCOS have high kisspeptin levels associated with increased insulin, LH, AMH, total and free testosterone, and DHEA-S, and lower SHBG levels. The HOMA-IR index, and circulating insulin, leptin, and triglycerides are also higher in patients with PCOS. The metaregression analysis did not identify factors participating in kisspeptin regulation and metabolism in PCOS patients.

Author contributions

Conceptualization of the study and PROSPERO protocol design: FRPL, MTLB, and GRPR. Data curation LO, RSC, and FRPL. Risk of bias assessment: MTLB, and GRPR. Meta-analyses and related methodology were performed by GRPR, JS, and FRPL. The initial manuscript was drafted by FRPL, MTLB, and GRPR, and all authors contributed with critical intellectual input, reviewing and approving the final manuscript.

Disclosure statement

The authors report no conflicts of interest and are alone responsible for the content and the writing of the article.

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Details of ethics approval

None required.

Data statement

The present meta-analysis was based on published articles. All summary data generated during this study are included in this published article. Raw data used for the analyses are available presented in the original reviewed articles

Declaration of Competing Interest

The authors report no conflicts of interest and are alone responsible for the content and the writing of the article.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/ ...

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- 1 Fig. 2. Forest plots comparing participants with and without polycystic ovary syndrome
- 2 (mean difference), from the top to the bottom, for age (Fig. 2A), BMI (Fig. 2B), and waist-
- 3 to-hip ratio ratio (Fig. 2C).
- 4
- 5 2A. Age

	Polycystic of	rome	Cont	rol gro	up		Mean Difference	Mean Difference			
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI		
Aljelawy ZH 2019	26.97	0.69	70	30.33	1	18	5.7%	-3.36 [-3.85, -2.87]	+		
Anim-Ankumah A 2019	32.8	0.9	41	33.55	1.12	40	5.7%	-0.75 [-1.19, -0.31]	-		
Branavan U 2019	24.67	0.88	55	33.8	0.53	110	5.7%	-9.13 [-9.38, -8.88]	+		
Celik N 2018	31	3.68	20	35.3	3.1	20	5.4%	-4.30 [-6.41, -2.19]			
Chen X 2010	22.42	2.69	42	18.6	0.68	20	5.7%	3.82 [2.95, 4.69]			
Daghestani MH 2020	25.06	3.88	104	24.96	5.56	109	5.6%	0.10 [-1.18, 1.38]			
El-Shehawy YM 2015	24.4	1.2	23	27.15	1.7	18	5.7%	-2.75 [-3.68, -1.82]			
Emekci Ozay O 2016	23.99	4.63	250	24.43	4.39	150	5.7%	-0.44 [-1.35, 0.47]			
Gorkem U 2018	27.76	4.65	60	27.92	4.67	57	5.5%	-0.16 [-1.85, 1.53]			
Ibrahim RO 2020	30.09	7.6	60	31.67	7.16	40	5.1%	-1.58 [-4.52, 1.36]			
Jeon YE 2013	23.72	5.32	54	24.92	2.94	36	5.5%	-1.20 [-2.91, 0.51]			
Kaya C 2019	28.75	3.49	29	30.25	4.66	27	5.4%	-1.50 [-3.67, 0.67]			
Mut A 2020	23.51	4.33	70	30.88	5.57	58	5.5%	-7.37 [-9.13, -5.61]			
Nyagolova PV 2016	24.99	0.49	87	26.65	0.71	42	5.7%	-1.66 [-1.90, -1.42]	•		
Panidis D 2006	23.97	0.93	56	26.85	1.06	13	5.7%	-2.88 [-3.51, -2.25]			
Rashad NM 2019	30.6	6.57	105	32.44	7.08	90	5.4%	-1.84 [-3.77, 0.09]			
Wang T 2019	27.53	5.08	73	31.96	3.96	63	5.5%	-4.43 [-5.95, -2.91]			
Yilmaz SA 2014	21	5.8	83	24.5	5.7	66	5.5%	-3.50 [-5.36, -1.64]			
Total (95% CI)			1282			977	100.0%	-2.38 [-4.32, -0.44]	◆		
Heterogeneity: Tau ² = 17.	06; Chi ^z = 265	3.06, df = 1	7 (P < 0.	.00001);	 ² = 99	9%					
Test for overall effect: Z =	2.41 (P = 0.02)							Polycystic ovary syndrome. Control group		
									r olycysic ovaly syndrome Control group		

6

7

8 2B. BMI

Polycystic ovary syndrome		rome	Cont	rol gro	que		Mean Difference	Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% CI
Aljelawy ZH 2019	28.5	0.55	70	26.59	0.86	18	7.2%	1.91 [1.49, 2.33]	
Anim-Ankumah A 2019	30.91	0.78	41	28.83	0.49	40	7,4%	2.08 [1.80, 2.36]	+
Branavan U 2019	26.89	0.72	55	25.25	0.34	110	7.4%	1.64 [1.44, 1.84]	
Celik N 2018	27.3	4.9	20	24.5	4.96	20	2.6%	2.80[-0.26, 5.86]	
Chen X 2010	21.52	3.7	42	20.11	2.31	20	5.2%	1.41 E-0.10, 2.92	
Daghestani MH 2020	29.45	5.99	104	29.89	7.31	109	4.6%	-0.44 [-2.23, 1.35]	
El-Shehawy YM 2015	23.4	1.7	23	22.1	1.5	19	6.3%	1.30 (0.32, 2.28)	
Emekci Ozay O 2016	24.32	3.4	250	23.44	4.08	150	6.7%	0.88 [0.10, 1.66]	
Gorkern U 2018	26.34	4.69	60	26.06	5.39	57	4.5%	0.28 [-1.55, 2.11]	
Ibrahim RO 2829	26.5	3.76	-60	25.93	37	40	5.2%	0.57[-0.92, 2.06]	
Jeon YE 2013	23.1	2.76	54	19.77	1.51	36	6.5%	3.33 [2.44, 4.22]	
Kaya C 2019	28.6	5.08	29	25.47	2	27	4.2%	313 [1.13, 5.13]	· · · · · · · · · · · · · · · · · · ·
Mut A 2028	24.53	5.22	70	22.78	5.48	58	4.4%	1.751-0.12, 3.621	
Nyagolova PV 2016	25.59	0.65	87	24.88	0.89	42	7.3%	0.71 [0.41, 1.01]	-
Panidis D 2006	26.83	0.44	56	32.13	1.85	13	6.2%	-5.30 [-6.31, -4.29]	
Rashad NM 2019	28.84	7.87	105	27.73	6.37	90	4.2%	1.11 [-0.89, 3.11]	
Wang T 2019	26.52	5.97	73	22.9	4.31	63	4.7%	3.62 [1.89, 5.35]	
Yilmaz SA 2014	24.5	4.17	83	23,1	4.2	66	5.5%	1.40 [0.05, 2.75]	
Total (95% CI)			1282			977	100.0%	1.16 [0.54, 1.78]	•
Heterogeneity: Tau ^a = 1.3	2, Chi# = 267	20, df = 17	(P + 0.00	001); P	= 93%			u - 2010/00/00/00/00/00/00/00/00/00/00/00/00/	-10 -5 0 5 10
rest for overall effect Z =	3.69 0° = 0.00	982)							Control group Polycystic every syndrome

9

10 2C. Waist-to-hip ratio

	Polycystic evary syndrome				trol gro	чир		Mean Difference	Mean Difference			
Study or Subgroup	Mean	5D	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, Ran	ndom, 95% Cl	
Branavan U 2019	0.84	0.01	65	0.82	.0.01	110	15.3%	0.02 (0.02, 0.02)		100000		
Daghestani MH 2020	0.84	0.07	104	0.77	0.06	109	13.4%	0.07 (0.05, 0.09)				
Emekci Ozay O 2016	0.85	0.12	250	0.79	80.0	150	13.0%	0.06 (0.04, 0.08)				
Gorkern U 2018	0.84	0.07	60	0.85	0.06	57	12.1%	-0.01 [-0.03, 0.01]		-		
Jeon YE 2013	0.83	0.05	54	0.76	0.05	36	12.7%	0.07 (0.05, 0.09)				
Panidis D 2006	0.8	0.01	56	0.8	0.02	13	14.5%	0.00 [-0.01, 0.01]			+	
Rashad NM 2019	1.23	0.26	105	1.16	0.19	90	5.2%	0.07 (0.01, 0.13)				-
Wang T 2019	0.88	0.05	73	0.85	0.04	63	13.8%	0.03 (0.01, 0.05)				
Total (95% CI)			757			628	100.0%	0.04 [0.02, 0.05]			+	
Heterogeneity: Tau ^a = 0	0.00: ChP = 89	73. df = 7.0	P + 0.000	101); (*=	92%				t			
Test for overall effect Z	= 3.91 (P ≤ 0)	0001)							-0.2	-0.1 Control grou	0 0.1 up Polycystic ovar	0.2 y syndrome

¹¹

- Fig. 3. Forest plots comparing participants with and without polycystic ovary syndrome
- (standardized mean difference), from the top to the bottom, for kisspeptin (Fig. 3A),
- luteinizing hormone (LH; Fig. 3B), follicle-stimulating hormone (FSH, Fig. 3C), anti-
- müllerian hormone (Fig. 3D), prolactin (Fig. 3E), and estradiol (Fig. 3F).

3A. Kisspeptin

Polycystic ovary syndrome		rome	Con	trol group	p		Std. Mean Difference	Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	50	Total	Weight	IV, Random, 95% CI	IV, Random, 95% Cl
Aljelawy ZH 2019	1,390	109.1	70	415.3	56,05	18	3.6%	9.58 [8.04, 11.12]	
Anim-Ankumah A 2019	20.21	1.74	41	12.15	2.51	40	5.2%	3.70 [2.98, 4.43]	
Branavan U 2019	4.87	0.24	55	4.13	0.13	110	5.5%	4.23 [3.66, 4.79]	+
Celik N 2018	644.27	486.15	20	555.09	401.02	20	5.4%	0.20 [-0.43, 0.82]	+
Chen X 2010	0.23	0.17	42	0.19	0.21	20	5.6%	0.22[-0.32, 0.75]	+
Daghestani MH 2020	0.41	0.16	104	0.39	0.07	109	5.9%	0.16[-0.11, 0.43]	+
El-Shehawy YM 2015	0.27	0.13	23	0.16	0.9	18	5.4%	0.18 [-0.44, 0.80]	+
Emekci Ozay O 2016	1.92	1.29	250	1.49	1.45	150	5.9%	0.32 [0.11, 0.62]	-
Gorkern U 2018	5.76	2.11	60	4,65	2.16	57	5.8%	0.52 [0.15, 0.09]	-
Ibrahim RO 2820	1.79	0.98	60	1.05	0.96	40	5.7%	0.79 [0.37, 1.20]	-
Jeon YE 2013	10.64	6.18	54	6.51	3.13	36	5.7%	0.79 [0.35, 1.23]	-
Kaya C 2019	525.49	164.17	29	354.31	111.38	27	5.5%	1.20 [0.62, 1.77]	
Mkit A 2020	474.77	362.07	70	421.46	338.63	58	5.8%	0.15 [-0.20, 0.60]	+
Nyagolova PV 2016	0.23	0.2	97	0.16	0.01	42	5.8%	0.42 [0.05, 0.79]	-
Panidis D 2008	2.95	0.26	56	3.04	0.55	13	5.5%	-0.27 [-0.87, 0.34]	-
Rashad NM 2019	0.31	0.1	105	0.27	0.08	90	5.9%	0.47 [0.19, 0.76]	-
Wang T 2019	265.85	214.67	73	219.11	165.69	63	5.8%	0.24 (-0.10, 0.58)	-
Yilmaz SA 2014	2.02	0.97	83	1.18	0.28	66	5.8%	1.15 [0.80, 1.50]	-
Total (95% CI)			1282			977	100.0%	1.15 [0.68, 1.62]	•
Heterogeneity Tau?=0.9	5: Chi# = 418	3.12. df=17	(P < 0.00	001); F=	98%			101-101-002-02-02-02-02-02-02-02-02-02-02-02-02	
Test for overall effect Z=	4.81 (P = 0.0	00001)							-10 -5 0 5 10 Control group Polycystic every syndrome

3B. LH

Polycystic ovary syndrome		rome	Cont	rol gro	quo		Std. Mean Difference	Std. Mean Difference			
Study or Subgroup	Mean	50	Total	Mean	SD.	Total	Weight	IV, Random, 95% CI	I IV, Random, 95% CI		
Aljelawy ZH 2019	4.6	0.5	70	3.78	0.34	18	6.2%	1.72[1.14, 2.30]	1		
Celik N 2018	7.98	5.47	20	6	2.31	20	6.1%	0.46 [-0.17, 1.09]	1 +-		
Chen X 2010	11.86	7.58	42	6.2	4.26	23	8.2%	0.85 (0.32, 1.38)	-		
Daghestani MH 2020	14.43	7.48	104	4.56	1.38	109	6.5%	1.85[1.53, 2.17]			
El-Shehawy YM 2015	8.42	3.2	23	4.95	2.3	18	6.0%	1.20 [0.52, 1.87]	1		
Emekci Ozay O 2016	7.75	4.31	250	8.65	6.14	150	6.6%	-0.18 [-0.38, 0.03]	-		
Oorkem U 2018	7.7	3.49	60	5.51	2.05	57	6.4%	0.76 [0.38, 1.13]	-		
Ibrahim RO 2020	8.92	5.47	60	4.57	1.02	40	6.4%	1.01 [0.58, 1.43]	1 +		
Jeon YE 2013	10.79	5.88	54	4.56	1.95	37	6.3%	1.32 [0.96, 1.78]	-		
Kaya C 2019	9.62	6.04	29	6.4	1.59	27	6.2%	0.71 [0.17, 1.25]	-		
Mut A 2020	8.14	4.21	70	6.41	2.81	58	6.5%	0.47 [0.12, 0.82]	-		
Nyagolova PV 2018	7.68	0.58	87	5.09	0.43	42	6.0%	4.80 [4.10, 5.50]			
Panidis D 2006	12.78	1.77	56	4.37	0.6	13	5.2%	5.12 [4.05, 6.19]			
Rashad NM 2019	6.5	1.2	90	7.1	1.28	105	6.5%	-0.48[-0.77, -0.19]			
Wang T 2019	12.14	7.46	73	5.58	3.8	63	6.4%	1.08 [0.72, 1.44]	1 ÷		
Yiimaz SA 2014	9.3	6.37	83	4,5	1.2	66	8.5%	0.99 [0.65, 1.33]	1 -		
Total (95% CI)			1171			846	100.0%	1.29 [0.76, 1.83]	i 🔶		
Heterogeneity: Tau ² = 1	11; Chi#= 41	8.70, df = 1	5 (P = 0.0	10001);	#= 96	%			ta ta da da da da		
Test for overall effect Z	= 4.76 (P = 0.)	00001)		endul M					Control group Polycystic ovary syndrome		

1 3C. FSH

	Polycystic ovary syndrome				rol gro	up		Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	50	Total	Mean	SD.	Total	Weight	IV, Random, 95% CI	IV, Random, 95% Cl
Aljelawy ZH 2019	5.51	0.78	70	3.19	0.23	18	6.2%	3.26 [2.54, 3.97]	
Celik N 2018	5.82	1.34	20	9.87	5.17	20	6.3%	-1.05[-1.72, -0.39]	
Chen X 2010	5.07	1.93	42	4.58	1.55	23	6.6%	0.27 [-0.24, 0.78]	
Daghestani MH 2020	5.09	1.54	104	4.83	1.38	109	7.0%	0.18 [0.09, 0.45]	+
El-Shehawy YM 2015	5.7	1.4	23	5.98	1.8	18	6.4%	-0.17 [-0.79, 0.45]	
Emekci Ozay O 2016	51	2.01	250	8.28	4.93	150	7.1%	-0.93 [-1.14, -0.72]	-
Gorkem U 2018	8.35	2.01	60	7.34	1.83	67	6.9%	-0.51 [-0.89, -0.14]	
Ibrahim RO 2020	5.64	1.33	60	6.25	2	40	6.8%	-0.74 [-1.15, -0.32]	
Jeon YE 2013	6.29	3.7	54	5.77	1.51	36	6.8%	0.17 [-0.25, 0.59]	
Kaya C 2019	6.06	4.3	29	6.37	1.69	27	6.6%	-0.09 [-0.62, 0.43]	
Mut.A 2028	5.47	1.4	70	6.26	2.82	68	6.9%	-0.36(-0.71, -0.01)	
Nyagolova PV 2016	6.55	0.19	87	7.09	0.34	42	6.7%	-2.16 [-2.62, -1.70]	
Panidis D 2006	10.68	1.53	56	5.64	0.63	13	5.9%	3.11 [2.30, 3.92]	
Rashad NM 2019	4.8	0.98	90	5.58	1.35	105	7.0%	-0.65[-0.94, -0.36]	
Yilmaz SA 2014	6.1	1.83	83	6.2	1.58	66	6.9%	-0.06 [-0.38, 0.27]	+
Total (95% CI)			1098			782	100.0%	-0.03 [-0.50, 0.44]	+
Heterogeneity: Tau ² = 0	80: Chi# = 30	11.0/=1	4 (P < 0.0	00001)	P= 95	%			t t 1 t t
Test for overall effect Z	= 0.12 (P = 0.1	31)	20201163	Sec. 17					-4 -2 0 2 4
	1985-12/07/12/2008								Polycysac ovary syndrome Control group

4 3D. Anti-müllerian hormone

	Polycystic (ovary synd	rome	Cont	rol gro	que		Std. Mean Difference	Std. Mean Difference			
Study or Subgroup	Mean	\$D	Total	Mean	50	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl			
El-Shebawy YM 2015	7.52	3.1	23	3.1	1.1	18	13.2%	1.78 [1.04, 2.51]				
Emekci Ozay O 2016	5.93	4.93	250	2.33	2.25	150	25.7%	0.87 (0.66, 1.08)	-			
Gorkern U 2018	7.42	4.57	60	2.95	1.87	57	21.1%	1.28 [0.88, 1.68]				
Kaya C 2019	2.55	2.39	30	2.2	2.1	27	18.0%	0.15[-0.37, 0.67]				
Mut A 2020	6.68	4.05	70	3.42	2.06	59	21.9%	0.98 [0.61, 1.35]	-			
Total (95% CI)			433			310	100.0%	0.97 [0.60, 1.34]	•			
Heterogeneity: Tau ^a = 0	13; Chi# = 16.	94, df = 4 (P = 0.003	$(5; 1^{\circ} = 7)$	896			+				
Test for overall effect Z	= 5.17 (P = 0.0	00001)		10				*4	Control group Polycystic ovary syndrome			

7 3E. Prolactin

	Polycystic	ovary synd	rome	Cont	rol grou	ip		Std. Mean Difference	Std. Mean Difference			
Study or Subgroup	Mean	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, Randor	n, 95% Cl		
Allelawy ZH 2019	25.41	3,6	70	10.97	0.76	18	19,1%	4.41 [3.57, 5.26]		200700000000		
Anim-Ankumah A 2019	2.84	0.1	41	2.93	0.09	40	20.2%	-0.94 [-1.40, -0.46]		-		
Kaya C 2019	13.86	6.74	29	19.37	7.74	27	20.0%	-0.75 [-1.29, -0.21]		-		
Nyagolova PV 2016	320.58	17.68	87	290.07	26.74	42	20.3%	1,44 (1.03, 1.85)			*	
Yilmaz SA 2014	12	7.87	83	12	3.7	66	20.4%	0.00 [-0.32, 0.32]		1		
Total (95% CI)			310			193	100.0%	0.80 [-0.55, 2.14]			•	
Heterogeneity: Tau ⁴ = 2.2	8; Chi# = 166.	43, df = 4 (F	< 0.000	01); P= 1	18%				10	1 1	1 1	
Test for overall effect Z =	1.16 (P = 0.2)	5)								Centrol group	Polycystic ovary syndrome	

10 3F. Estradiol

Polycystic ovary syndrome				Cont	rol gros	ip		Std. Mean Difference	Std. Mean Difference			
Study or Subgroup	Mean 5D Total		Mean	tan 5D		Weight	IV, Random, 95% CI	IV, Random, 95% CI				
Aljelawy ZH 2019	38.44	3.36	70	34.14	7.15	18	12.0%	0.97 [0.44, 1.51]	3 14 A * A *	jana grand.		
Anim-Anikumah A 2019	246.8	13.32	41	275.2	14.95	40	11.8%	-2.41 -2.99, -1.83[
Daghestani MH 2020	203.74	93.12	104	141.57	71.51	109	13.1%	0.75 (0.47, 1.03)				
Gorkern U 2018	41.34	33.33	60	45.61	25.52	57	12.8%	-0.14 [-0.51, 0.22]				
Ibrahim RO 2020	47.76	42	60	45.85	15	40	12.6%	0.06 [-0.34, 0.46]				
Kaya C 2019	45.13	23.58	29	33.51	12.07	27	12.0%	0.61 (0.07, 1.14)				
Mut A 2020	38.19	14.37	70	40.57	13.24	68	12.8%	-0.17 [-0.52, 0.18]				
Yilmaz SA 2014	41	12.5	83	43	10	66	12.9%	-0.17 [-0.50, 0.15]		-		
Total (95% CI)			517			415	100.0%	-0.05[-0.81, 0.51]		•		
Heterogeneity Tau? = 0.8	0: Ch#= 113	40. df = 7 (P = 0.000	01); F=1	9495			그 아님 않는 것	+ t	1 1	- 1	
Test for overall effect Z =	0.18 (P = 0.8	5)		2.221	02/30				 -47 Polycystic ovary syndror 	ne Control group	4	

- 1 Fig. 4. Forest plots comparing participants with and without polycystic ovary syndrome
- 2 (standardized mean difference), from the top to the bottom, for total testosterone (Fig.
- 4A), free testosterone (Fig. 4B), dehydroepiandrosterone sulphate (DHEA-S, Fig. 4C), sex
- 4 hormone-binding globulin (Fig. 4D), and modified Ferriman-Gallweig score (Fig. 4E).
- 5

6 Fig. 4A. Total testosterone

	Polycystic	ovary synd	frome	Con	trol gro	up		Std. Mean Difference	Std. Mean Difference		
Study or Subgroup	Mean	\$0	Total	Mean	\$0	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI		
Anim-Ankumah A 2019	12.37	0.37	41	11.27	0.62	40	7.4%	2.42 [1.84, 3.00]			
Branavan U 2019	4.713	D.459	55	3.415	0.258	110	7.4%	3.84 [3.31, 4.37]	-		
Celik N 2018	74.4	3.32	20	34.1	6.72	20	5.3%	7.45 [5.62, 9.28]			
Chen X 2010	2.43	0.92	42	2.04	0.47	20	7.4%	0.48 [-0.06, 1.02]	+-		
EI-Shehawy YM 2015	0.78	0.31	23	0.37	0.12	18	7.2%	1.63 (0.91, 2.36)			
Emekci Ozay O 2016	50.51	27.93	250	49.6	20.5	150	7.7%	0.04 [-0.17, 0.24]	+		
Gorkern U 2018	30.62	14.61	60	23.1	12.22	57	7.6%	0.55 [0.18, 0.92]	-		
Jeon YE 2013	51,1	24.9	54	21.4	11.44	36	7.5%	1.43 (0.95, 1.90)	-		
Mut A 2020	44.41	17.36	70	29.84	13.2	-58	7.6%	0.93 (0.56, 1.29)	÷		
Nyagolova PV 2016	0.73	0.03	87	0.51	0.04	42	7.0%	6.52 [5.63, 7.41]			
Panidis D 2006	83.86	4.56	56	41.59	2.74	13	5.4%	9.74 [7.96, 11.52]			
Rashad NM 2019	0.78	0.22	105	0.5	0.14	90	7.8%	1.49 [1.17, 1.81]	-		
Wang T 2019	69.12	27.34	73	44.82	16.48	63	7.6%	1.05 (0.69, 1.41)	-		
Yilmaz SA 2014	1.9	0.89	83	1	0.76	66	7.6%	1.07 (0.73, 1.42)	-		
Total (95% CI)			1019			783	100.0%	2.48 [1.73, 3.23]	•		
Heterogeneity: Tau ² = 1.8	39; Ch≇= 532	.68, df = 13	(P < 0.00	001); P	= 98%						
Test for overall effect Z =	6.50 (P < 0.0	0001)	n: 1990	29934					-10 -5 0 5 10 Control group Polycystic ovary syndrome		

7

9

8 Fig. 4B. Free testosterone

	Polycystic	tic ovary syndrome Control group				oup		Std. Mean Difference	e Std. Mean Difference				
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV,	Random, 95%	CI	
Chen X 2010	4.28	3.06	42	3.21	1.36	20	14.2%	0.40 [-0.14, 0.94]					
Emekci Ozay O 2016	2.52	1.05	250	2.18	0.96	150	15.0%	0.33 [0.13, 0.54]			-		
lbrahim RO 2020	0.66	0.75	60	0.37	0.22	40	14.6%	0.48 [0.08, 0.89]					
Jeon YE 2013	5.56	4.09	54	1.36	0.97	36	14.4%	1.29 [0.82, 1.75]					
Nyagolova PV 2016	8.83	0.88	87	4.69	0.93	42	13.7%	4.59 [3.91, 5.27]					
Panidis D 2006	11.22	3.84	56	3.35	0.28	13	13.5%	2.24 [1.52, 2.95]				_	
Yilmaz SA 2014	3.2	4.52	83	1.4	1.6	66	14.7%	0.51 [0.18, 0.83]			-		
Total (95% CI)			632			367	100.0%	1.37 [0.56, 2.17]			•		
Heterogeneity: Tau ² = 1 Test for overall effect: Z	.12; Chi² = 16 = 3.32 (P = 0.	6.47, df = 6 0009)	(P < 0.0I	0001); P	°= 969	6			+ -10	-5 Control	group Polycy	5 stic ovary syr	10 ndrome

10 Fig. 4C. DHEA-S

	Polycystic	ovary synd	rome	Contr	ol group	9		Std. Mean Difference		Std. Mean Difference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, Random, 95% Cl	
Chen X 2010	1,543	1,058	42	2,435	1,034	20	10.5%	-0.84 [-1.39, -0.28]		+	
El-Shehawy YM 2015	284.6	78.2	23	190.3	34.1	18	9.3%	1.47 [0.77, 2.17]			
Emekci Ozay O 2016	252.7	60.3	250	221.84	105	150	12.6%	0.38 (0.18, 0.59)		-	
Gorkern U 2018	253.19	112.57	60	216.76	85.73	57	11.7%	0.36 -0.01, 0.73		-	
Mut A 2020	337.45	133.3	70	250.85	66.6	58	11.8%	0.80 [0.44, 1.16]		-	
Panidis D 2006	2.62	0.2	56	1,88	0.19	13	8.1%	3 69 [2.82, 4.57]			
Rashad NM 2019	1.26	0.814	105	0.93	0.51	90	12.2%	0.48 (0.19, 0.76)		+	
Wang T 2019	3,181	849.47	73	2,861.23	694.9	63	11.9%	0.41 [0.07, 0.75]		+	
Vilmaz SA 2014	303	101.6	83	230.5	107.7	66	11.9%	0.69 (0.36, 1.02)		*	
Total (95% CI)			762			535	100.0%	0.72 [0.32, 1.13]		· · · · ·	
Heterogeneity Tau ² = 0	1.33; ChF = 8	6.81. df = 9 (P < 0.000	01); P=9;	196				+	-t-1t	
Test for overall effect 2	= 3 49 (P = 0	0005)	C						-10	Control group Polycystic ovary syndh	ome

1 Fig. 4D. Sex hormone-binding globulin

	Polycystic	ovary synd	rome	Con	trol gro	up		Std. Mean Difference		Std. Mean D	ofference	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI.		IV, Randon	n, 95% CI	
Chen X 2010	67.61	40.88	42	88.05	55.68	20	14.2%	-0.44 [-0.98, 0.10]			personal to	
Emekci Ozay O 2018	50.1	22.8	250	63.74	45.62	150	15.0%	-0.41 [-0.61, -0.21]		-		
brahim RO 2020	77.86	55	60	80	13.2	40	14.6%	-0.05 [-0.45, 0.35]		-	-	
Jeon YE 2013	50.83	36.8	54	66.17	31.73	36	14.5%	-0.44 [-0.86, -0.01]				
Nyagolova PV 2016	40.65	3.01	87	57.98	6.64	42	14.0%	-3.79 [-4.39, -3.19]				
Panidis D 2006	33.84	2.79	56	47.55	5.89	13	12.8%	-3.82 [-4.71, -2.93]				
Yiimaz SA 2014	31	15.27	83	45.1	19.35	66	14.8%	-0.82 [-1.15, -0.48]		-		
Total (95% CI)			632			367	100.0%	-1.34 [-2.15, -0.52]		-		
Heterogeneity Tau ^a = 1	14: Chi#=1)	70.75. df = 8	(P < 0.0)	00013-P	= 96%				-	1	- +	- +
Test for overall effect. Z	= 3.22 (P = 0	.001)		222,004					-4 Polycystic ov	-2 0 ary syndrome	2 Control gro	4

4 Fig. 4E. Modified Ferriman-Gallweg score

	Polycystic	ovary synd	rome	Cont	rol gro	up		Std. Mean Difference		Std. Me	an Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, Ran	idom, 95% Cl		
Branavan U 2019	8	0.45	55	3	0.22	110	18.4%	15.78 [14.03, 17.54]				_	-
Emekci Ozay O 2016	14.49	1.78	250	6.01	1.22	150	20.4%	5.31 [4.89, 5.73]			•		
Mut A 2020	10.02	6.04	70	4.72	2	58	20.4%	1.13 [0.75, 1.50]			-		
Rashad NM 2019	8.86	4.36	105	5.44	0.75	90	20.5%	1.05 [0.75, 1.35]			-		
Yilmaz SA 2014	9	3.75	83	0.01	0.17	66	20.3%	3.19 [2.70, 3.68]			-		
Total (95% CI)			563			474	100.0%	5.08 [2.76, 7.39]			•		
Heterogeneity: Tau ² = 6	6.80; Chi ^z = 53	8.30, df = 4	(P < 0.0	0001); P	e = 999	6			+		<u> </u>	+	
Test for overall effect: Z	Z = 4.30 (P ≤ 0.	0001)							-20	Control gro	up Polycystic	ovary syn	drome

- 1 Fig. 5. Forest plots comparing participants with and without polycystic ovary syndrome
- 2 (standardized mean difference), from the top to the bottom, for glycemia (Fig. 5A),
- 3 HOMA-IR index (Fig. 5B), insulin (Fig. 5C), and leptin (Fig. 5D).
- 4

5 5A. Glucose

	Polycystic	ovary synd	tome	Cont	rol grou	ip		Std. Mean Difference	Std. Mea	n Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Rand	lom, 95% Cl
Anim-Ankumah A 2019	6.82	0.06	41	5.37	0.14	40	7.9%	4.16 [3.37, 4.94]	4000000	
Branavan U 2019	98.81	2.08	55	108.69	2.74	110	8.3%	-3.87 [-4.40, -3.34]		
Celik N 2018	93.4	3.41	20	87.9	6.41	20	8.1%	1.05 [0.38, 1.72]		
Chen X 2010	4.86	0.32	42	4.68	0.29	20	8.3%	0.57 [0.03, 1.11]		
Daghestani MH 2020	5.06	0.44	104	4.78	0.48	109	8.5%	0.61 (0.33, 0.99)		-
Emekci Ozay O 2016	80.68	13.8	250	78.7	13.4	150	8.6%	0.14 [-0.06, 0.35]		-
Jaon YE 2013	87.35	8.09	54	79.33	10.76	36	8.4%	0.86 [0.42, 1.30]		
Mut A 2020	84.23	9.79	70	80.4	9.76	58	8.5%	0.39 [0.04, 0.74]		
Nyagolova PV 2015	5.24	0.08	87	4.99	0.08	42	8.3%	3.11 [2.57, 3.64]		
Panidis D 2006	95.91	2.45	56	99.54	3.54	13	8.1%	-1.34 [-1.99, -0.69]		
Wang T 2019	.5.46	0.6	73	5.14	0.53	63	8.5%	0.56 [0.22, 0.90]		
Yilmaz SA 2014	89	11.25	83	91	5,43	66	8.5%	-0.22 [-0.54, 0.11]	5	+
Total (95% CI)			935			727	100.0%	0.49 [-0.27, 1.24]		•
Heterogeneity: Tau ² = 1.7	1: Chi#= 490	45. df = 11	(P = 0.00	001); P=	98%			St. 11 - 200	- t - t -	1 1 1
Test for overall effect Z =	1.27 (P = 0.2	1)	0	201011-0	1000				-4 -2 Control grou	p Polycystic ovary syndromy

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8 5B. HOMA-IR

	Polycystic	ovary synd	rome	Cont	rol gro	up 🦾		Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	\$0	Total	Mean	\$D	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Cellk N 2018	4.22	3.4	20	2.12	1.52	20	8.8%	0.78 [0.14, 1.43]	
Chen X 2010	2.28	1.64	42	1.43	0.73	20	8.4%	0.59 [0.05, 1.14]	
Emekci Ozay O 2016	2.76	2.34	250	1.41	1.5	150	11.0%	0.65 (0.44, 0.86)	+
Jeon YE 2013	3.14	2.95	54	1.24	0.67	36	10.0%	0.81 [0.37, 1.25]	
Mut A 2020	2.56	2.04	70	1.61	0.93	58	10.4%	0.68 [0.22, 0.93]	
Nyagolova PV 2016	1.82	0.13	87	1.39	0.2	42	9.7%	2.74 [2.24, 3.24]	
Panidis D 2006	2.95	0.26	56	3.84	0.55	13	9.1%	-0.27 [-0.87, 0.34]	
Rashad NM 2019	3.31	2	105	1.42	0.33	90	10.6%	1.27 [0.98, 1.58]	
Wang T 2019	4.15	2.68	73	3.15	3.4	63	10,5%	0.33 [-0.01, 0.67]	177
Vilmaz SA 2014	2	2.12	83	1.8	2.57	66	10.6%	0.09 [-0.24, 0.41]	-
Total (95% CI)			840			558	100.0%	0.76 [0.35, 1.17]	•
Heterogeneity: Tau ² = 0	0.39: Ch₽ = 10	5.93. df = 9	P + 0.0	0001); P	= 929	6			+
Test for overall effect. Z	= 3.62 (P = 0	0003)	<i>CO 11253</i>		10000	2.93			-4 -2 0 2 4 Control group Polycystic ovary syndrome

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11 5C. Insulin

	Polycystic	ovary synd	rome	Con	trol gro	up		Std. Mean Difference		Std. Mean	Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI		IV, Rande	om, 95% Cl		
Daghestani MH 2020	97.71	58.15	104	79.51	39.65	109	13.2%	0.37 [0.10, 0.64]					
Jeon YE 2013	14.24	11.9	54	6.26	3.08	36	12.2%	0.84 [0.40, 1.28]					
Mut A 2020	12.4	8.44	70	8.37	4.4	58	12.8%	0.58 [0.22, 0.94]					
Nyagolova PV 2016	7.62	0.49	82	6.19	0.53	42	11.7%	2.82 [2.31, 3.34]					
Panidis D 2006	12.25	0.97	56	11.9	1.98	13	11.1%	0.28 [-0.32, 0.89]		-			
Rashad NM 2019	5.57	1.35	105	4.8	0.97	90	13.1%	0.64 [0.36, 0.93]					
Wang T 2019	19.56	11.55	73	14.8	15.66	63	12.9%	0.35 [0.01, 0.69]			⊢ ⊷		
Yilmaz SA 2014	9.5	8.72	83	7.7	5.43	66	13.0%	0.24 [-0.08, 0.56]			+		
Total (95% CI)			627			477	100.0%	0.75 [0.30, 1.19]			•		
Heterogeneity: Tau ² = 0.	.37; Chi² = 83	.30, df = 7 (l	P < 0.000)01); I ² =	92%				+	<u> t </u>		1	-+
Test for overall effect: Z	= 3.30 (P = 0.	0010)							-4	-2 Control group	U Polycystic	ovary syndroi	me

13 5D. Leptin

	Polycystic	ovary synd	rome	Con	trol gro	up	mound	Std. Mean Difference		Std. Mean Difference
Study or Subgroup	Mean	SD.	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl.	5	IV, Random, 95% CI
Aljelawy ZH 2019	145.7	3.985	70	98.5	5,766	18	19.9%	10.65 [8.96, 12.34]		
Ernekci Ozay 0 2016	112.95	46.99	250	83.5	18.3	150	26.9%	0.76 [0.55, 0.97]		
Rashad NM 2019	302.6	85.15	105	179.4	11.27	90	26.6%	1.95 [1.61, 2.29]		
Vilmaz SA 2014	90	59.5	83	93.5	43.82	66	26.7%	-0.07 [-0.39, 0.26]		+
Total (95% CI)			508			324	100.0%	2.82 [1.35, 4.29]		•
Heterogeneity: Tau* = 2	2.08; Chi*= 19	99.65, tf = 3	(P < 0.0)	0001); P	*= 98%				tan	1. 1. 1
fest for overall effect Z	= 3.77 (P = 0	0002							-20	

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- 1 Fig. 6. Forest plots comparing participants with and without polycystic ovary syndrome
- 2 (standardized mean difference), from the top to the bottom, for total cholesterol (Fig.
- 6A), HDL-cholesterol (Fig. 6B), LDL-cholesterol (Fig. 6C), and triglycerides (Fig. 6D).
- 4

5 6A Total cholesterol

	Polycystic	ovary synd	rome	Cont	rol grou	p		Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	50	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Daghestani MH 2020	4.41	0.84	104	3.74	0.56	109	25.0%	0.94 [0.66, 1.22]	
Emekci Ozay O 2816	214.85	39.27	250	214.85	39.27	150	25.5%	0.00 [-0.20, 0.20]	+
Rashad NM 2019	195.65	17.3	105	166.6	20.6	90	24.8%	1.53 [1.21, 1.85]	
Yilmaz SA 2014	163	48,5	83	165	28.67	66	24.7%	-0.05[-0.37, 0.27]	
Total (95% CI)			542			415	100.0%	0.60 [-0.12, 1.33]	
Heterogeneity: Tau ^a = 0	1.53; Chi#= 83	23, df = 3 (P < 0.000	101); P=	96%			5	<u>t 1 t</u>
Test for overall effect Z	= 1.62 (P = 0	10)						-2	Control group Polycystic ovary syndrome

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7 6B HDL-Cholesterol

	Polycystic	ovary synd	Irome	Con	trol gro	up		Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Daghestani MH 2020	1.14	0.34	104	1.21	0.33	109	25.1%	-0.21 [-0.48, 0.06]	
Emekci Ozay O 2016	51.36	12.15	250	54.5	14.07	150	25.8%	-0.24 [-0.45, -0.04]	
Rashad NM 2019	34.7	4.736	105	41.26	4.3	90	24.6%	-1.44 [-1.76, -1.12]	_ _
Yilmaz SA 2014	47	11.5	83	47.5	8	66	24.5%	-0.05 [-0.37, 0.27]	_ _
Total (95% CI)			542			415	100.0%	-0.48 [-1.04, 0.08]	
Heterogeneity: Tau² = 0 Test for overall effect: Z	.30; Chi² = 50 = 1.69 (P = 0.	.20, df = 3 (09)	P < 0.000)01); I²=	94%				-2 -1 0 1 2 Polycystic ovary syndrome Control group

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10 6C LDL-Cholesterol

	Polycystic	ovary synd	rome	Cont	rol gro	up		Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Daghestani MH 2020	2.41	0.71	104	1.82	0.66	109	25.1%	0.86 [0.58, 1.14]	
Emekci Ozay O 2016	102.56	23.45	250	108.1	29.6	150	25.3%	-0.21 [-0.42, -0.01]	-
Rashad NM 2019	137.9	17.43	105	105.9	4.36	90	24.7%	2.43 [2.05, 2.80]	
Yilmaz SA 2014	99.5	24.1	83	101.2	27.8	66	24.9%	-0.07 [-0.39, 0.26]	
Total (95% CI)			542			415	100.0%	0.75 [-0.32, 1.81]	
Heterogeneity: Tau ² = 1.	.15; Chi ² = 16 - 1.37 (P - 0	7.16, df = 3	(P < 0.00	0001); I r	= 98%)			-4 -2 0 2 4
Testion overall effect. 2	- 1.57 (1 - 0.	10							Control group Polycystic ovary syndrome

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12 6D Triglycerides

	Polycystic	ovary synd	rome	Con	trol gro	up		Std. Mean Difference		Std. Mean	Differen	ce	
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl		IV, Rando	m, 95% (3	
Aljelawy ZH 2019	145.7	3.98	70	98.5	5.77	18	14.2%	10.65 [8.96, 12.34]					
Daghestani MH 2020	1.08	0.39	104	0.93	0.42	109	21.5%	0.37 [0.10, 0.64]			•		
Emekci Ozay O 2016	112.95	46.88	250	83.5	18.3	150	21.6%	0.76 [0.55, 0.97]			-		
Rashad NM 2019	302.6	85.15	105	179.4	11.27	90	21.3%	1.95 [1.61, 2.29]			•		
Yilmaz SA 2014	90	59.5	83	93.5	43.82	66	21.4%	-0.07 [-0.39, 0.26]					
Total (95% CI)			612			433	100.0%	2.15 [1.08, 3.23]			•		
Heterogeneity: Tau ² = 1	.38; Chi² = 21	0.67, df = 4	(P < 0.00)001); l ^a	= 98%				+	10		10	
Test for overall effect: Z	= 3.93 (P < 0.	0001)							-20	Control group	Polycys	tic ovary syn	drome



Table 1. Studies comparing serum kisspeptin levels in women with and without polycystic ovary syndrome (PCOS): Study location and period, aim, number of participants, age, body mass index (BMI), PCOS diagnosis criteria, and main findings.

Author	Location and period of study. Aim of the	PCOS participants; age; BMI and	Participants without	Main findings
[reference]	study	diagnosis criteria	PCOS; age and BMI	
Al-Jelawy ZH	Location: Najaf City, Iraq; December 2017	n = 70 women with infertility and	n = 18 fertile women.	Significant higher circulating kisspeptin, cholesterol,
2019 [29,30]	to September 2018.	PCOS.	Age: 33 ± 1.24; BMI	triglyceride, LDL-C, VLDL-C, hemoglobin and prolactin
	Aim: To study kisspeptin and interleukin	Age: 26.97 ± 0.69; BMI: 28.50 ±	26.59 ± 0.86.	levels in women with PCOS. Significant lower serum
	37 in patients with infertility and PCOS.	0.55.		HDL-C and interleukin-37 levels in women with PCOS.
		Diagnosis: Rotterdam criteria.		
Anim-	Location: Accra, Ghana; 2018.	n = 41 women with PCOS. Age: 32.8	n = 40 women.	Significant differences in kisspeptin, LH, FSH,
Ankumab A	Aim: To study kisspeptin and other	± 0.9; BMI 30.91 ± 0.78. Diagnosis:	Age 33.55 ± 1.12; BMI:	testosterone and glucose levels.
2019 [31]	hormones in women > 18 years with	Rotterdam criteria.	28.83 ± 0.49	
	PCOS.			
Branavan U	Location: Colombo, Sri Lanka; period of	n = 55 women with PCOS. Age:	n = 110 women. Age	Serum kisspeptin and testosterone concentrations
2019 [32]	study not stated.	24.67 ± 0.88; BMI: 26.89 ± 0.72.	33.80 ± 0.53; BMI: 25.25	were significantly higher in women with PCOS than
	Aim: To study circulating kisspeptin and its	Diagnosis: Rotterdam criteria.	± 0.34	controls. Sequencing the Kiss1 gene revealed 2 single
	polymorphisms.			nucleotide polymorphisms.
Celik N 2018	Location: Istanbul, Turkey; period of study	n = 20 infertile women with PCOS.	n = 20 infertile women	Serum kisspeptin levels were negatively correlated with the number
[33]	not stated.	Age: 35.3 ± 3.1. BMI = 27.3 ± 4.9	with poor ovarian	of retrieved oocytes and pregnancy rates. Amylin and adiponutrin
	Aim: To assess adiponutrin, amylin,	kg/m². Diagnosis Rotterdam	response. Age: 35.3 ±	seems to participate in follicle developmental in PCOS women.
	preptin and kisspeptin in women	criteria.	3.1. BMI: 24.5 ± 5.0.	
	undergoing in vitro fertilization.		PCOS diagnosis:	
Chen X 2010	Location: Guangdong, China; period of	n = 42 (adolescent n = 19, adult n =	n = 20 participants.	Circulating kisspeptin levels are increased in
[34]	study not stated.	23). Age: adolescents: 17.89 ± 1.24,	Age: 18.6 ± 0.68.	participants with PCOS compared to the control group.
	Aim: To study kisspeptin and correlations	adults: 26.17 ± 3.45.	BMI: 20.1 ± 2.3.	Kisspeptin levels were positively correlated with serum
	with reproductive and metabolic	BMI: 21.52 ± 3.7 kg/m ²		LH and testosterone levels.
	outcomes.	PCOS diagnosis: Rotterdam criteria.		
Daghestani	Location: Makkah, Saudi; period of study	n = 104.	n = 109. Age: 25.0 ± 5.6;	Waist-hip ratio, LH, and LH-FSH ratio were significantly
MH 2020 [35]	not stated. Aim: To study kisspeptin, LH,	Age: 25.1 ± 3.9;	BMI: 29.9 ± 7.3.	higher in PCOS women than controls. BMI, kisspeptin
	FSH and KISS1 polymorphisms in women	BMI: 29.5 ± 6.0. PCOS diagnosis:		and FSH levels exhibited no significant difference
	with PCOS.	Rotterdam criteria.		between groups.
El-Shehawy	Location: Kuwait; 2010-2012. Aim: To	n = 23. Age = 24.4± 1.2; BMI:	n = 19. Age: 27.15 ± 1.7;	Serum kisspeptin, AMH, LH, testosterone and DHEA-S
YM 2016 [36]	study kisspeptin and AMH levels in	23.4±1.7.	BMI: 22.1 ± 1.5.	levels were higher in women with PCOS. There were
	women with PCOS.	PCOS diagnosis: Rotterdam criteria.		correlations between kisspeptin and AMH in women
				with and without PCOS.

Emekci Ozay	Location: Izmir, Turkey; December 2011 to	n = 250. Age: 23.99 ± 4.63; BMI	n = 150.	Serum kisspeptin and leptin levels do not differ
OE 2016 [37]	September 2013.	24.32 ± 3.40. PCOS diagnosis:	Age: 24.43 ± 4.39;	between PCOS and control women while LH levels and
	Aim: To study kisspeptin, AMH, insulin,	Rotterdam criteria.	BMI: 23.44 ± 4.08.	LH/FSH ratio were higher in PCOS patients compared
	LH, FSH and steroid hormones.			with controls.
Gorkem U,	Location: Corum, Turkey; January to	N = 60. Age 27.76 ± 4.65; BMI:	N = 57. Age: 27.9 ± 4.7;	Kisspeptin, AMH, LH, total testosterone and DHEA
2018 [38]	September 2016.	26.34 ± 4.69. PCOS diagnosis:	BMI:	levels were higher in infertile women with PCOS as
	Aim: To investigate kisspeptin levels in	Rotterdam criteria.	26.1 ± 5.4.	compared to women without the syndrome and
	infertile women with different ovarian			normal ovarian reserve.
	reserve patterns.			
Ibrahim RO	Location: Sulaymaniyah, Iraq; April 2018	n = 60 infertile women with PCOS.	n = 40. Age: 31.6 ± 7.16.	Serum kisspeptin levels were higher in PCOS patients
2020 [39]	to March 2019.	Age: 30.09 ± 7.60; BMI: 26.05 ±	BMI: 25.93 ± 3.7.	than in those without the syndrome. In women
	Aim: To study kisspeptin levels in women	3.76. PCOS diagnosis: Roterdam		without PCOS preovulatory kisspeptin levels were
	with and without PCOS, and the effect of	criteria:		higher than in the follicular phase; this difference was
	obesity and age on this hormone.			not present in women with PCOS. The BMI difference
				was not associated with different kisspeptin levels.
Jeon YE 2013	Location: Yongin, Korea; period of study	n = 54. Age: 23,72 ± 5,32. BMI:	n = 36. Age: 24.92 ±	Kisspeptin, leptin and RBP4 levels were significantly
[40]	not stated.	23,10 ± 2,76. PCOS diagnosis:	2.94. BMI: 19.77 ± 1.51.	higher in women with PCOS. In women with PCOS,
	Aim: to evaluate circulating kisspeptin,	Rotterdam criteria.		kisspeptin levels were positively correlated with RBP4
	leptin and retinol binding protein 4 (RBP4)			levels.
	levels in women with and without PCOS.			
Kaya C 2018	Location: Istanbul, Turkey; August 2016 to	n = 29 infertility and PCOS. Age:	n = 27. Age: 30.25 ±	Women with PCOS display higher levels of kisspeptin,
[41]	June 2017.	28.75 ± 3.49; BMI : 28.6 ± 5.08 kg.	4.66; BMI 26.4 ± 3.32.	increased antral follicle count and higher BMI.
	Aim: To study circulating kisspeptin in	PCOS diagnosis: Rotterdam criteria.		
	infertile women with PCOS.			
Mut A 2020	Location: Istanbul, Turkey; June 2017 to	n = 70. Age: 23.51 ±4.33	n = 58. Age: 30,88 ±	There were not significant differences in kisppeptin
[42]	June 2018.	BMI: 24,53 ± 5,22	5,57; BMI: 22,78 ± 3,48	and FSH levels between women with and without
	Aim: To asses serum kisspeptin and AMH	PCOS diagnosis: Rotterdam criteria.		PCOS. Age, BMI, LH, AMH, DHEA-S, total testosterone,
	levels in women with PCOS.			glucose and insulin, and HOMA-IR were significantly
				higher in women with PCOS.
Nyagolova PV	Location: Plovdiv, Bulgaria; period of	n = 87 (BMI ≥ 25, n = 40; BMI <25, n	n = 42 (BMI ≥ 25, n = 20;	Kkisspeptin and GALP are increased in women with
2016 [43]	study not stated.	= 47).	BMI < 25, n =22)	PCOS and positively associated with
	Aim: To study kisspeptin and galanin-like	Age: 24.99 ± 0.49. BMI: 25.59 ±	Age: 26.65 ± 0.71	hyperandrogenism. In overweight PCOS women
	peptide (GALP) roles in the development	0.65. PCOS diagnosis: Rotterdam	BMI: 24.88 ± 0.89.	kisspeptin correlated positively with insulin,
	of PCOS.	criteria.		testosterone and SHBG.

Panidis D 2006 [44]	Location: Thessaloniki; Greece; period not stated.	n = 56. Age: 23.97 ± 0.93. BMI = 26.83 ± 0.44.	n = 13. Age: 26.85 ± 1.06. BMI: 32.13 ± 1.85	There were not significant difference in circulating kisspeptin in women with PCOS compared to controls.
	and without PCOS and related metabolic disturbances.	anovulation (< 6 cycles in 12 months) and hyperandrogenemia.		and increased free androgens.
Rashad NM 2019 [45]	Location: Zagazig, Egypt; period of study not stated. Aim: To estimate kisspeptin levels in women with and without PCOS.	n = 105. Age: 30.6 ± 6.57. BMI: 28.4 ± 7.87 PCOS diagnosis: Rotterdam criteria.	n = 90. Age: 32.44 ± 7.08. BMI 27.73 ± 6.37	Kisspeptin levels were higher in PCOS patients, decreasing with increasing of BMI. Moreover, it was negatively correlated to anthropometric measures, glycemic and lipid profile.
Wang T 2019 [46]	Location: Tianjin, China; December 2014 to July 2017. Aim: to study kisspeptin and its relationship with abnormal metabolism in PCOS.	n = 73 PCOS cases seeking treated for menstrual disorders or infertility. Age: 27.53 ± 5.08. BMI: 26.52 ± 5.97. PCOS diagnosis: Rotterdam criteria.	n = 63 women seeking assisted reproductive techniques due to male factors or tubal factors Age: 31.96 ± 3.96. BMI: 22.29 ± 4.31.	Kisspeptin levels were higher in PCOS women than in the control group. Kisspeptin correlated with LH levels, and negatively correlated with triglyceride levels.
Yilmaz SA 2014 [47]	Location: Konya, Turkey; period of study not stated. Aim: to study circulating kisspeptin in relation with hormonal and metabolic measurements in women with PCOS.	n = 83 (BMI < 25, n = 42; BMI ≥ 25; n = 41). Age: 21.0 ± 5.0. BMI: 24.5 ± 4.17. PCOS diagnosis: Rotterdam criteria.	$\label{eq:masses} \begin{array}{l} n = 66 \; (BMI < 25, n = 41; \\ BMI \geq 25, n = 25). \\ Age: 24.5 \pm 3.5 \;\; n = 66 \\ BMI: 23.1 \;\; \pm 4.2. \end{array}$	Women with PCOS had higher kisspeptin levels than controls even after controlling for BMI. Kisspeptin had positive correlations with glucose, testosterone, DHEA-S, and LH; however, kisspeptin negatively correlated with SHBG.

AMH: anti-mullerian hormone; BMI: Body mass index; DHEA-S: dehydroepiandrosterone sulphate; FSH: Follicle-stimulating hormone; HOMA-IR: homeostasis model assessments of insulin resistance; LH: Luteinizing hormone; N: numer of participants; SHBG: sex hormone-binding globulin; SHBG: sex hormone-binding globulin; DHEA-S: dehydroepiandrosterone sulphate.

Table 2. Pooled effects reported as mean differences (MDs) or standardized MDs (SMDs) and 95 % confidence interval (CI) using random effect models and heterogenity (I²) in women with and without polycystic ovary syndrome (PCOS).

Outcome (Figures)	Included Studies	Participants PCOS / control	MD or SMD and 95% CI	l² (%)	р
Age (Fig 2A)	18	1282 / 977	MD -2.38 [-4.32, -0.44]	99	0.02
BMI (Fig 2B)	18	1282 / 977	MD 1.16 [0.54, 1.78]	93	0.0002
Waist-to-hip ratio (Fig 2 C)	8	757 / 628	MD 0.04 [0.02, 0.05]	92	<0.00001
Kisspeptin (Fig 3A)	18	1286 / 977	SMD 1.15 [0.68, 1.62]	96	< 0.00001
LH (Fig 3B)	16	1171 / 846	SMD 1.29 [0.76, 1.83]	96	< 0.00001
FSH (Fig 3C)	15	1098 / 782	SMD -0.02 [-0.49, 0.45]	95	0.94
Anti-müllerian hormone (Fig 3D)	5	433 / 310	SMD 0.97 [0.60, 1.34]	76	< 0.00001
Prolactin (Fig 3E)	5	310 / 193	SMD 0.80 [-0.55, 2.14]	98	0.25
Estradiol (Fig 3F)	8	517 / 415	SMD -0.05 [-0.61, 0.51]	94	0.80
Total testosterone (Fig 4A)	14	1019 / 783	SMD 2.58 [1.82, 3.35]	98	< 0.00001
Free testosterone (Fig 4B)	7	632 /367	SMD 1.37 [0.56, 2.17]	96	0.0009
DHEA-S (Fig 4C)	9	762 / 535	SMD 0.72 [0.32, 1.13]	91	0.0005
SHBG (Fig 4 D)	7	632 / 367	SMD -1.34 [-2.15, -0.52]	96	0.001
Ferriman-Gallweg score (Fig 4E)	5	563 / 474	SMD 5.08 [2.76, 7.39]	99	<0.0001
Glucose (Fig 5A)	11	879 / 714	SMD 0.66 [-0.13, 1.43]	98	0.10
HOMA-IR (Fig 5B)	10	840 / 558	SMD 0.76 [0.35, 1.17]	92	0.0003
Insulin (Fig 5C)	8	627 / 477	SMD 0.75 [0.30, 1.19]	92	0.001
Leptin (Fig 5D)	4	508 / 324	SMD 2.82 [1.35, 4.29]	98	0.0002
Total cholesterol (Fig 6A)	4	542 / 415	SMD 0.60 [-0.12, 1.33]	96	0.10
HDL-cholesterol (Fig 6B)	4	542 / 415	SMD -0.43 [-1.02, 0.16]	95	0.15
LDL-cholesterol (Fig 6C)	4	542 / 415	SMD 0.75 [-0.32, 1 81]	98	0.17
Triglycerides (Fig 6 D)	5	612 / 433	SMD 38.83 [9.00, 68.66]	100	0.01

Table 3. Sensitivity analyses (by excluding one trial at one time) reporting SMD and 95%confidence interval (CI), and I² for circulating kisspeptin, HOMA-IR index and AMH whencomparing women with and without PCOS.

Deleted publication [reference]	Kisspeptin SMD (95%Cl); I ²	HOMA-IR index SMD (95%Cl); I ²	AMH SMD (95%CI); I ²
Al-Jelawy ZH 2019 [29,30]	0.82 (0.43, 1.22); 94%	-	-
Anim-Ankumah A 2019 [31]	0.99 (0.54, 1.43); 95%	-	-
Branavan U 2019 [32]	0.91 (0.52, 1.29); 94%	-	-
Celik N 2018 [33]	1.21 (0.72, 1.70); 96%	0.75 (0.31, 1.19); 92%	-
Chen X 2010 [34]	1.21 (0.72, 1.70); 96%	0.77 (0.33, 0.41); 92%	-
Daghestani MH 2020 [35]	1.23 (0.72, 1.73); 96%	-	-
El-Shehawy YM 2016 [36]	1.21 (0.72, 1.70); 96%	-	0.85 (0.50, 1.21) 74%
Emekci Ozay OE [37]	1.23 (0.70, 1.75); 96%	0.77 (0.26, 1.27) 92%	1.02 (0.46, 1.58) 82%
Gorkem U, 2018 [38]	1.20 (0.70, 1.70); 96%	-	0.89 (0,46, 1.33) 78%
Ibrahim RO 2020 [39]	1.18 (0.68, 1.68); 96%	-	-
Jeon YE 2013 [40]	1.18 (0.68, 1.67); 96%	0.75 (0.30, 1.20) 92%	-
Kaya C 2018 [41]	1.15 (0.66, 1.64); 96%	-	1.1. (0.81, 1.42) 61%
Mut A 2020 [42]	1.22 (0.72, 1.72); 96%	0.78 (0.21, 1.24) 92%	0.98 (0.48, 1.48) 82%
Nyagolova PV 2016 [43]	1.20 (0.71, 1.70); 96%	0.55 (0.28, 0.83) 80%	-
Panidis D 2006 [44]	1.23 (0.75, 1.72); 96%	0.86 (0.44, 1.28) 92%	-
Rashad NM 2019 [45]	1.21 (0.70, 1.72); 96%	0.70 (0.26, 1.13) 91%	-
Wang T 2019 [46]	1.22 (0.72, 1.72); 96%	0.81 (0.35, 1.26) 92%	-
Yilmaz SA 2014 [47]	1.16 (0.66, 1.65); 96%	0-84 (0.40, 1.27) 91%	-
All available studies	1.15 (0.68, 1.62); 96%	0.76 (0.35, 1.17); 92%	0.97 (0.60, 1.34) 76%

Supplementary Material Online

Title: Circulating kisspeptin levels and insulin resistance in women with polycystic ovary syndrome: A systematic review, meta-analysis, and meta-regression.

This supplementary material has been provided by the authors to give readers additional information about their work.

<u>eMethod</u>

eMethods 1. Pubmed earch strategy.

<u>eTables</u>

eTable 1. Risk of bias of included observational studies using the Newcastle-Otawa Risk of Bias Scale.

eTable 2. Publication bias assessment for outcomes reported in at least 10 studies, Egger's regression, and trim and fill number of studies.

eSupplementary figures

eSupplementary figure 1. Subgroup analysis of circulating kisspeptin levels including studies matched and non-matched by mean age.

eSupplementary figure 2. Subgroup analysis of circulating kisspeptin levels including studies matched and non-matched by BMI.

eSupplementary figure 3. Subgroup analysis of circulating kisspeptin levels including studies matched and non-matched by mean HOMA-IR (< 0.60 versus > 0.70 values).

eSupplementary figure 4. Sensitivity analysis of circulating kisspeptin levels, deleting 4 articles the heterogeneity was reduced ($I^2 = 67\%$).

eSupplementary figure 5. Sensitivity analysis for kisspeptin levels.

eSupplementary figure 6. Publication bias assessment for circulating kisspeptin in women with and without PCOS.

eMethods 1. Pubmed search strategy.

"polycystic ovary syndrome"[MeSH Terms] OR "polycystic ovary syndrome"[Tiab] OR "Polycystic Ovarian Syndrome"[tiab] OR "sclerocystic ovary syndrome"[Tiab] OR "ovary polycystic disease"[tiab] OR "polycystic ovarian syndrome"[Tiab] OR "syndrome polycystic ovary"[Tiab] OR "stein leventhal syndrome"[Tiab] OR ("polycystic"[Tiab] AND "ovary"[Tiab] AND "syndrome"[Tiab]) OR ("stein"[Tiab] AND "leventhal"[Tiab] AND "syndrome"[Tiab]) AND "kisspeptins"[MeSH Terms] OR "kisspeptins"[Tiab] OR "metastasis suppressor kiss 1"[Tiab] OR "kisspeptin s"[Tiab] OR "kisspeptin"[Tiab] OR "kiss 1 metastasis suppressor"[Tiab] OR "metastin"[tiab] OR "receptors, kisspeptin-1"[MeSH Terms] OR "kiss1 receptor"[All Fields]

<u>eTables</u>

eTable 1. Risk of bias of included studies using the Newcastle-Otawa Scale (NOS).

		Selectio	on				Outcome		
Author, year	Case definition	Representativeness	Selection of controls	Definition of	Comparability	Ascertainment	Same method of	Statistical test	Total
[reference]	adequate				matched	ojexposure	uscertainment	rate	score
Aljelawy ZH 2019 [29,30]	1	1	1	1	0	1	0	0	5
Anim-Ankumah A 2019 [31]	1	1	0	1	0	1	1	0	5
Branavan U 2019 [32]	1	1	0	1	0	1	1	0	5
Celik N 2018 [33]	1	1	1	1	0	1	1	0	6
Chen X 2010 [34]	1	1	1	1	0	1	1	0	6
Daghestani MH 2020 [35]	1	1	1	1	0	1	1	1	7
El-Shehawy YM 2016 [36]	1	1	1	1	1	1	1	0	7
Emekci Ozay OE 2016 [37]	1	1	1	1	1	1	1	1	8
Gorkem U, 2018 [38]	1	1	1	1	1	1	1	0	7
Ibrahim RO 2020 [39]	1	1	1	1	1	1	1	0	7
Jeon YE 2013 [40]	1	1	1	1	0	1	1	1	7
Kaya C 2018 [41]	1	1	1	1	0	1	1	1	7
Mut A 2020 [42]	1	1	1	1	0	1	1	1	7
Nyagolova PV 2016 [43]	1	1	1	1	0	1	1	1	7
Panidis D 2006 [44]	1	1	1	1	0	1	1	1	7
Rashad NM 2019 [45]	1	1	1	1	1	1	1	1	8
Wang T 2019 [46]	1	1	1	1	0	1	1	1	7
Yilmaz SA 2014 [47]	1	1	1	1	1	1	1	0	7

Outcomes	Studies (<i>n</i>)	Egger's test; p	Kendall's tau; p
Age	18	0.122; 0.903	-0.359; < 0.001
ВМІ	18	0.371; 0.711	-0.371; 0.881
Kisspeptin	18	7.535; <0.001	0.373; 0.032
Kisspeptin (^)	15	- 0.172; 0.863	0.124; 0.559
LH	16	5.042; <0.001	0.367; 0.052
FSH	15	3.461; <0.001	0.219; 0.282
Estradiol	15	-1.022; 0.307	0.071; 0.905
Testosterone	14	10.607; <0.001	0.429; 0.036
Glucose	12	1.145; 0.252	0.182; 0.459
HOMA-IR	10	0.136; 0.892	0.111; 0.727

eTable 2. Publication bias assessment for outcomes reported in at least 10 studies, Egger's regression, and Kendall's tau.

(^) Deleted references [29, 31, 32]

eSupplementary figures

eSupplementary figure 1. Subgroup analysis of circulating kisspeptin levels including studies matched and non-matched by mean age.

	Polycystic	c ovary synd	rome	Con	trol grou	p		Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD.	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
.3.1 Kisspeptin matche	d by mean a	quoto equ	1000	10000	1.00	100	1000		
Daghestani MH 2020	0.41	0.16	104	0.39	0.07	109	5.9%	0.16 [-0.11, 0.43]	
imekci Ozay O 2016	1.92	1.29	250	1.49	1.46	150	5.9%	0.32 [0.11, 0.52]	+
korkem U 2018	5.76	2.11	60	4.85	2.16	57	5.8%	0.52 (0.15, 0.99)	-
trahim RO 2020	1.79	0.98	60	1.65	0.86	40	5.8%	0.15 [-0.25, 0.55]	+
eon YE 2013	10.64	6.18	54	6.51	3.13	36	5.7%	0.79 [0.35, 1.23]	-
aya C 2019	525.49	164.17	29	354.31	111.38	27	5.5%	1.20 [0.62, 1.77]	
Rashad NM 2019 Subtotal (95% CI)	0.31	0.1	105	0.27	0.06	90 509	5.9% 40.5%	0.47 [0.19, 0.76] 0.45 [0.24, 0.66]	ī
Heterogeneity: Tau* = 0:0 Fest for overall effect: Z =	5; Chi#= 16. 4.27 (P < 0.0	23, df = 6 (P 1001)	= 0.01); (* = 63%					
.3.2 Kisspeptin not mat	ched by me	an age grou	p						
ilelawy ZH 2019	1,390	109.1	70	415.3	56.06	18	3.7%	9.58 [8.04, 11.12]	
nim-Ankumah A 2019	20.21	1.74	41	12.15	7.51	40	5.2%	3.70 [2.98, 4.43]	
Iranavan U 2019	4.87	0.24	55	4.13	0.13	110	5.5%	4.23 [3.66, 4.79]	
etik N 2018	644.27	486.15	20	555.09	401.02	20	5.4%	0.20 [-0.43, 0.82]	+
hen X 2010	0.23	0.17	42	0.19	0.21	20	5.6%	0.22 [-0.32, 0.75]	+
i-Shehawy YM 2015	0.27	0.13	23	0.16	0.9	18	5.4%	0.18 [-0.44, 0.80]	+
lut A 2020	474.77	362.07	70	421.46	338.63	58	5.8%	0.15 [-0.20, 0.50]	- +
hagolova PV 2016	0.23	0.2	87	0.16	0.01	42	5.8%	0.42 [0.05, 0.79]	
anidis D 2006	2.95	0.26	56	3.04	0.55	13	5.5%	-0.27 [-0.87, 0.34]	-
Yang T 2019	265.85	214.67	73	219,11	165,69	63	5.8%	0.24 [-0.10, 0.59]	-
Nmaz SA 2014 Subtotal (95% CI)	2.02	0.97	83 620	1,16	0.26	-56 468	5.8% 59.5%	1.15[0.80, 1.50] 1.65[0.74, 2.57]	•
leterogenieity: Tau* = 2.2 est for overall effect. Z =	6; Chi² = 383 3.55 (P = 0.0	3.55, df= 10 1004)	(P < 0.00	001); i* =	97%				
fotal (95% CI)			1282			977	100.0%	1.11 [0.65, 1.58]	
Heterogeneity: Tau ^a = 0.9 Fest for overall effect Z = Test for suburnum differen	6; Chi [#] = 421 4.65 (P < 0.0 sces: Chi [#] =	1.84, df= 17 00001) 6.37 df= 1.0	(P < 0.00 P = 0.01)	001); P=	96%				-10 -5 0 5 10 Control group Polycystic evary syndror

eSupplementary figure 2. Subgroup analysis of circulating kisspeptin levels including studies matched and non-matched by BMI.

	Polycystic	: ovary synd	rome	Con	trol grou	p		Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
2.1 Studies matched b	y mean 8MI	group	10.000		1.1.2.2.2	1000	1000		
Cellk N 2018	644.7	486.15	20	555.09	401.02	20	5.4%	0.20 [-0.42, 0.82]	+
Chen X 2010	0.23	0.17	42	0.19	0.21	20	5.6%	0.22 [-0.32, 0.75]	+
Daghestani MH 2020	0.41	0.16	104	0.39	0.07	109	5.9%	0.16 [-0.11, 0.43]	+
Sorkern U 2018	5.76	211	60	4.65	216	57	5.8%	0.52 (0.15, 0.89)	-
brahim RO 2020.	1.79	0.98	60	1.05	0.86	40	5.7%	0.79 [0.37, 1.20]	-
Aut A 2020	474.77	362.07	70	421.46	338.63	58	5.8%	0.15 [-0.20, 0.50]	+
Rashad NM 2019 Subtotal (95% CI)	0.31	0.1	105	0.27	0.06	90 394	5.9%	0.47 (0.19, 0.76) 0.36 (0.18, 0.54)	-
Heterogeneity: Tau* = 0.0 Test for overall effect. Z =	2; Chi#= 9.3 3.99 (P < 0.0	5, df = 6 (P =)001)	0.16); 7	= 38%					
3.2.2 Studies not match	ed by mean l	BMI group							
Vielawy ZH 2019	1,390	109.1	70	415.3	56.06	18	3.7%	9.58 [8.04, 11.12]	
nim-Ankumah A 2019	20.21	1.74	41	12.15	7.51	40	5.2%	3.70 [2.98, 4.43]	
Iranavan U 2019	4.87	0.238	55	4.127	0.13	110	5.5%	4.27 (3.70, 4.83)	
I-Shehawy YM 2015	0.27	0.13	23	0.16	0.9	18	5,4%	0.18 [-0.44, 0.80]	+
imekci Ozay O 2018	1.92	1.29	250	1.49	1.46	150	5,9%	0.32 (0.11, 0.52)	-
eon YE 2013	10.64	5.18	54	6.51	3.13	36	5.7%	0.79 (0.35, 1.23)	
aya C 2019	525.49	164.17	29	354.31	111.38	27	5.5%	1.20 [0.62, 1.77]	-
hagolova PV 2016	0.23	0.2	87	0.16	0.01	42	5.8%	0.42 (0.05, 0.79)	-
anidis D 2006	2.95	0.26	56	3.04	0.55	13	5.5%	-0.27 [-0.87, 0.34]	-
Vang T 2019	265.85	214.67	73	219,11	165,69	63	5.8%	0.24 [-0.10, 0.59]	-
1imaz SA 2014 Subtotal (95% CI)	2.02	0.97	83 821	1.16	0.26	66 583	5.8% 59.9%	1.15[0.80, 1.50] 1.79[0.97, 2.60]	•
leterogeneity: Tau* = 1.7 'est for overall effect. Z =	9; Chi ^a = 383 4 30 (P = 0.0	7.18, df = 10 1001)	(P < 0.00	001); i* =	97%				1-12 T 1 T
(otal (95% CI)			1282			977	100.0%	1.15 [0.68, 1.67]	- 10 IV - 10 IV
leterogeneity: Tau ^a = 0.9	6; Chi#= 419	9.57, df = 17	(P = 0.00	001); P=	96%				- <u>ta</u> <u>t</u> <u>t</u> <u>t</u> <u>t</u>
est for overall effect Z =	4.82 (P = 0.0	10001)	0.4004-00	~0.0101010					-10 -5 0 5 10
est for subaroup differen	nces: Chi*=	11 24. df = 1	dP = 0.00	1085 P*+	91.1%				counce Brand - Loniciane availy shudeou

eSupplementary figure 3. Subgroup analysis of circulating kisspeptin levels including studies matched and non-matched by mean HOMA-IR (< 0.60 versus > 0.70 values).

	Polycystic	ovary synd	rome	Com	trol grou	p		Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD.	Total	Mean	5D	Total	Weight	IV, Random, 95% CI	IV, Random, 95% Cl
4.1 Kisspeptin mate	hed by HOM/	A-IR		en bist iv				eres in his weight an and show	and the second state of the second state
Chen X 2010	0.23	0.17	42	0.19	0.21	20	7.9%	0.22 [-0.32; 0.75]	
anidis D 2006	2.95	0.26	56	3.04	0.55	13	6.9%	-0.27 [-0.87, 0.34]	
Vang T 2019	265.85	214.67	73	219.11	165.69	63	11.1%	8.24 [-0.10, 0.68]	
filmaz SA 2014 Subtotal (95% CI)	2.02	0.97	83 254	1.16	0.26	66 162	10.9% 36.8%	1.15 (0.80, 1.50) 0.36 [-0.24, 0.96]	
leterogeneity: Tau*=	0.37; Chi*= 2	2.82, df = 3	P < 0.00	01); F = E	7%				
lest for overall effect 1	Z = 1.18 (P = 1	0.24)							
.4.2 Kisspeptin non-I	natched by h	IOMA-IR							
elik N 2018	644.27	486.15	20	555.09	401.02	20	6.7%	0.20 [-0.43, 0.82]	
mekci Ozay O 2016	1.92	1.29	250	1.49	1.48	150	13.6%	0.32 (0.11, 0.62)	
eon YE 2013	10.64	6.18	54	6.51	3.13	36	9.4%	0.79 [0.35, 1.23]	
fut A 2020	474.77	362.07	70	421.46	338.63	58	10.9%	0.15 [-0.20, 0.50]	
hagolova PV 2016	0.23	0.2	87	0.16	0.01	42	10.5%	0.42 (0.05, 0.79)	
Rashad NM 2019 Rubtotal (95% CI)	0.31	0.1	105	0.27	0.06	90 396	12.1%	0.47 (0.19, 0.76) 0.38 (0.23, 0.53)	•
leterogeneity Tau*= fest for overall effect 2	0.01; Chi*= 6 Z= 4.95 (P × 1	.20, df = 5 (F 0.00001)	e = 0.29),	l*= 19%					0.000
fotal (95% CI)			840			558	100.0%	0.40 [0.18, 0.61]	•
leterogeneity: Tau* = fest for overall effect 2 fest for subgroup diffe	0.08; Chi#= 2 2= 3.64 (P = 1 rentes: Chi#	9.98, df = 9 0.0003) = 0.00, df = 1	(P=0.00	04); (* = 7	0%				Control group Polycystic evary syndrom

Test for subaroup differences: Chi# = 0.00, df = 1 (P = 0.95), I# = 0%

eSupplementary figure 4. Meta-regression analyses on the effect of age (Figure 4A, p = 0.356), HOMA-IR index (Figure 4B, p = 0.930), insulin (Figure 4C, p = 0.898), AMH (Figure 4D, p = 0.64), and LH (Figure 4E, p = 0.998) on circualting kisspeptin levels.

5

n

(4A) Effect of age

÷. 14

Model Resul	ts				
Covariate	Coefficients	Lower bound	Upper bound	Std. error	p-Valu
Intercept	0.915	-0.327	2.158	0.634	0.149
SMD_Age	-0.159	-0.498	0.179	0.173	0.356
2		e			1
2 2 e -		e			
2 e -		e			1
2 - 2 - 4 - 4 -	0	e	o		

SMD_Ape



0

-10

-5





(4C) Effect of circulating insulin

Meta-Regression

Metric: Standardized Mean Difference

Model Results					
Covariate	Coefficients	Lower bound	Upper bound	Std. error	p-Value
Intercept	0.392	0.032	0.752	0.184	0.033
SMD_insulin	8.021	-0.304	0.346	0.166	0.898



(4D) Effect of anti-müllerian hormone

Meta-Regression					
Metric: Standardized Mean	Difference				
Model Menults					
Covariate	Coefficients	Lower bound	Upper bound	Std. error	p-Value
Intercept	0.879	0.334	1.424	0.278	0.002
SHD_Antimullerianhormone	-0.488	-1,001	0.028	0.263	0.064



(4E) Effect of circulating LH

Meta-Regression

Retrict Standardized Mean Difference

Hodel Resul	1.0				
Covariate	coefficienss	tower bound	upper bound	Bid, error	p-value
Intercept	0.942	-0.392	2.277	0.681	0.347
SHD_LE	0.001	-0.664	0.665	0.339	0.938



eSupplementary figure 5. Sensitivity analysis for kisspeptin levels by deleting 4 studies. The heterogenity was reduced to to $I^2 = 67\%$.

	Polycystic ovary syndrome			Control group			Std. Mean Difference	Std. Mean Difference	
Study or Subgroup	Mean	5D	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% CI
Vielawy ZH 2019	1,390	109.1	70	415.3	56.06	19	0.0%	9.58 [8.04, 11.12]	2. 10. 10. 10. 10. 10. 10. 10. 10. 10. 10
nim-Ankumati A 2019	20.21	1.74	41	12.15	2.51	40	0.0%	3.70 [2.98, 4.43]	
ranavan U 2019	4.87	0.24	55	4.13	0.13	110	0.0%	4 23 (3.66, 4.79)	
elik N 2018	644.27	486.15	20	555.09	401.02	20	4.6%	0.20 [-0.43, 0.82]	
hen × 2010	0.23	0.17	42	0.19	0.21	20	5.5%	0.22 [-0.32, 0.75]	
aghestani MH 2020	0.41	0.16	104	0.39	0.07	109	9.0%	0.16 [-0.11, 0.43]	
Shehawy YM 2015	0.27	0.13	23	0.16	0.9	18	4.7%	0.18 [-0.44, 0.80]	
meikol Ozay O 2016	1.92	1.29	250	1.49	1.46	150	9.9%	0.32[0.11, 0.52]	
orkem U 2018	5.76	2.11	60	4.65	2.16	57	7.6%	0.52 (0.15, 0.89)	
rahim RO 2020	1.79	0.98	60	1.05	0.86	40	6.9%	0.79 [0.37, 1.20]	
eon YE 2013	10.64	6.18	54	6.51	3.13	36	8.6%	0.79 [0.35, 1.23]	
aya C 2019	525.49	164.17	29	354.31	111.38	27	5.1%	1.20 [0.62, 1.77]	
ut A 2020	474.77	362.07	70	421.46	338.63	58	7.9%	0.15[-0.20, 0.50]	
yagolova PV 2016	0.23	0.2	87	0.16	0.01	42	7.5%	0.42 [0.05, 0.79]	
anidis D 2006	2.95	0.26	56	3.04	0.55	13		Not estimable	
ashad NM 2019	0.31	0.1	105	0.27	0.66	90	8.8%	0.47 (0.19, 0.76)	
ang T 2019	265.85	214.67	73	219.11	165.69	63	8.0%	0.24 [-0.10, 0.58]	
ilmaz 8A 2014	2.02	0.97	83	1.16	0.26	66	7.8%	1.15 (0.80, 1.50)	
otal (95% CI)			1060			796	100.0%	0.48 [0.30, 0.65]	•
eterogeneity: Tau ^a = 0.0	7; Chi#= 39.	29. df = 13.6	P = 0.000	(2), P = 6)	196				- t - t -
est for overall effect Z =	5.40 (P = 0.0	10001)						-2	Control group Polycystic ovary syndro

eSupplementary figure 6. Publication bias assessment for circulating kisspeptin in women with and without PCOS (n = 18 studies; top) and after deleting 3 studies (bottom) with publication bias [29, 31, 32]. The table summarizes Kendall's tau and Egger's test values.



(6a) 18 studies

(6b) Deleting 3 studies [29, 31, 32]



	Kisspeptir studi	n n= 18 es	Kisspeptin deletting 3 studies [29, 31, 32] (with suspected publication bias)		
Test name	Value	р	Value	р	
Fail-safe n	1847.0	< 0.001	404.0	< 0.001	
Kendalls Tau	0.373	0.032	0.124	0.559	
Egger's Regression	7.535	< 0.001	-0.172	0.863	
Trim and Fill Number of Studies	0		3		

eSupplementary figure 7. Publication bias assessment for HOMA-IR index in women with and without PCOS (n = 10 studies). The table summarizes Kendall's tau and Egger's test values.



	HOMA-IR index n = 10 studies		
Test name	Value	р	
Fail-safe n	522.0	< 0.001	
Kendalls Tau	0.111	0.727	
Egger's Regression	0.136	0.892	
Trim and Fill Number of Studies	0		