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

Faustino R. Pérez-López[a,b,*,1], Lía Ornat[a,1], María T. López-Baena[b], Javier Santabárbara[b,c], Ricardo Savirón-Cornudella[d], Gonzalo R. Pérez-Roncero[b]

[a] *Department of Obstetrics and Gynecology, University of Zaragoza Faculty of Medicine, Zaragoza 50009, Spain*

[b] *Aragón Health Research Institute, Zaragoza 50009, Spain*

[c] *Department of Microbiology, Preventive Medicine and Public Health, University of Zaragoza Faculty of Medicine, Zaragoza 50009, Spain*

[d] *Department of Obstetrics and Gynecology, Villalba General Hospital, Madrid 28400, Spain*

[1] These authors have contributed equally to this manuscript.

*Corresponding author at Department of Obstetrics and Gynecology, Facultad de Medicina, Universidad de Zaragoza, Domingo Miral s/n, Zaragoza 50009, Spain; email: faustino.perez@unizar.es

Short title: Kisspeptin, HOMA-IR and PCOS

Word count of the main text: 3642 words; **Figures:** 6; **Tables:** 3

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.

Declaration of Interest: None.

ORCID identifications

Faustino R. Pérez-López	0000-0002-2801-416X
Lía Ornat	0000-0001-9056-2143
María T. López-Baena	0000-0002-9890-8003
Javier Santabárbara	0000-0002-7297-6104
Ricardo Savirón-Cornudella	0000-0001-9585-0187
Gonzalo R. Pérez-Roncero	0000-0001-8137-4837

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. Co-primary 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; Anti-müllerian hormone

Abbreviations

AMH: Anti-müllerian hormone; BMI: Body mass index; CI: Confidence interval; DHEA-S: dehydroepiandrosterone sulfate; FSH: Follicle-stimulating hormone; GnRH: gonadotrophin-releasing 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 ≥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 fixed-effect 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 I^2: An I^2 value of 0-30% define low heterogeneity, 30-65 % moderate heterogeneity, and >65 % substantial heterogeneity [24]. A $p < 0.1$ for the Chi^2 test was defined as an indicator of heterogeneity; a $Tau^2 > 1$ 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 (I^2 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 (I^2 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 (I^2 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 (I^2 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 I^2 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 waist-to-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 meta-analyzed 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 meta-regression 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.

Funding / Source of support

This research did not receive any specific grant or was funded by any commercial or non-profit organization or public agency.

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.

Acknowledgments

The authors thank Ms. M. Salas Valero for assistance with the study. Dr. Zahraa H Al-Jelawy provided complementary information of the article cited as reference [29]. Dr. Huiying Zang provided the mean ages ± SD results not included in the full-publication cited as reference [47]. Dr Xiaoli Chen informed about a preliminary publication in Chinese language cited as reference [48].

Appendix A. Supplementary data

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

References

1. Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiological reviews 2912;92: 92:1235-316. doi: 10.1152/physrev.00037.2010 1235-1316, doi:10.1152/physrev.00037.2010.
2. West A, Vojta PJ, Welch DR, Weissman BE. Chromosome localization and genomic structure of the KiSS-1 metastasis suppressor gene (KISS1). Genomics 1998;54:145-8. doi:10.1006/geno.1998.5566.
3. Ke R, Ma X, Lee LTO. Understanding the functions of kisspeptin and kisspeptin receptor (Kiss1R) from clinical case studies. Peptides. 2019;120:170019. doi:10.1016/j.peptides.2018.09.007
4. Tolson KP, Garcia C, Yen S, Simonds S, Stefanidis A, Lawrence A, Smith JT, Kauffman AS. Impaired kisspeptin signaling decreases metabolism and promotes glucose intolerance and obesity. J Clin Invest. 2014;124:3075-9. doi: 10.1172/JCI71075.
5. Harter CJL, Kavanagh GS, Smith JT. The role of kisspeptin neurons in reproduction and metabolism. J Endocrinol 2018;238(3):R173-83. doi: 10.1530/JOE-18-0108.
6. Hu KL, Zhao H, Min Z, He Y, Li T, Zhen X, Ren Y, Chang HM, Yu Y, Li R. Increased expression of KISS1 and KISS1 receptor in human granulosa lutein cells-potential pathogenesis of polycystic ovary syndrome. Reprod Sci. 2019;26(11):1429-1438. doi: 10.1177/1933719118818899.
7. Tang R, Ding X, Zhu J. Kisspeptin and polycystic ovary syndrome. Front Endocrinol (Lausanne). 2019;10:298. doi: 10.3389/fendo.2019.00298.
8. Araújo BS, Baracat MCP, Dos Santos Simões R, de Oliveira Nuñes C, Maciel GAR, Lobo RA, Soares-Jr JM, Baracat EC. Kisspeptin influence on polycystic ovary syndrome-a mini review. Reprod Sci. 2020;27(2):455-60. doi: 10.1007/s43032-019-00085-6.
9. Behboudi-Gandevani S, Ramezani Tehrani F, Rostami Dovom M, Farahmand M, Bahri Khomami M, Noroozzadeh M, et al. Insulin resistance in obesity and polycystic ovary syndrome: systematic review and meta-analysis of observational studies. Gynecol Endocrinol. 2016;32(5):343-53. doi: 10.3109/09513590.2015.1117069.
10. Li Y, Chen C, Ma Y, Xiao J, Luo G, Li Y, Wu D. Multi-system reproductive metabolic disorder: significance for the pathogenesis and therapy of polycystic ovary syndrome (PCOS). Life Sci. 2019;228:167-175. doi: 10.1016/j.lfs.2019.04.046.
11. Wang J, Wu D, Guo H, Li M. Hyperandrogenemia and insulin resistance: the chief culprit of polycystic ovary syndrome. Life Sci. 2019;236:116940.

12. Jones MR, Goodarzi MO. Genetic determinants of polycystic ovary syndrome: progress and future directions. Fertil Steril. 2016;106(1):25-32. doi: 10.1016/j.fertnstert.2016.04.040.
13. Randeva HS, Tan BK, Weickert MO, Lois K, Nestler JE, Sattar N, et al., Cardiometabolic aspects of the polycystic ovary syndrome. Endocr Rev 2012;33:812-41.
14. Cassar S, Misso ML, Hopkins WG, Shaw CS, Teede HJ, Stepto NK. Insulin resistance in polycystic ovary syndrome: a systematic review and meta-analysis of euglycaemic-hyperinsulinaemic clamp studies. Hum Reprod 2016;31:2619-31
15. Zhao Y, Zhao Y, Wang C, Liang Z, Liu X. Diagnostic value of anti-müllerian hormone as a biomarker for polycytic ovary syndrome: A meta-analysis update. Endocr Pract. 2019;25(10):1056-1066. doi: 10.4158/EP-2019-0098.
16. Dewailly D, Barbotin AL, Dumont A, Catteau-Jonard S, Robin G. Role of anti-müllerian hormone in the pathogenesis of polycystic ovary syndrome. Front Endocrinol (Lausanne). 2020;11:641. doi: 10.3389/fendo.2020.00641.
17. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. For the meta-analysis of observational studies in epidemiology (MOOSE) group.Meta-analysis of observational studies in epidemiology. A proposal for reporting. JAMA 2000;283:2008–12. doi.org/10.1001/jama.283.15.2008.
18. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81(1):19-25. doi: 10.1016/j.fertnstert.2003.10.004.
19. Goodman NF, Cobin RH, Futterweit W, Glueck JS, Legro RS, Carmina E. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society Disease State Clinical Review: Guide to the Best Practices in the Evaluation and Treatment of Polycystic Ovary sydnrome. Part 1. Endocr Pract. 2015;21(11):1291-1300. doi:10.4158/EP15748.DSC
20. Dewailly D, Lujan ME, Carmina E, et al. Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. Hum Reprod Update. 2014;20(3):334-352. doi:10.1093/humupd/dmt061
21. Wells G, Shea B, O'Connell D, Peterson G, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at http://www.ohri.ca/progr ams/clini cal_epide miolo gy/oxfor d.asp.
22. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Methodol 2005;5:13.
23. Higgins JPT, Green S. Cochrane handbook for systematic reviews of interventions version 5.1.0, 2011.
24. Higgins JP. Heterogeneity in meta-analysis should be expected and appropriately quantified. Int J Epidemiol 2008;37(5):1158–1160.
25. Tobías A, Campbell MJ. Modelling influenza epidemics in the relation between black smoke and total mortality. A sensitivity analysis. J Epidemiol Community Health 1999;53(9):583–584.
26. Patsopoulos NA, Evangelou E, Ioannidis JPA. Sensitivity of between-study heterogeneity in meta-analysis: proposed metrics and empirical evaluation. International Journal of Epidemiology 2008;37:1148–1157. doi:10.1093/ije/dyn065
27. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50: 1088-1101.
28. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629e34.
29. Al-Jelawy ZH, Al-Sallami ASM. Role of kisspeptin in polycystic ovary disease in patients of ALNajaf ALAshrif City, Iraq. J Pharm Sci Res 2019;11:169-73.
30. Al-Jelawy ZH. Assessment of kisspeptin and inteleukin-37 levels and some hormonal and biochemical parameters in women with polycystic ovary syndrome. Master degree Thesis in Biology. University of Kufa Faculty of Science, Republic of Iraq, 2018.
31. Anim-Ankumah A. The association of kisspeptin with polycystic ovarian syndrome. Thesis, University of Ghana for the award of Chemical Pathology Degree. University of Ghana, July 2019.
32. Branavan U, Muneeswaran K, Wijesundera WSS, Senanayake A, Chandrasekharan NV, Wijeyaratne CN. Association of KISS1 and GPR54 gene polymorphisms with polycystic ovary syndrome among sri lankan women. Biomed Res Int 2019:6235680. doi.org/10.1155/2019/62356 80

33. Celik N, Aydin S, Ugur K, Yardim M, Acet M, Yavuzkir S, et al. Patatin-like phospholipase domain containing 3-gene (adiponutrin), preptin, kisspeptin and amylin regulates oocyte developmental capacity in PCOS. Cell Mol Biol (Noisy-le-grand). 2018;64(15):7-12.
34. Chen X, Mo Y, Li L, Chen Y, Li Y, Yang D. Increased plasma metastin levels in adolescent women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2010;149(1):72-6. doi: 10.1016/j.ejogrb.2009.11.018.
35. Daghestani MH, Daghestani MH, Daghistani M, Ambreen K, Almuammar MN, Al Neghery LM, et al. Relevance of *KISS1* gene polymorphisms in susceptibility to polycystic ovary syndrome and its associated endocrine and metabolic disturbances. Br J Biomed Sci. 2020; 77(4):185-190. doi: 10.1080/09674845.2020.1726662.
36. El-Shehawy, Y.M., Safan, M.A., Evaluation of Serum Anti-Mullerian Hormone (AMH) and Plasma Metastin Levels in Women with Polycystic Ovary Syndrome (PCOS). Med. J. Cairo Univ. 2015; 83:399–406.
37. Emekci Ozay O, Ozay AC, Acar B, Cagliyan E, Seçil M, Küme T. Role of kisspeptin in polycystic ovary syndrome (PCOS). Gynecol Endocrinol. 2016;32(9):718-722. doi: 10.3109/09513590.2016.1161019.
38. Gorkem U, Togrul C, Arslan E, Sargin Oruc A, Buyukkayaci Duman N. Is there a role for kisspeptin in pathogenesis of polycystic ovary syndrome? Gynecol Endocrinol. 2018;34(2):157-60. doi: 10.1080/09513590.2017.1379499.
39. Ibrahim RO, Omer SH, Fattah CN. The Correlation between Hormonal Disturbance in PCOS Women and Serum Level of Kisspeptin. Inter J Endocrinol 2020; 2020 D 6237141. doi.org/10.1155/2020/6237141
40. Jeon YE, Lee KE, Jung JA, Yim SY, Kim H, Seo SK, et al. Kisspeptin, leptin, and retinol-binding protein 4 in women with polycystic ovary syndrome. Gynecol Obstet Invest. 2013;75(4):268-74. doi:10.1159/000350217
41. Kaya C, Alay İ, Babayeva G, Gedikbaşı A, Ertaş Kaya S, Ekin M, et al. Serum Kisspeptin levels in unexplained infertility, polycystic ovary syndrome, and male factor infertility. Gynecol Endocrinol. 2019;35(3):228-32. doi: 10.1080/09513590.2018.1519792.
42. Mut A, Erel CT, İnan D, Öner YÖ. Serum kisspeptin levels correlated with anti-mullerian hormone levels in women with and without polycystic ovarian syndrome. Gynecol Endocrinol. 2020 Sep 23:1-5. doi: 10.1080/09513590.2020.1825670. PMID: 32964765.
43. Nyagolova PV, Mitkov MD, Orbetzova MM, Terzieva DD. Kisspeptin and galanin-like peptide (GALP) levels in women with polycystic ovary syndrome. International J Pharmaceutical Medical Research. 2016;4:7-12.
44. Panidis D, Rousso D, Koliakos G, Kourtis A, Katsikis I, Farmakiotis D, et al. Plasma metastin levels are negatively correlated with insulin resistance and free androgens in women with polycystic ovary syndrome. Fertil Steril. 2006;85(6):1778-83. doi: 10.1016/j.fertnstert.2005.11.044.
45. Rashad NM, Al-Sayed RM, Yousef MS, Saraya YS. Kisspeptin and body weight homeostasis in relation to phenotypic features of polycystic ovary syndrome; metabolic regulation of reproduction. Diabetes Metab Syndr. 2019;13(3):2086-2092. doi: 10.1016/j.dsx.2019.04.017.
46. Wang T, Han S, Tian W, Zhao M, Zhang H. Effects of kisspeptin on pathogenesis and energy metabolism in polycystic ovarian syndrome (PCOS). Gynecol Endocrinol. 2019;35(9):807-810. doi: 10.1080/09513590.2019.1597343.
47. Yilmaz SA, Kerimoglu OS, Pekin AT, Incesu F, Dogan NU, Celik C, et al. Metastin levels in relation with hormonal and metabolic profile in patients with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 2014;180:56–60. doi.org/10.1016/j.ejogr b.2014.06.004
48. Chen XL, Mo YQ, Li L, Chen YX, Li Y, Zhang QX, et al. [Plasma metastin in adolescent polycystic ovary syndrome.]. Zhonghua Fu Chan Ke Za Zhi. 2009;44(10):745-9. PMID: 20078960
49. Albalawi FS, Daghestani MH, Daghestani MH, Eldali A, Warsy AS. rs4889 polymorphism in KISS1 gene, its effect on polycystic ovary syndrome development and anthropometric and hormonal parameters in Saudi women. J Biomed Sci. 2018;25(1):50. doi: 10.1186/s12929-018-0452-2.
50. Daghestani MH. Evaluation of biochemical, endocrine, and metabolic biomarkers for the early diagnosis of polycystic ovary syndrome among non-obese Saudi women. Int J Gynaecol Obstet. 2018;142(2):162-169. doi: 10.1002/ijgo.12527.
51. Daghestani MH, Daghestani MH, Daghistani M, Ambreen K, Albalawi FS, AlNeghery LM, et al. Influence of KISS1 gene polymorphisms on the risk of polycystic ovary syndrome and its associated variables, in Saudi women. BMC Endocr Disord. 2020;20(1):59. doi: 10.1186/s12902-020-0537-2.
52. Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM,Weissman BE, Welch DR. KiSS-1, a novel human

malignant melanoma metastasissuppressor gene. J Natl Cancer Inst. 1996;88(23):1731–1737.
53. Ohkura S, Uenoyama Y, Yamada S, Homma T, Takase K, Inoue N, Maeda K, Tsukamura H. Physiological role of metastin/kisspeptin in regulating gonadotropin-releasing hormone (GnRH) secretion in female rats. Peptides. 2009;30(1):49-56. doi: 10.1016/j.peptides.2008.08.004.
54. Greenhill C. Kisspeptin receptor agonist shows promise. Nature Reviews Endocrinology 2021;17:68.
55. Navarro VM. Metabolic regulation of kisspeptin - the link between energy balance and reproduction. Nat Rev Endocrinol. 2020;16(8):407-420. doi: 10.1038/s41574-020-0363-7.
56. Comninos AN, Wall MB, Demetriou L, Shah AJ, Clarke SA, Narayanaswamy S, et al. Kisspeptin modulates sexual and emotional brain processing in humans. J Clin Invest. 2017;127(2):709-19. doi: 10.1172/JCI89519
57. Han SK, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, et al. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. J Neurosci. (2005) 25:11349–56. 10.1523/jneurosci.3328-05.2005
58 Sithinamsuwan K, Mahachoklertwattana P, Wankanit S, Chanprasertyothin S, Pongratanakul S, Khlairit P, Poomthavorn P. Serum Kisspeptin and Its Relation to Metabolic Parameters and Glucose Metabolism in Prepubertal and Pubertal Obese Children. Int J Endocrinol. 2020;2020:8826401. doi: 10.1155/2020/8826401.
59. Blasco V, Pinto FM, Fernández-Atucha A, Prados N, Tena-Sempere M, Fernández-Sánchez M, Candenas L. Altered expression of the kisspeptin/KISS1R and neurokinin B/NK3R systems in mural granulosa and cumulus cells of patients with polycystic ovarian syndrome. J Assist Reprod Genet. 2019;36(1):113-120. doi: 10.1007/s10815-018-1338-7.
60. Romero-Ruiz A, Skorupskaite K, Gaytan F, Torres E, Perdices-Lopez C, Mannaerts BM, et al. Kisspeptin treatment induces gonadotropic responses and rescues ovulation in a subset of preclinical models and women with polycystic ovary syndrome. Hum Reprod. 2019;34(12):2495-2512. doi: 10.1093/humrep/dez205.
61. Abdolahian S, Tehrani FR, Amiri M, Ghodsi D, Yarandi RB, Jafari M, et al. Effect of lifestyle modifications on anthropometric, clinical, and biochemical parameters in adolescent girls with polycystic ovary syndrome: a systematic review and meta-analysis. BMC Endocr Disord. 2020;20(1):71. doi: 10.1186/s12902-020-00552-1.
62. Fanchin R, Schonäuer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Müllerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod. 2003;18(2):323-7. doi: 10.1093/humrep/deg042.
63. Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod. 2011;26(11):3123-9. doi: 10.1093/humrep/der297.
64. Tal R, Seifer CM, Khanimov M, Seifer DB, Tal O. High serum Antimullerian hormone levels are associated with lower live birth rates in women with polycystic ovarian syndrome undergoing assisted reproductive technology. Reprod Biol Endocrinol. 2020 Mar 10;18(1):20. doi: 10.1186/s12958-020-00581-4.
55. Phylactou M, Clarke SA, Patel B, Baggaley C, Jayasena CN, Kelsey TW, Comninos AN, Dhillo WS, Abbara A. Clinical and biochemical discriminants between functional hypothalamic amenorrhoea (FHA) and polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf). 2020 Dec 23. doi: 10.1111/cen.14402.
56. Andreozzi F, Mannino GC, Mancuso E, Spiga R, Perticone F, Sesti G. Plasma kisspeptin levels are associated with insulin secretion in nondiabetic individuals. PLoS One. 2017;12(6):e0179834. doi: 10.1371/journal.pone.0179834.
57. Welt CK, Duran JM. Genetics of polycystic ovary syndrome. Semin Reprod Med. 2014;32(3):177-82. doi: 10.1055/s-0034-1371089.
58. Castillo-Higuera T, Alarcón-Granados MC, Marin-Suarez J, Moreno-Ortiz H, Esteban-Pérez CI, Ferrebuz-Cardozo AJ, et al. A Comprehensive Overview of Common Polymorphic Variants in Genes Related to Polycystic Ovary Syndrome. Reprod Sci. 2020 Nov 10. doi: 10.1007/s43032-020-00375-4.
59. Jones MR, Goodarzi MO. Genetic determinants of polycystic ovary syndrome: progress and future directions. Fertil Steril. 2016;106(1):25-32. doi: 10.1016/j.fertnstert.2016.04.040.
60. Skorupskaite K, George JT, Veldhuis JD, Millar RP, Anderson RA. Kisspeptin and neurokinin B interactions in modulating gonadotropin secretion in women with polycystic ovary syndrome. Hum Reprod. 2020;35(6):1421-1431. doi: 10.1093/humrep/deaa104.

61. Romero-Ruiz A, Skorupskaite K, Gaytan F, Torres E, Perdices-Lopez C, Mannaerts BM, et al. Kisspeptin treatment induces gonadotropic responses and rescues ovulation in a subset of preclinical models and women with polycystic ovary syndrome. Hum Reprod. 2019;34(12):2495-2512. doi: 10.1093/humrep/dez205.
62. Abdolahian S, Tehrani FR, Amiri M, Ghodsi D, Yarandi RB, Jafari M, et al. Effect of lifestyle modifications on anthropometric, clinical, and biochemical parameters in adolescent girls with polycystic ovary syndrome: a systematic review and meta-analysis. BMC Endocr Disord. 2020;20(1):71. doi: 10.1186/s12902-020-00552-1.
63. Vilmann LS, Thisted E, Baker JL, Holm JC. Development of obesity and polycystic ovary syndrome in adolescents. Horm Res Paediatr. 2012;78(5-6):269-78. doi: 10.1159/000345310.
64. Li X, Li L, Ouyang D, Zhu Y, Yuan T. The abnormal expression of kisspeptin regulates pro-inflammatory cytokines, cell viability and apoptosis of macrophages in hyperandrogenism induced by testosterone. Gynecol Endocrinol. 2021; 37(1):72-77.doi: 10.1080/09513590.2020.1811964.
65. de Assis Rodrigues NP, Laganà AS, Zaia V, Vitagliano A, Barbosa CP, de Oliveira R, et al. The role of Kisspeptin levels in polycystic ovary syndrome: a systematic review and meta-analysis. Arch Gynecol Obstet. 2019;300(5):1423-34. doi: 10.1007/s00404-019-05307-5.
66. Varikasuvu SR, Prasad VS, Vamshika VC, Satyanarayana MV, Panga JR. Circulatory metastin/kisspeptin-1 in polycystic ovary syndrome: a systematic review and meta-analysis with diagnostic test accuracy. Reprod Biomed Online. 2019;39(4):685-97. doi: 10.1016/j.rbmo.2019.04.018.
67. Marin CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in polycystic ovary syndrome (PCOS) patients using the homeostatic measurement assessment (HOMA-IR). Fertil Steril 2003;80 Suppl 3:274-5.
68. Csajbók E, Gyói A, Magony S, Sepp K, Valkusz Z. How to estimate insulin resistance in PCOS patients- HOMA-IR or QUICKI. Endocrine Abstracts 2014;35: P645. DOI: 10.1530/endoabs.35.P645. https://www.endocrine-abstracts.org/ea/0035/eposters/ea0035p645_eposter.pdf
69. Andreozzi F, Mannino GC, Mancuso E, Spiga R, Perticone F, Sesti G. Plasma kisspeptin levels are associated with insulin secretion in nondiabetic individuals. PLoS One. 2017;12(6):e0179834. doi: 10.1371/journal.pone.0179834.
70. Izzi-Engbeaya C, Comninos AN, Clarke SA, Jomard A, Yang L, Jones S, et al. The effects of kisspeptin on β-cell function, serum metabolites and appetite in humans. Diabetes Obes Metab. 2018;20(12):2800-2810. doi: 10.1111/dom.13460.
71. Fanchin R, Schonäuer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Müllerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod. 2003;18(2):323-7. doi: 10.1093/humrep/deg042.
72. Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod. 2011;26(11):3123-9. doi: 10.1093/humrep/der297.
73. Tata B, N.E.H. Mimouni, A.L. Barbotin, S.A. Malone, A. Loyens, P. Pigny, D. Dewailly, S. Catteau-Jonard, I. SundstromPoromaa, T.T. Piltonen, F. Dal Bello, C. Medana, V. Prevot, J. Clasadonte, P. Giacobini, Elevated prenatal anti-Mullerian hormone reprograms the fetus and induces polycystic ovary syndrome in adulthood. Nat. Med. 2018;24(6);834–46 (2018). doi. org/10.1038/s41591-018-0035-5
74. Bansal P, Sardana K, Arora P, Khurana A, Garga UC, Sharma L. A prospective study of anti-mullerian hormone and other ovarian and adrenal hormones in adult female acne. Dermatol Ther. 2020 Jul 5:e13974. doi: 10.1111/dth.13974.
75. Qin L, Zhao S, Yang P, Cao Y, Zhang J, Chen ZJ, et al. Variation analysis of anti-Müllerian hormone gene in Chinese women with polycystic ovary syndrome. Endocrine. 2020 Nov 9. doi: 10.1007/s12020-020-02538-4.
76. Goldman AL, Bhasin S, Wu FCW, Krishna M, Matsumoto AM, Jasuja R. A Reappraisal of Testosterone's Binding in Circulation: Physiological and Clinical Implications. Endocr Rev. 2017;38(4):302-324. doi: 10.1210/er.2017-00025.
77. Deswal R, Yadav A, Dang AS. Sex hormone binding globulin - an important biomarker for predicting PCOS risk: A systematic review and meta-analysis. Syst Biol Reprod Med. 2018;64(1):12-24. doi: 10.1080/19396368.2017.1410591.
78. Li Y, Fang L, Yan Y, Wang Z, Wu Z, Jia Q, et al. Association between human SHBG gene

polymorphisms and risk of PCOS: a meta-analysis. Reprod Biomed Online. 2020:S1472-6483(20)30568-X. doi: 10.1016/j.rbmo.2020.10.003.

79. Harrison CL, Lombard CB, Moran LJ, Teede HJ. Exercise therapy in polycystic ovary syndrome: A systematic review. Hum Reprod Update 2011;17:171-83.
80. Lim SS, Hutchison SK, Van Ryswyk E, Norman RJ, Teede HJ, Moran LJ. Lifestyle changes in women with polycystic ovary syndrome. Cochrane Database Syst Rev. 2019;3(3):CD007506. doi: 10.1002/14651858.CD007506.pub4.

Figure 1. Flowchart of study selection.

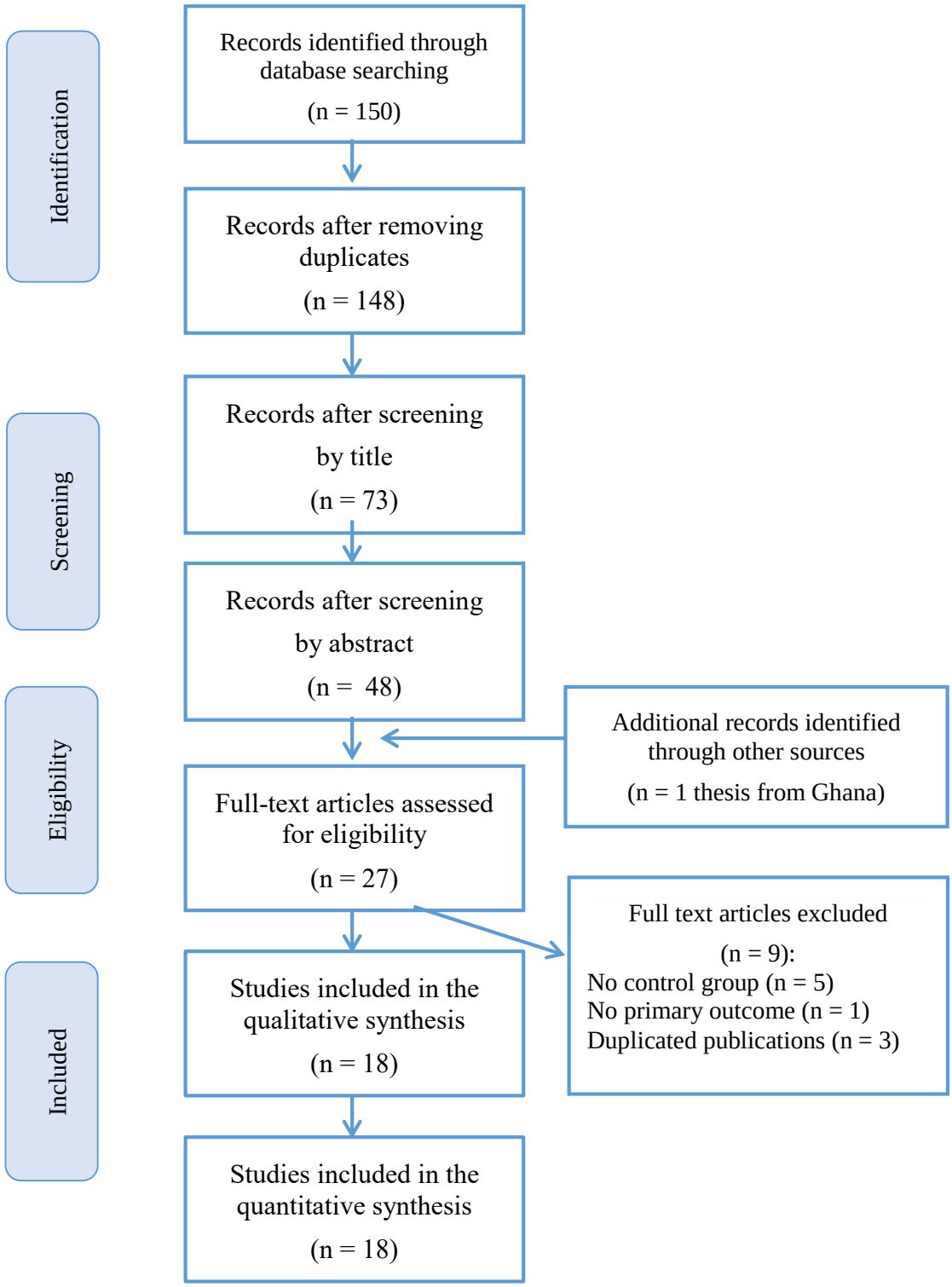
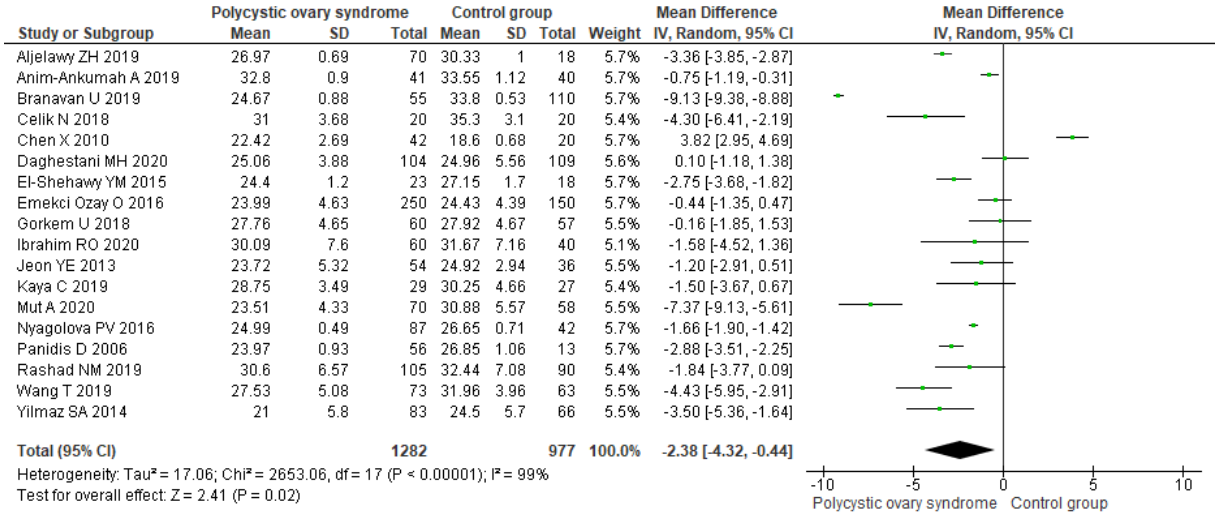
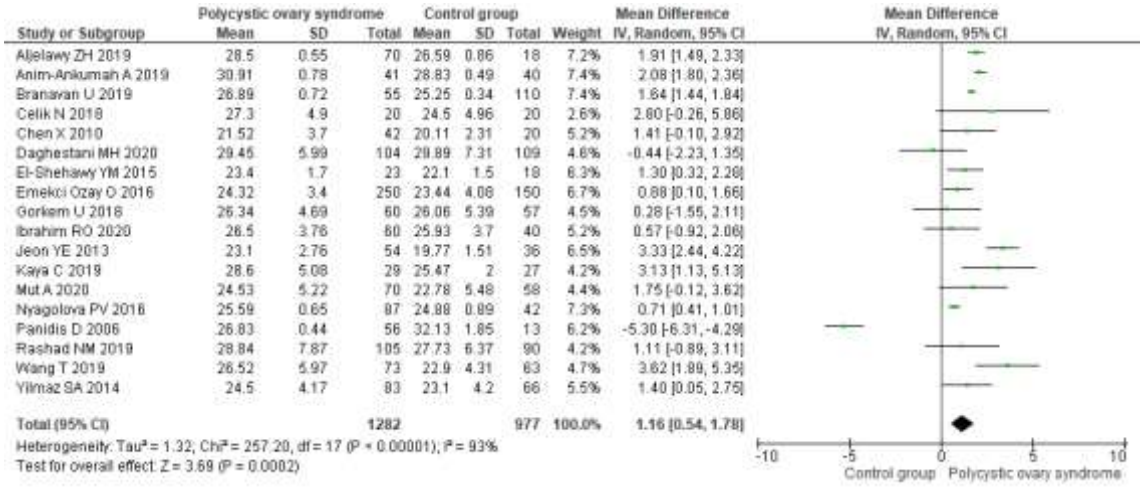


Fig. 2. Forest plots comparing participants with and without polycystic ovary syndrome (mean difference), from the top to the bottom, for age (Fig. 2A), BMI (Fig. 2B), and waist-to-hip ratio ratio (Fig. 2C).

2A. Age



2B. BMI



2C. Waist-to-hip ratio

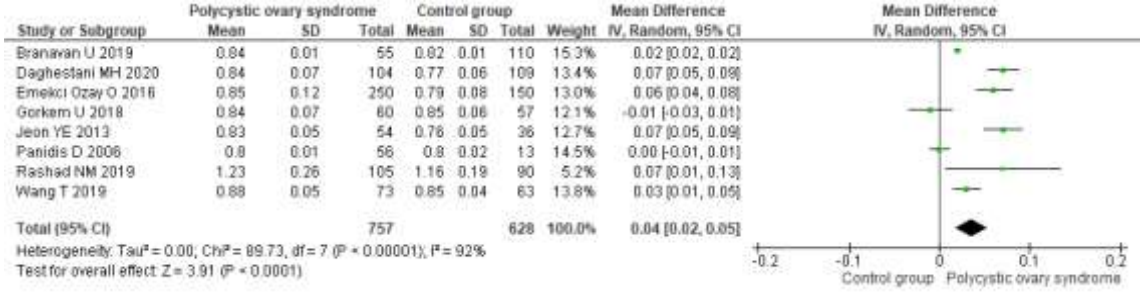
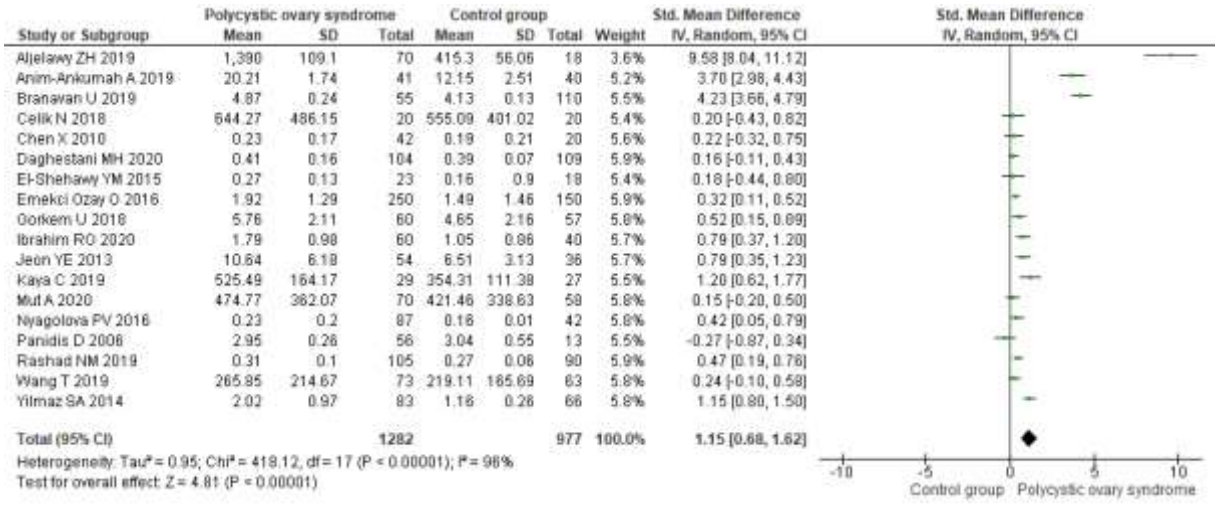
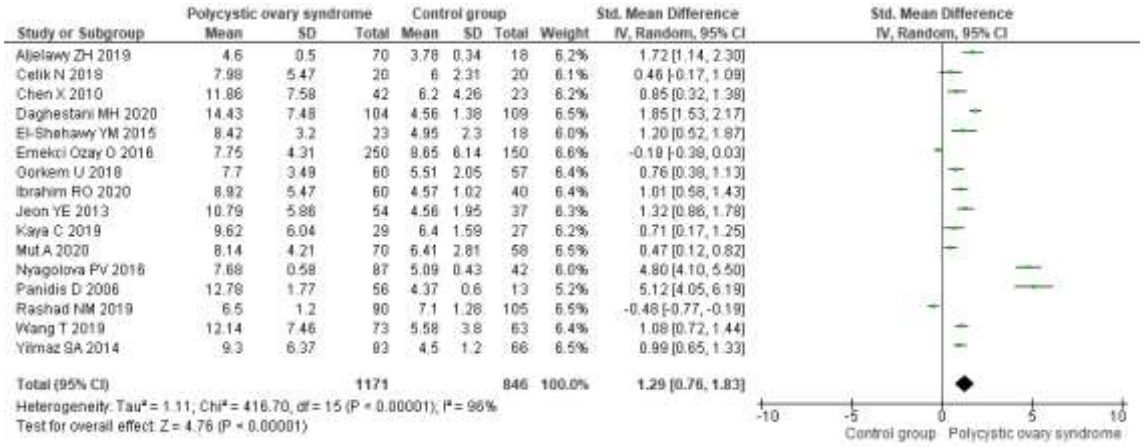


Fig. 3. Forest plots comparing participants with and without polycystic ovary syndrome (standardized mean difference), from the top to the bottom, for kisspeptin (Fig. 3A), luteinizing hormone (LH; Fig. 3B), follicle-stimulating hormone (FSH, Fig. 3C), anti-müllerian hormone (Fig. 3D), prolactin (Fig.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

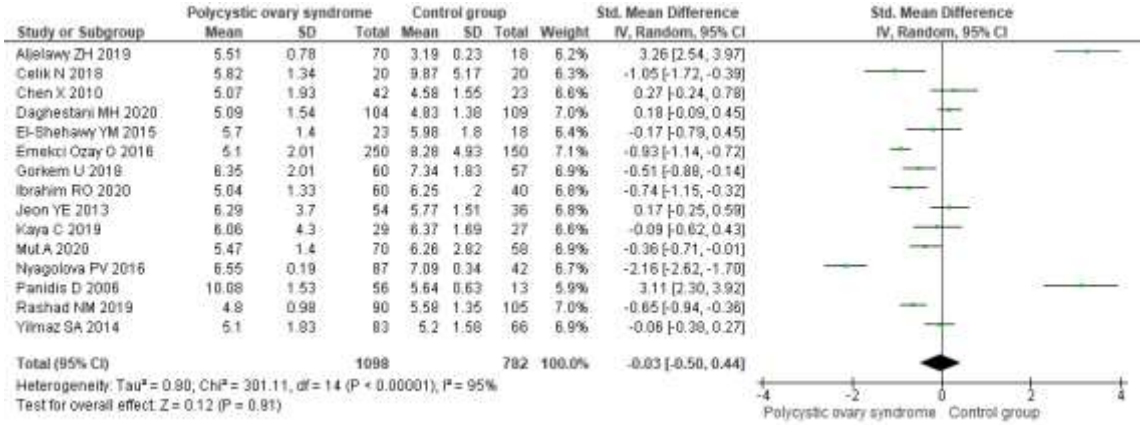
3A. Kisspeptin



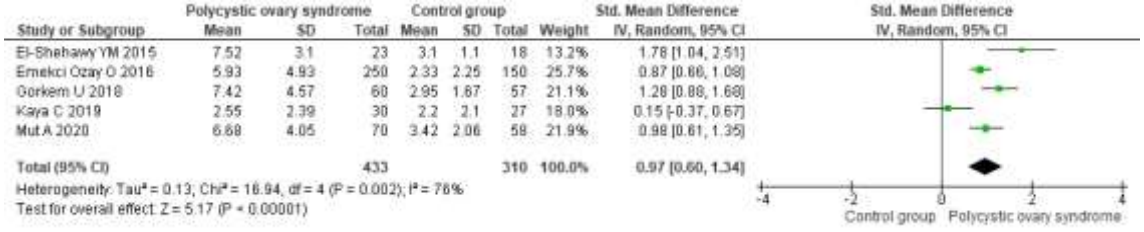
3B. LH



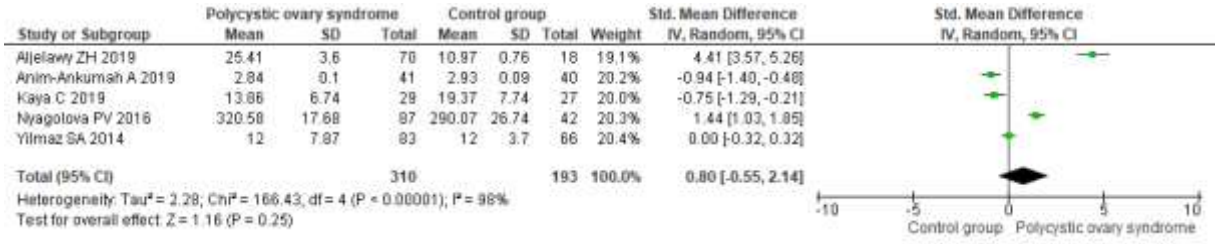
3C. FSH



3D. Anti-müllerian hormone



3E. Prolactin



3F. Estradiol

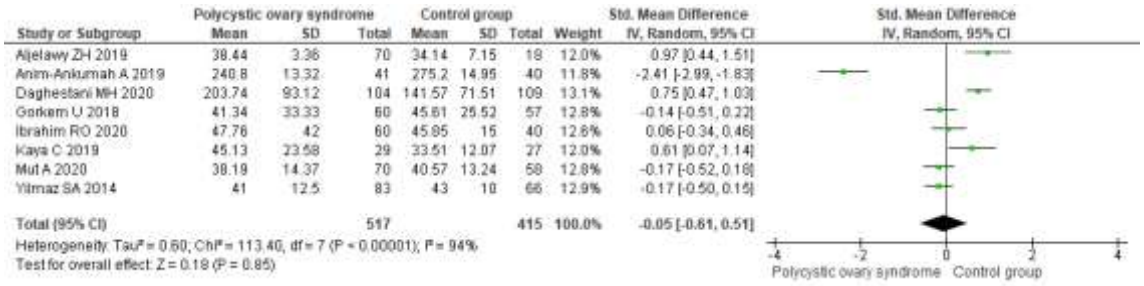


Fig. 4. Forest plots comparing participants with and without polycystic ovary syndrome (standardized mean difference), from the top to the bottom, for total testosterone (Fig. 4A), free testosterone (Fig. 4B), dehydroepiandrosterone sulphate (DHEA-S, Fig. 4C), sex hormone-binding globulin (Fig. 4D),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

Fig. 4A. Total testosterone

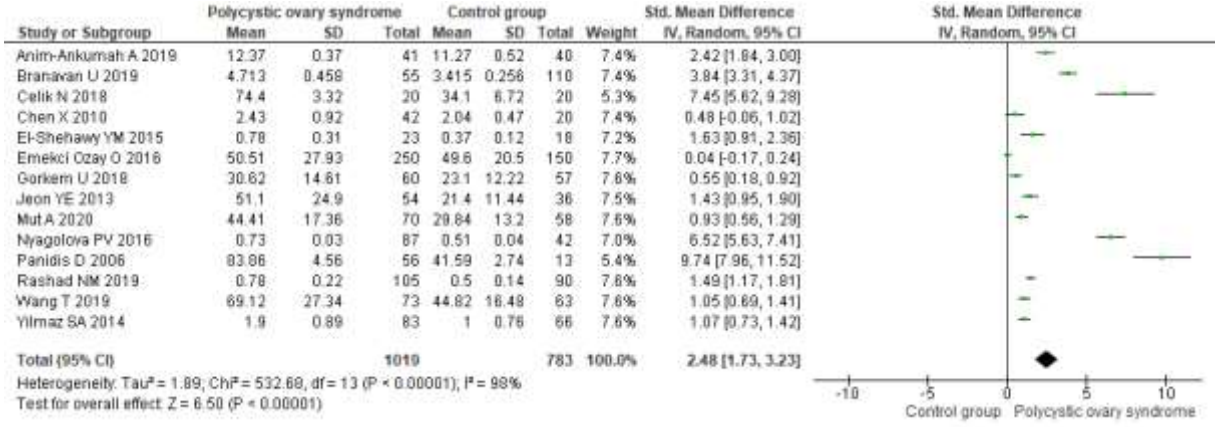


Fig. 4B. Free testosterone

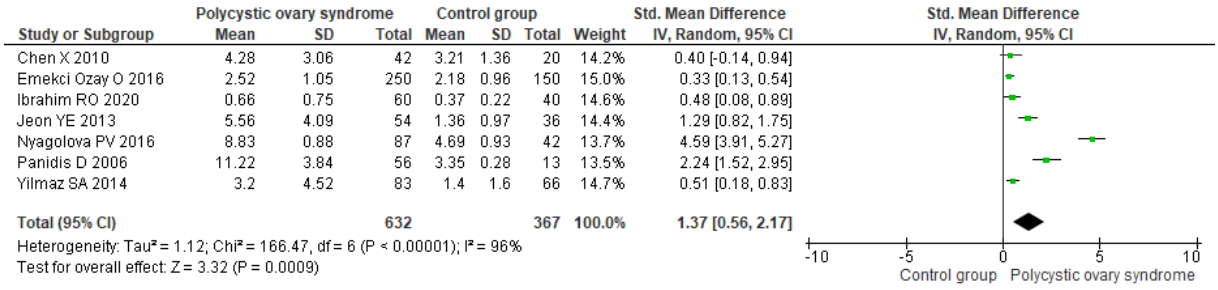


Fig. 4C. DHEA-S

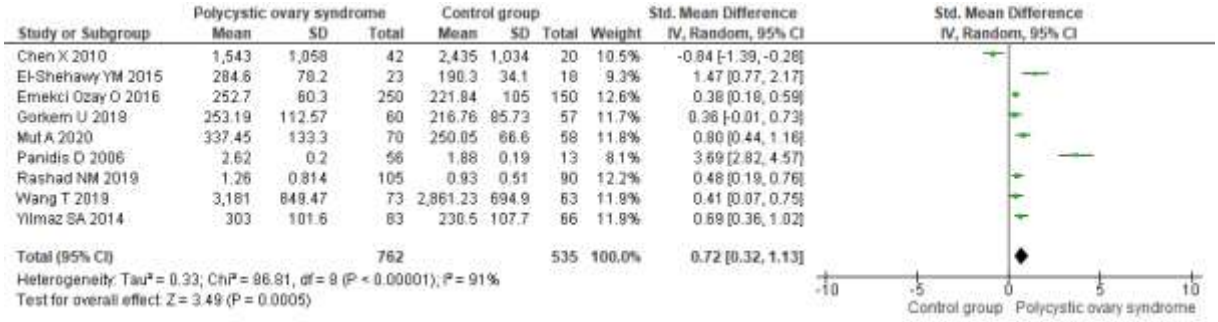


Fig. 4D. Sex hormone-binding globulin

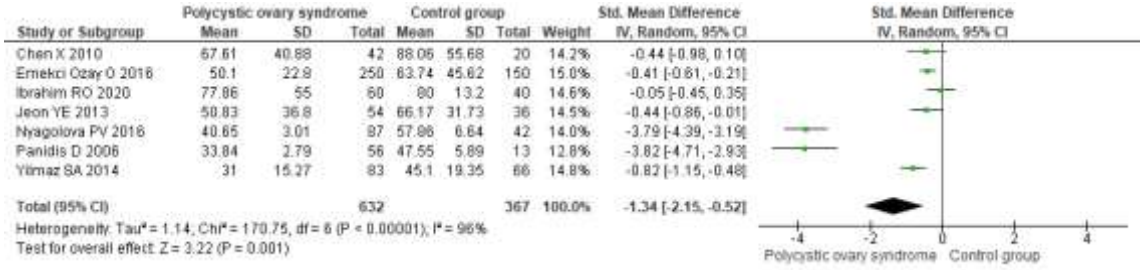


Fig. 4E. Modified Ferriman-Gallweg score

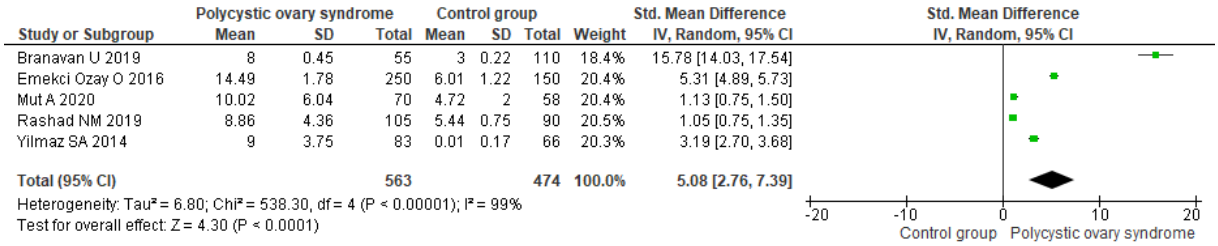
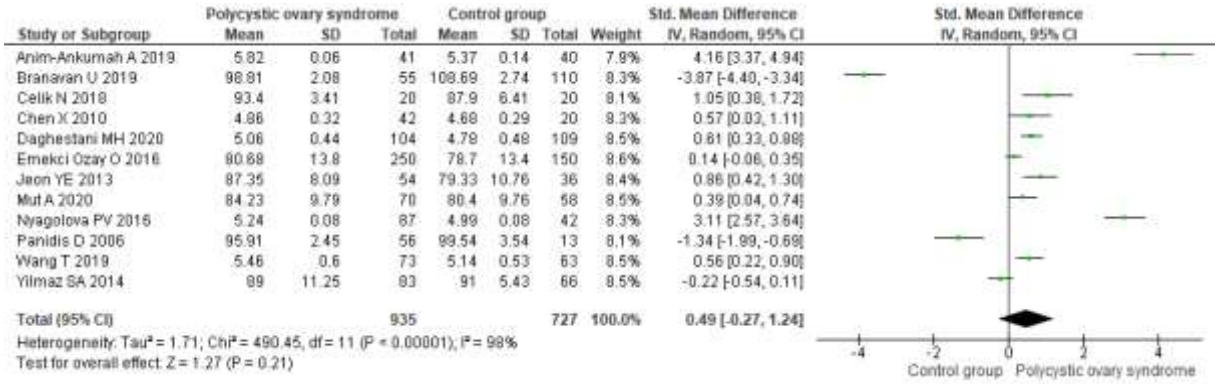
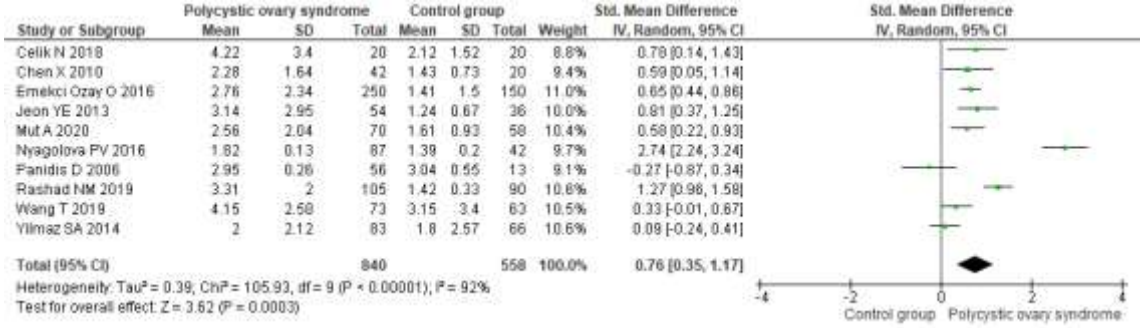


Fig. 5. Forest plots comparing participants with and without polycystic ovary syndrome (standardized mean difference), from the top to the bottom, for glycemia (Fig. 5A), HOMA-IR index (Fig. 5B), insulin (Fig. 5C), and leptin (Fig. 5D).

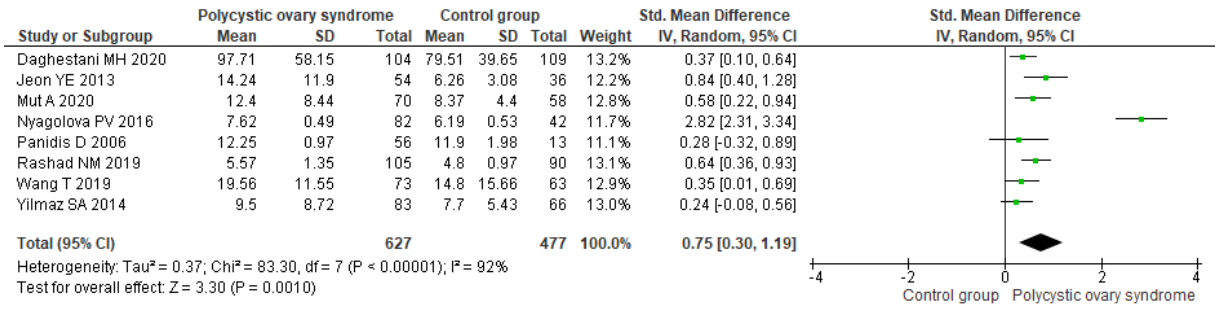
5A. Glucose



5B. HOMA-IR



5C. Insulin



5D. Leptin

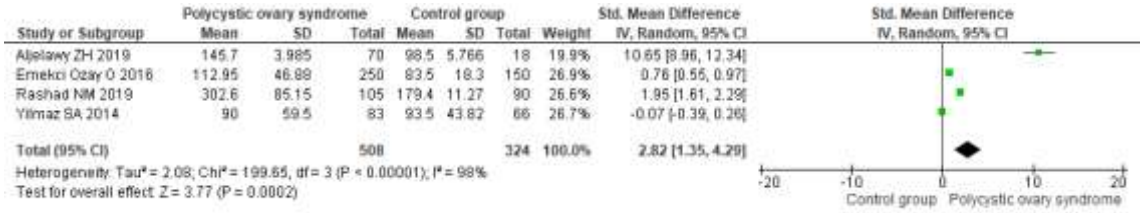
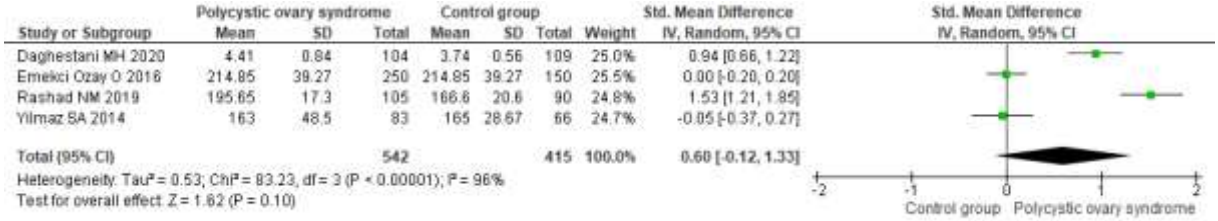
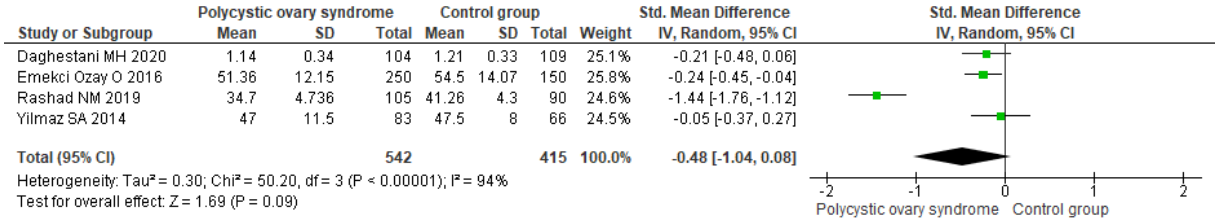


Fig. 6. Forest plots comparing participants with and without polycystic ovary syndrome (standardized mean difference), from the top to the bottom, for total cholesterol (Fig. 6A), HDL-cholesterol (Fig. 6B), LDL-cholesterol (Fig. 6C), and triglycerides (Fig. 6D).

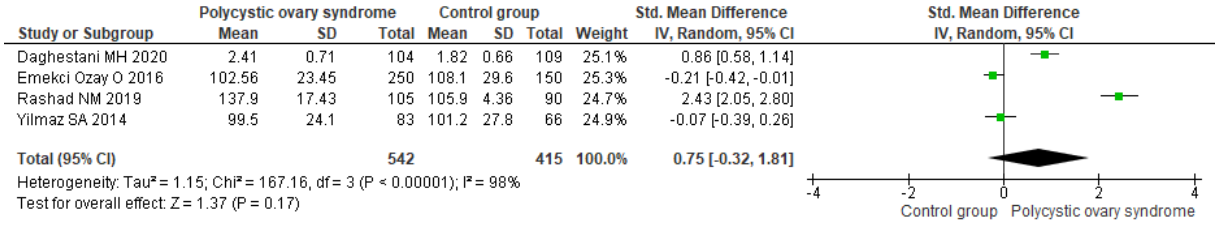
6A Total cholesterol



6B HDL-Cholesterol



6C LDL-Cholesterol



6D Triglycerides

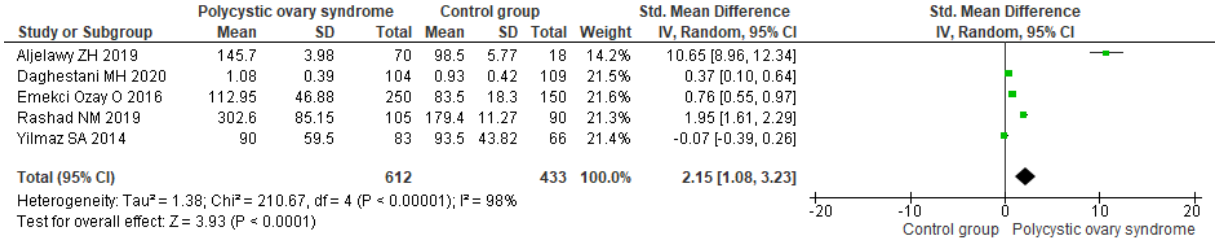


Table 1. Studies comparing serum kisspeptin levels in women with and without polycystic ovary syndrome (PCOS): Study location and period, aim, number of participants, age, body mass index (BMI), PCOS diagnosis criteria, and main findings.

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

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

Panidis D 2006 [44]	Location: Thessaloniki; Greece; period not stated. Aim: To study kisspeptin in women with and without PCOS and related metabolic disturbances.	n = 56. Age: 23.97 ± 0.93. BMI = 26.83 ± 0.44. PCOS diagnosis criteria: chronic anovulation (< 6 cycles in 12 months) and hyperandrogenemia.	n = 13. Age: 26.85 ± 1.06. BMI: 32.13 ± 1.85 (all BMI ≥ 25)	There were not significant difference in circulating kisspeptin in women with PCOS compared to controls. Kisspeptin negatively correlated with insulin resistance 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.	n = 66 (BMI < 25,n = 41; BMI ≥ 25, n = 25). Age: 24.5 ± 3.5 n= 66 BMI: 23.1 ± 4.2.	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 (I^2) in women with and without polycystic ovary syndrome (PCOS).

Outcome (Figures)	Included Studies	Participants PCOS / control	MD or SMD and 95% CI	I^2 (%)	p
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, 1l81]	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 I^2 for circulating kisspeptin, HOMA-IR index and AMH when comparing women with and without PCOS.

Deleted publication [reference]	**Kisspeptin SMD (95%CI); I^2**	**HOMA-IR index SMD (95%CI); 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.

eMethod

eMethods 1. Pubmed earch strategy.

eTables

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]

eTables

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

Author, year [reference]	Selection				Comparability Adjusted or matched	Outcome			Total NOS score
	Case definition adequate	*Representativeness of the cases*	*Selection of controls*	*Definition of the controls*		*Ascertainment of exposure*	*Same method of ascertainment*	*Statistical test Non-response rate*	
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

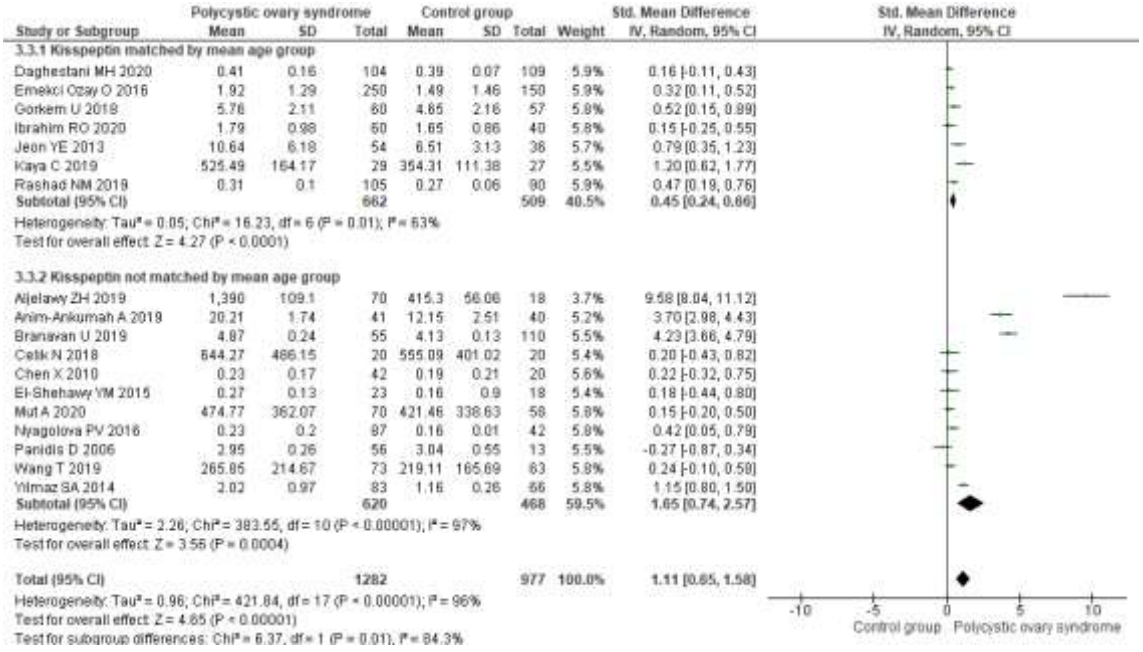
eTable 2. Publication bias assessment for outcomes reported in at least 10 studies, Egger's regression, and Kendall's tau.

Outcomes	Studies (*n*)	Egger's test; p	Kendall's tau; p
Age	18	0.122; 0.903	-0.359; < 0.001
BMI	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

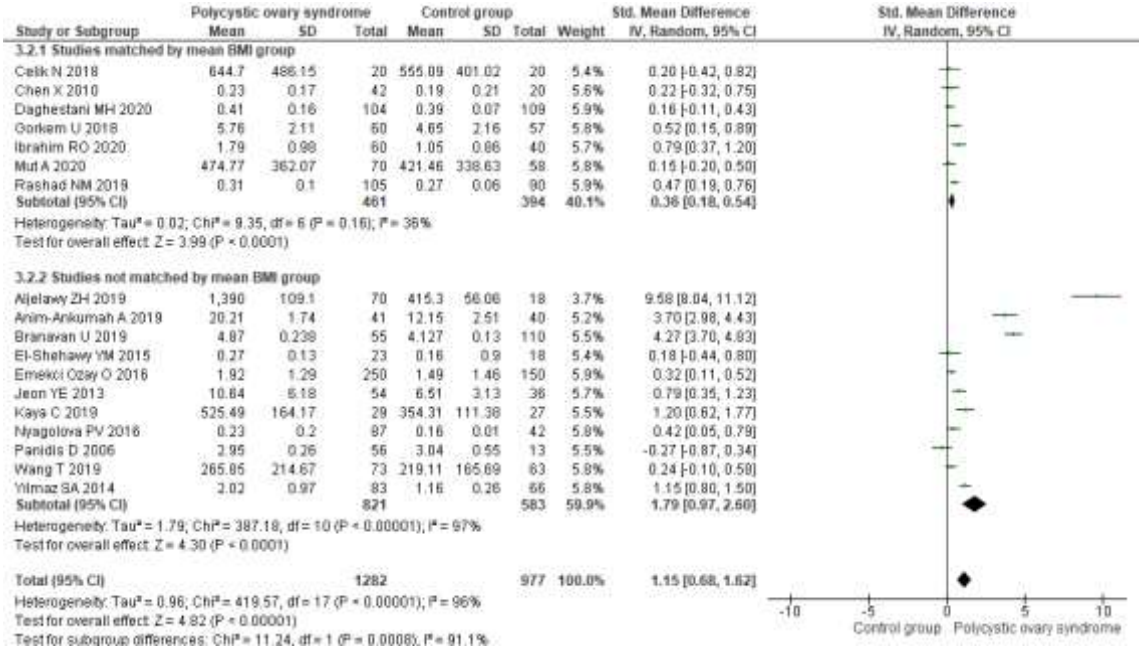
(^) Deleted references [29, 31, 32]

eSupplementary figures

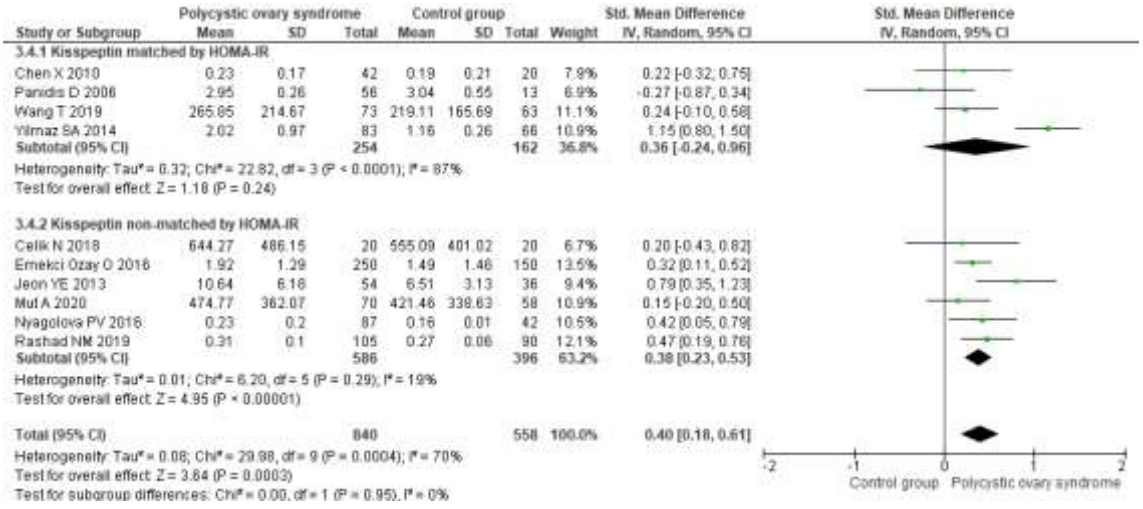
eSupplementary figure 1. Subgroup analysis of circulating kisspeptin levels including studies matched and non-matched by mean age.



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. 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.

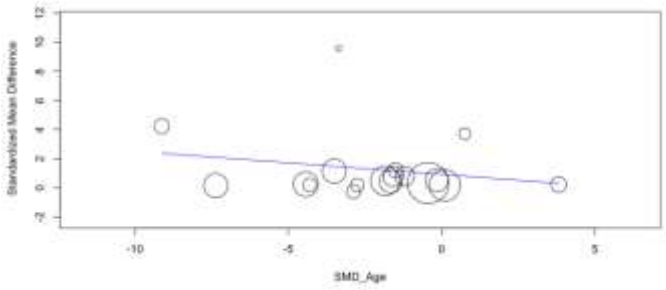
(4A) Effect of age

Meta-Regression

Metric: Standardized Mean Difference

Model Results

Covariate	Coefficients	Lower bound	Upper bound	Std. error	p-Value
Intercept	0.915	-0.327	2.158	0.634	0.149
SMD_Age	-0.159	-0.498	0.179	0.173	0.356



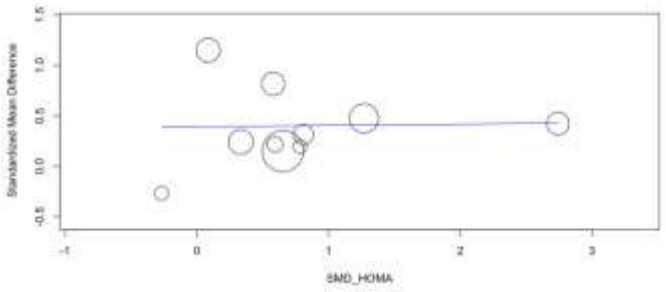
(4B) Effect of HOMA-IR index

Meta-Regression

Metric: Standardized Mean Difference

Model Results

Covariate	Coefficients	Lower bound	Upper bound	Std. error	p-Value
Intercept	0.392	0.072	0.711	0.163	0.016
SMD_HOMA	0.013	-0.276	0.302	0.147	0.930



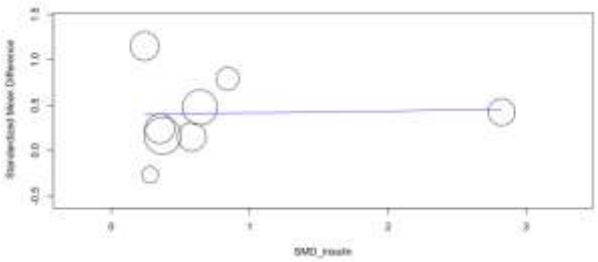
(4C) Effect of circulating insulin

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	0.021	-0.304	0.346	0.166	0.898



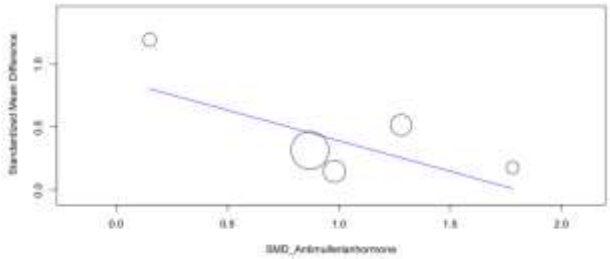
(4D) Effect of anti-müllerian hormone

Meta-Regression

Metric: Standardized Mean Difference

Model Results

Covariate	Coefficients	Lower bound	Upper bound	Std. error	p-Value
Intercept	0.879	0.334	1.424	0.278	0.002
SMD_Antimullerianhormone	-0.486	-1.001	0.028	0.263	0.064



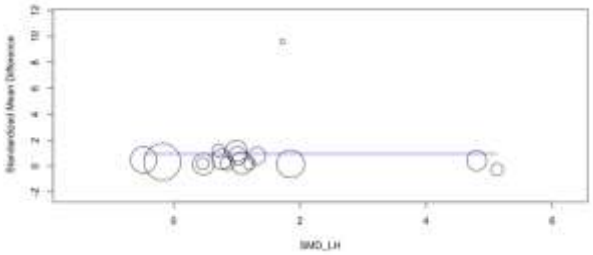
(4E) Effect of circulating LH

Meta-Regression

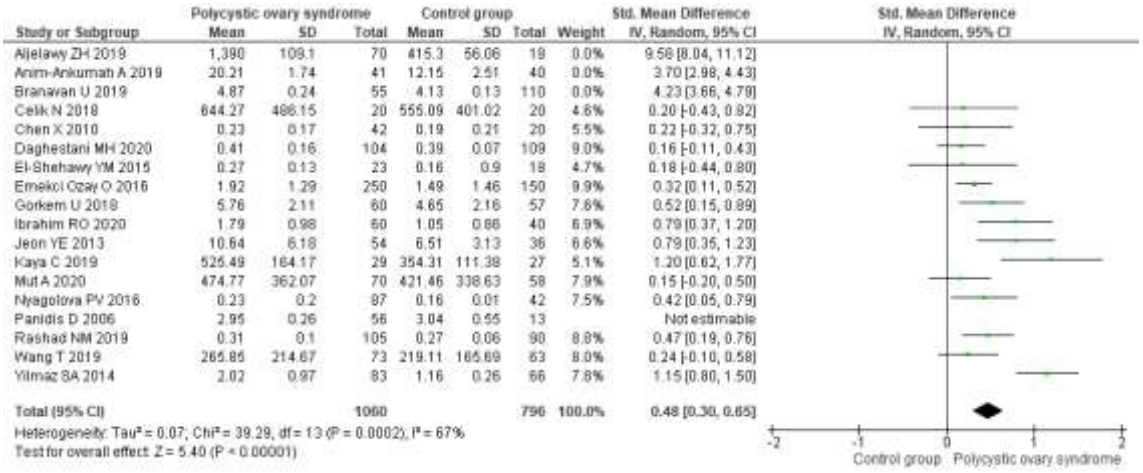
Metric: Standardized Mean Difference

Model Results

Covariate	Coefficients	Lower bound	Upper bound	Std. error	p-Value
Intercept	0.942	-0.393	2.277	0.681	0.167
SMD_LH	0.051	-0.664	0.666	0.339	0.938

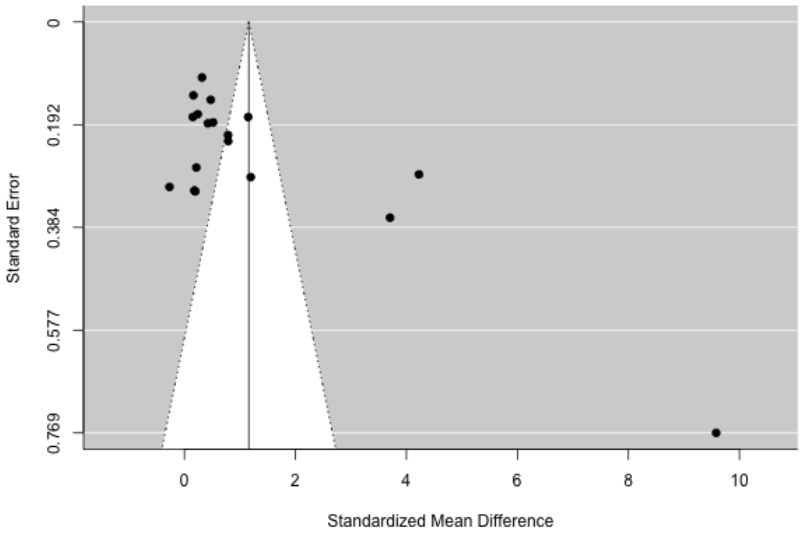


eSupplementary figure 5. Sensitivity analysis for kisspeptin levels by deleting 4 studies. The heterogenity was reduced to to I^2 = 67%.

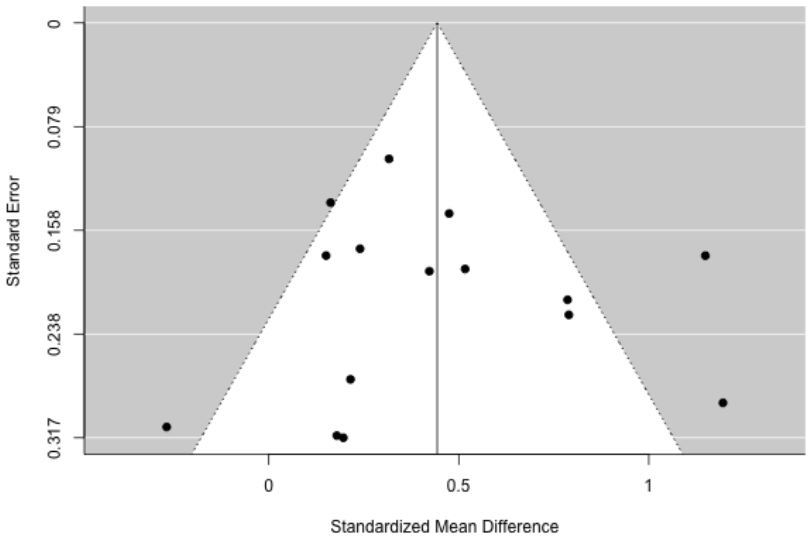


eSupplementary figure 6. Publication bias assessment for circulating kisspeptin in women with and without PCOS (n = 18 studies; top) and after deleting 3 studies (bottom) with publication bias [29, 31, 32]. The table summarizes Kendall's tau and Egger's test values.

(6a) 18 studies

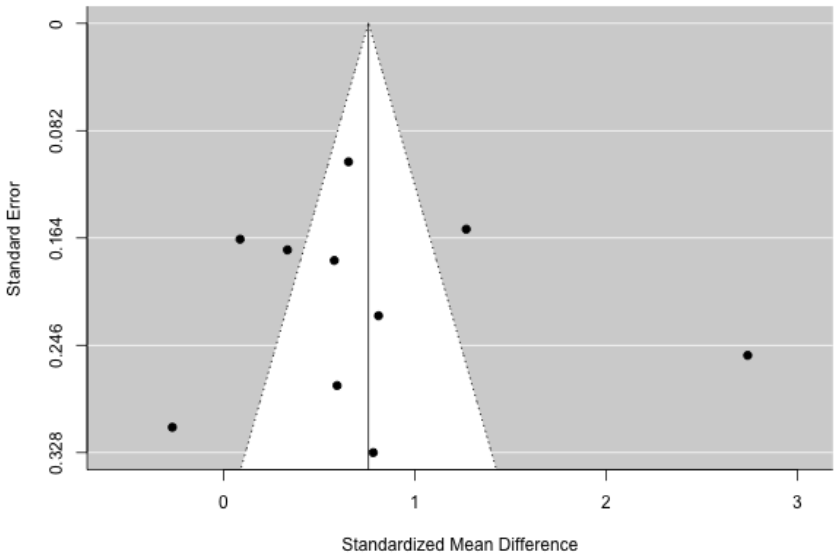


(6b) Deleting 3 studies [29, 31, 32]



	Kisspeptin n= 18 studies		Kisspeptin deletting 3 studies [29, 31, 32] (with suspected publication bias)	
Test name	Value	p	Value	p
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	p
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	