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Review

Molecular role of peptides/proteins in subfertility of polycystic ovarian syndrome

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Abstract: Obesity and hyperandrogenemia are known to have adverse effects on both developing follicle and endometrium receptivity in polycystic ovarian syndrome (PCOS). Insulin resistance also contributes to this dilemma as a cause or a consequence and leads to worsening of the clinical picture. The difficulty in obtaining pregnancy despite the presence of a large number of oocyte has concentrated our attention on oocyte quality and development. However, the occurence of subfertility has also caused us to investigate the presence of different etiologic agents in non-obese PCOS women with normal androgen and insulin levels. In this context peptides have become the most accused and investigated molecules in cases of impaired fertility due to PCOS. Most of the studies investigating the relationship between PCOS and peptide did not support each other. The difficulties in measuring peptide levels as well as the individual variations in peptide synthesis and release are possible causes of this incongruity. For all these reasons, the incorporation of studies investigating the relationship between PCOS, peptide and subfertility in an article has become critical to pioneering future work. Understanding the association between peptides and subfertility will help us to understand the effects of peptides on failed fertility in PCOS. Moreover, updating our knowledge about peptides may allow us designing new drugs to to treat subfertility in PCOS. This review provides a general summary of the mechanisms of action of neuroendocrine peptides in regulating reproductive events. Since it is not usual to discuss all peptides in this context, only the effects of key central and peripheral peptides on fertility in PCOS have been extensively addressed.

Key words: Peptides; PCOS; Subfertility.

Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder characterized by three key features including hyperandrogenism, oligo-anovulation, and insulin resistance (1). This complex human syndrome affects up to one in five women during reproductive period (5–12%) (2,3). At least half of the women affected by PCOS are obese indicating adipose tissue dysfunction (4,5). Concordantly, dysregulated secretion of some peptides in addipose tissues of obese women has been reported (6). Likewise, close relationship between subcutaneous or perirenal fat tissues, inflammation, oxidative stress and metabolic syndrome has been noted (7,8).

In addition to hyperandrogenism and failed expressions of receptivity molecules, many studies have demonstrated abnormal secretion of peptides in PCOS (9-12). Indeed, there is a complex interaction between central and/or peripheral peptides, hypothalamo-pituitary neurons and ovary. Both negative and positive energy balance cause fluctuations in circulating levels of peptides and leads to defective release of GnRH and gonadotropins in PCOS (13-17).

Elevated androgen levels can be considered the main culpritin subfertility due to PCOS. More specifically, PCOS women with hyperandrogenism have low HOXA-10 and β3-integrin expression suggesting that high levels of androgen may be detrimental on receptivity (11,18,19). Since all PCOS patients have not high levels of serum testosterone or DHT, hyperandrogenemia cannot be the only etiological factor underlying decreased fertility in PCOS (20). One possible additional reason for subfertility in PCOS may be peptides (21,22). Many clinical studies have showed that circulating peptide levels regulate reproductive events (23-26). Physiological interactions between peptides, adipose tissue, gonads, and the central nervous system may be impaired in PCOS (23-28). In a recent review we comprehensively discussed the possible relationship between peptides and their impact on reproductive functions (10). So far, there is no comprehensive review regarding role of peptides in subfertility due to PCOS. Actually, impaired production and release of peptides could account for the reduced implantation rates in women with PCOS. However, it is not clear whether subfertility in PCOS is due to excessive androgen or impaired peptide release. In this

Table 1. Evidence showing role of adipose tissue on metabolic and reproductive events (Adapted from References in the text).

Condition	Clinical and laboratory results
Adipose tissue dysfunction	⇧ Chemokine (C-C motif) ligand 2 ⇧ Heme oxygenase Large waistline ⇩ Adiponectin receptor 2 ⇩ Lipoprotein lipase ⇩ Twist-related protein 1
Adipose tissue and follicle maturation	⇧ Apoptosis in follicle cells smaller size oocyte ⇧ Granulosa cell apopitosis Lipid accumulation and lipotoxicity in oocyte Malfunctioning mitochondria in zygote and oocyte ⇩ Oocyte number
Adipose tissue and type 2 diabetes	⇧ Adipocyte hypoxia Hypertrophic adipocytes Insulin resistance ⇩ Adipocyte differentiation ⇩ Chronic inflammation
Adipose tissue and the endometrium	⇧ Endometrial leukemia inhibitory factor ⇧ Glutathione peroxidase 3 ⇩ Endometrial interleukin-15 ⇩ Mucin1
Adipose tissue and insulin resistance	⇧ Adipocyte hypoxia Enlarged adipocytes Hypertrophic adipocytes ⇩ Adipocyte differentiation
Adipose tissue and cardiovasculary disease	Disturbances of coagulation Dyslipidemia Fibrinolysis
Adipose tissue and obesity	⇧ Myotonic dystrophy ⇩ Adiponectin
Adipose tissue and ovarian dysfunction	⇧ Adiposity ⇧ Obesity
Adipose tissue and pregnancy	⇧ Adiposity ⇧ Subscapular skinfold thickness
Adipose tissue and implantation markers	⇩ Integrin, HOXA-10 and IGFBP-1

review we will try to discuss the possible effects of key peripheral and central peptides on reproductive events in PCOS patients suffering from subfertility (Tables 1 and 2). To discuss all peptides is not within the scope of this review.

Leptin in PCOS

Close association between subcutaneous adipose tissue, circulating leptin, and reproductive events have been reproted (52). As well as regulating metabolic processes, leptin is an important mediator of reproductive functions (53-55). Relative leptin deficiency in cases with lipodystrophy supports that main production area of leptin is adipose tissue (56). Granulosa cells in newborn and adult females can also produce and store leptin (57).

Leptin regulates the activities of hypothalamic and pituitary neurons in response to energy status (58). Since GnRH neurons do not express leptin receptors leptin impact on arcuate nucleus may occur indirectly (59). Most probably, leptin stimulates GnRH production by affecting kisspeptin neurons (13). Concordantly, admnistration of exogenous leptin improves menstrual dysfunction in lean women with amenorrhea or hypogonadotrophic hypogonadism (60,61). Consistent with this prediction, significantly increased hypothalamic Kiss1 mRNA expression was detected in prenatally androgenized rats (36). On the other hand, pretreatment with a kisspeptin antagonist failed to inhibit induction of LH by leptin suggesting that the stimulatory effect of leptin could be transmitted to GnRH neurons via receptors other than the kisspeptin (36).

Since reproductive dysfunction is the main characteristic of PCOS, leptin could be implicated in subfertility due to PCOS. Concordantly, high levels of leptin have been reported in PCOS subjects (62). While physiological levels of leptin induce ovarian steroidogenesis and follicle development, supraphysiological concentrations may induce ovarian cyst formation (15). Zheng et al. showed that leptin could be associated with insulin resistance and subfertility in PCOS (62). Nevertheless, recent study showed that leptin levels in women with PCOS were similar to those in controls with similar body weight (23). Surprisingly, central leptin resistance has been reported in the rat model of PCOS (63). Chronic low grade inflammation in the hypothalamus could account

for the central leptin resistance in PCOS (64). Celik et al. showed the presence of leptin resistance at the blood-brain barrier in preeclamptic pregnant women (65). Gararuti et al. reported a significant decline in serum and follicular fluid leptin levels in PCOS (66). Their results were compatible with the study conducted by De Placido et al (67). They highlighted a correlation between follicular fluid leptin levels and fertilization rates (67). Conversely, Li et al. reported that follicular fluid and serum leptin levels were higher in PCOS subjects than controls (68). In conclusion, while physiological levels of leptin contribute to ovarian steroidogenesis and follicle development, either high or low levels of leptin in lean or obese women with PCOS could lead to subfertility. Leptin may modulate the reproductive axis by acting on nonspecific hypothalamic receptors that express *Kiss1* and could participate indirectly in stimulating the GnRH pulse generator and initiation of LH release. Together, impaired leptin secretion could be involved in the pathogenesis of subfertility due to PCOS (69).

Adiponectin in PCOS

Adiponectin is a protein hormone produced by adipocytes and enhances insulin sensitivity (70). Multimeric isoform of adiponectin has a more potent effect than the smaller isoform (70,71). A decreased level of adiponectin is the most obvious change in PCOS (72,73). This might be result of altered adipose tissue distribution and function in PCOS (23,73). Notwithstanding, the role of adiponectin in subfertility due to PCOS is controversial. Since adiponectin increase insulin sensitivity it may affect reproductive functions in PCOS. As cumulus oocyte complexes express insulin and adiponectin receptors, either insulin or adiponectin could contribute to follicle development and dominance (21,22). In keeping with this, adiponectin production has been reported in the granulosa cells of PCOS subjects undergoing ovarian stimulation (74). The corroborating evidence regarding impact of adiponectin in subfertility comes from study of Garcia et al (75). Authors of this study showed that adiponectin signals of endometrium cells in obese PCOS subjects not normal (75). They emphasized close association between circulating adiponectin and endometrial function. Likewise, increased adipokine receptor expression has been reported in endometrial cells during decidualization (76). Together, because adiponection is a hormone that contributes to follicle development as well as endometrium function defective production or release of this peptide may lead to subfertility due to PCOS (Figure 1).

Ghrelin and GnIH in PCOS

Both ghrelin and gonadotropin inhibitory hormone (GnIH) are two agonistic peptides regulating reproductive events (14). Each peptide inhibits gonadotropin and sex steroid secretion (77-79). Both peptides have opposite effects on follicle survival. While ghrelin acts as a survival factor for developing follicles, GnIH inhibits germ cell maturation (80,81).

Although ghrelin inhibits GnRH neurons it is still not clear how ghrelin do that. In a recent review, Celik et al. hypothesized that unacylated ghrelin exerts its central effect through an unidentified receptor (14). Ghrelin could therefore contribute to PCOS-related subfertility by regulating the GnRH pulse generator and the energy demand of growing follicle. Since ghrelin inhibits GnRH and LH secretion decreased ghrelin concentrations in PCOS could abolish inhibitory impact of ghrelin on gonadotropins. Together, decreased faasting gherelin in PCOS may lead to excess LH release which could account for the subfertility due to PCOS (Table 2 and Figure 1).

Resistin and amylin in PCOS

Resistin is produced by adipocytes and ovary. While resistin regulates steroid production from ovary gonadotropins induce its synthesis (82). The risk of PCOS increases in the presence of resistin gene polymorphism (73). Concordantly, high levels of resistin have been reported in PCOS (23). Likewise, BMI of PCOS subjects correlated positively with serum resistin levels (73). Notwithstanding, Gul et al. showed that plasma resistin levels of PCOS women were normal (83). When corrected according to BMI normal levels

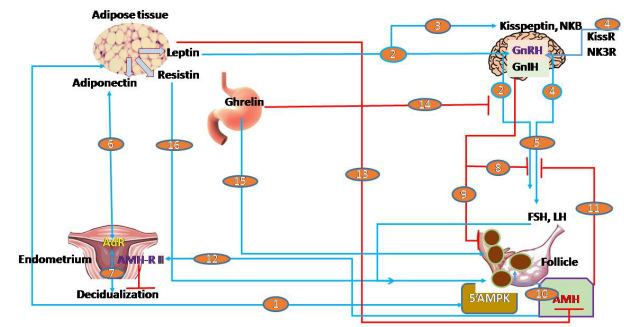

Figure 1. Schematic representation of basic peptides mediating reproductive events in PCOS. The ovarian follicle receives signals from peripheral adipose tissues via a 5' AMP-activated protein kinase (AMPK) that regulates both follicle development and steroidogenesis in cumulus cells [1]. By affecting arcuate neurons, leptin stimulates GnRH production [2]. Since GnRH neurons do not express leptin receptors, thestimulatory effect of leptin could be transmitted indirectly to the GnRH neurons via KissR [3]. The kisspeptins stimulate GnRH secretion from the arcuate neurons and regulate LH release. Neurokinin B is co-expressed with kisspeptin in the hypothalamus and regulates GnRH release [4]. The interaction of kisspeptin, NKB, and leptin could account for the increased LH pulse frequency in PCOS [5]. As cumulus oocyte complexes and endometrium express the adiponectin receptor (AdR), adiponectin contributes to follicle development and implantation [6]. Decidualization leads to increased AdR expression in endometrial cells [7]. GnIH inhibits gonadotropin release and gonadal steroid secretion [8] and also germ cell maturation [9]. By regulating steroid hormone synthesis in granulosa cells, AMH regulates folliculogenesis [10]. Early stages of follicle development [11] and decidualization [12] are also blocked by AMH. Increase in body and visceral fat accumulation leads to a reduction of serum AMH levels [13]. Ghrelin inhibits GnRH neurons and blocks LH secretion [14]. It also acts as a survival factor for follicle cells [15]. While resistin induces steroid production from granulosa cells, gonadotropins induce resistin production in the growing follicle [16]. (Adapted from References in the text).

Table 2. Possible impact of peptides/proteins on reproductive events in PCOS.

Adipokines (29-33)	Low grade inflammation⇨IR⇨metabolic syndrome⇨PCOS⇨Diabetes Adiponectins⇨gonadal 5'AMPK⇨maturation of oocyte and spermatozoa ⇧saturated free fatty acids⇨defect in granulosa cell morphology	Adipocyte hyperplasia⇨NEFA⇧⇨macrophage accumulation⇨TNFα⇧⇨NEFA and cytokines⇧⇨testosterone⇩ Overweight men⇨testosterone, sperm count and motility⇩ High-fat and energy diets⇨defective spermatogenesis
Leptin (34-36)	Leptin⇨upregulates Kiss1 mRNA⇨GnRH, LH⇧ Leptin⇧ (ob/ob) mice⇨infertility Exogenous leptin⇨kisspeptin⇧⇨GnRH⇧	Leptin⇩⇨GnRH⇩⇨LH, FSH⇩⇨E2⇩⇨infertility ⇩Leptin⇨JAK/STAT⇩⇨sex steroid hormones⇩ ⇧Food⇨⇩Leptin⇨hypogonadism
Kisspeptin, Neurokinin B (NKB) (10,13,36-39)	Kisspeptin⇨GnRH⇧⇨FSH, LH⇧ NKB⇨GnRH, LH⇧⇩⇨steroidogenesis and follicle growing Male ob/ob mice⇨⇩kisspeptin ⇧Estrogen, progesterone⇨kisspeptin⇧⇨GnRH⇧⇨LH, FSH⇧	Kisspeptin knock-out or food restriction⇨infertility PCOS⇨down regulated NK3R in granulosa cells Kisspeptin receptor (GPR54) deletion⇨hypogonadism
Adiponectin (40-44)	LH peak⇨follicular fluid adiponectin and adiponectin receptor⇧	⇧LH, FSH⇨Adiponectin receptor-2⇧⇨IGF-1⇧⇨progesteron, estrogen⇧ Adiponectin⇨NF-κB⇩, AMPK⇧⇨protects leydig cells
Visfatin (45,46)	Granulosa cells⇨visfatin⇧	Follicular fluid visfatin⇨correlated with the number of mature oocyte Visfatin⇨Leydig cells⇨steroidogenesis⇧
Resistin, Chemerin (47-49)	Sex steroids, FSH, LH⇨ovarian resistin⇧⇨PCOS	Chemerin and resistin⇨ovarian steroidogenesis⇩ Chemerin⇨aromatase⇩ Chemerin⇨PCOS⇧
Irisin (27)	Muscle derived irisin⇨adipose tissue brwoning⇨UCP1⇧⇨regulates follicular fluid temparature⇨follicle maturation	
Cerebellin and Betatrophin (50,51)	Betatrophin⇧⇨pancreatic beta cell proliferation⇨obesity and PCOS Cerebellin⇨cAMP⇨insulinostatic activity⇨PCOS	

of resistin have been reported in PCOS (84). The neuropeptide amylin is co-secreted with insulin (85). Increased amylin secretion has been reported in PCOS (86). Nevertheless, no study has investigated the effect of both resistin and amylin on subfertility due to PCOS.

AMH in PCOS

The anti-Müllerian hormone (AMH), known as Müllerian inhibiting substance, is a 140-kDa-homodimeric glycoprotein hormone secreted from both male and female gonads. AMH is a member of the transforming growth factor-beta superfamily (87). The AMH gene is located on the small arm of chromosome 19 (88). A physiological effect of AMH occurs through the AMH type II receptor (89). Firstly, it is produced in granulosa cells of primary follicles during third trimester of gestation (90). Its levels increase slowly from birth to adolescence, and then a steady-state low circulating level persists throughout the reproductive period. Circulating AMH becomes undetectable after natural or surgical menopause (91,92).

Although AMH blocks the early stage of follicle development and contributes to steroid hormone production by granulosa cells. Its precise role in subfertility due to PCOS is not yet clears (93-95). Correspondingly, a study on AMH null or knockout models showed that in the absence of AMH increase in primordial follicle recruitment may lead to early exhaustion of the primordial follicle pool (96). Insulin is an important regulator of AMH release. Indeed, AMH expression is increased in PCOS subjects with insulin resistance (97). PCOS women taking insulin sensitizers showed reduced serum levels of AMH, indicating that insulin resistance could affect AMH secretion (98). Likewise, amenorrheic PCOS subjects showed higher levels of AMH than oligomenorrheic PCOS subjects (99). At the cellular level, granulosa cells of PCOS women produce more AMH than those in healthy subjects without PCOS (100). Regardless of the phenotype of PCOS adiposity can also dysregulate AMH secretion (101). In accordance with this, hypogonadotrophic patients with myotonic dystrophy showed increased body and visceral fat accumulation which affect serum circulating AMH levels adversely (102).

AMH receptor mRNA and protein expressions have been identified in normal human endometrium (103,104), Notwithstanding, no study has reported presence of AMH receptor expression in PCOS subjects.

Inhibitory effect of AMH on human endometrial cell proliferation (103) indicates that circulating AMH may affect endometrium function in PCOS. In conclusion, physiological levels of AMH can contribute to sustained reproductive function in PCOS by regulating both follicle selection and endometrial proliferation. Nevertheless, high levels of AMH in PCOS can lead to deviations from normal physiology in follicle selection and decidualization. Clinically, increased incidences of endometrial shape anomalies in PCOS cases with high AMH are also striking and worth investigating. All these abnormalities can be considered probable causes of fertility decline in PCOS cases with high AMH.

Kisspeptin in PCOS

The kisspeptins stimulate GnRH secretion from the arcuate neurons and regulate LH release (17,105). By increasing LH release overactivation of kisspeptin neurons causes PCOS-like appearance. The concentrations of kisspeptin in adolescent girls with PCOS were significantly higher than in adolescent girls without PCOS (106). Panidis et al. reported that both normal-weighted and obese PCOS women had significantly higher kisspeptin levels than obese and overweight women without PCOS (107). They also noted a negative correlation between kisspeptin, BMI, free androgen index, and insulin resistance (107). Conversely, Yilmaz et al. reported that kisspeptin positively correlated with LH, total testosterone, dehydroepiandrosterone sulphate, and the free androgen index (108). Emekci et al. showed a positive correlation between kisspeptin and leptin levels (109). In accordance with this, Jeon et al. reported high kisspeptin and leptin levels in PCOS (110). Another evidence supporting role of kisspeptin in PCOS comes from an animal study. Kondo et al. reported that arcuate nucleus of RU486-treated rats shows higher kisspeptin expression than in healthy controls (111).

In addition to its direct effect on GnRH neurons, kisspeptin also mediates the functions of other peptides in the arcuate nucleus. For example, interaction between leptin and the Kiss1-expressing neurons induces GnRH release. We therefore propose that dysregulated synthesis and release of kisspeptin are implicated in the etiology of PCOS-related subfertility by directly affecting GnRH neurons or regulating the effects of other peptides in the arcuate nucleus (112).

Neurokinin B in PCOS

Neurokinin B (NKB) was recently discovered as an upstream regulator of GnRH release. It is co-expressed with kisspeptin in the arcuate nucleus and ovary. The precise physiological function of NKB in PCOS is not clear. By regulating GnRH secretion NKB might be implicated in follicle growing and implantation in PCOS. Correspondingly, downregulated NKB receptor mRNA expression has been reported in the granulosa cells of PCOS women (38). They also noted that NKB induces both follicle growing and estradiol production in zebrafish. Likewise, Osuka et al. reported that prenatal exposure to androgens resulted in higher NKB expression in the hypothalamus which could be associated with PCOS (113). Together, in addition to its central effects NKB regulates ovarian steroidogenesis and follicle development directly. These findings raise the possibility that dysregulated synthesis or release of the NKB may contribute to occurence of subfertility in women with PCOS.

Irisin in PCOS

Irisin is a newly-discovered muscle-derived factor that regulates energy expenditure in several tissues and biological fluids including ovarian follicles (27,114,115). Exercise induce its release and mediates brown adipose tissue differentiation (27,114-116). Irisin mediates the beneficial effects of exercise on metabolism by activating uncoupling protein 1, PPARγ coactivator-1 α, and insulin (117,118). Abnormal irisin metabolism has been reported in metabolic disorders (119,120). Concordantly, an increased level of irisin has been noted in women with PCOS undergoing IVF/ICSI (119,121). Because irisin raises energy expenditure in brown and beige adipose tissues, it is most likely that the high irisin in PCOS protects follicle against excess energy inflow (115). Moreover, high irisin levels could represent an "irisin resistance" state in PCOS resembling that of insulin resistance (122,123).

In a recent study, for the first time that, presence of irisin in follicular fluid of PCOS women has been reported (27). Authors of this study noted a positive correlation between follicular fluid and serum irisin levels. By regulating energy demand and local temperature in the follicle, irisin may contribute to follicle development in PCOS (27). Hence, in addition to basic culprits of PCOS such as obesity, hyperandrogenemia, and insulin resistance altered metabolism of irisin in PCOS could provide a basis for subfertility.

ANP and BNP in PCOS

Natriuretic peptides are produced by cardiomyocytes and regulate hemodynamic homeostasis (124). Both atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) stimulate lipolysis in human adipocytes (58,125). Since intravenous administration of ANP induces adiponectin secretion, adiponectin could be a mediator of ANP (126). Recent study showed lower plasma ANP levels in PCOS (127). These data suggest that low levels of ANP could be a part of the etiology of PCOS (127).

BNP is produced in heart ventricle walls (128). The biologically active 32-amino acid BNP is separated from the N-terminal part of the prohormone (NT-proBNP) (129). In a previous study we found higher NT-proBNP levels in PCOS subjects suggesting it may be related to PCOS etiology (130).

Granulosa cells of developing follicle produce ANP and release it into antrum (131). Although ANP regulates cyclic guanosine monophosphate (cGMP) and steroidogenesis in granulosa cells (131,132) it inhibits oocyte maturation (133). Similar to ANP, BNP induces generation of cGMP in porcine oocytes to maintain prophase I arrest emphasizing that both peptides are important in follicle development (134). Because natriuretic peptides have an impact on granulosa cell

differentiation and follicle development dysregulated secretion of both peptides may compromise follicle development and cause PCOS-related subfertility (135,136).

Cholecystokinin, peptide YY, incretins, visfatin in PCOS

Duodenal proteins and fat induce cholecystokinin production by the small intestine (58). Decreased postprandial cholecystokin response has been reported in obese women with PCOS (137). Likewise, peptide YY is produced in the gastrointestinal tract and its secretion is impaired in overweight subjects (28,138). Fasting or postprandial peptide YY and cholecystokinin concentrations were similar in both PCOS and controls (139).

Although disturbed secretion of incretin may be responsible for some metabolic components of PCOS precise role of incretins on subfertility due to PCOS are not yet clear (140,141). Visfatin is an enzyme involved in several metabolic conditions and human granulosa cells have ability to release this peptide. Exogenous visfatin induces follicle response during ovarian stimulation (142). However, Garruti et al. showed normal levels of serum visfatin in PCOS (66). As a conclusion, comprehensive studies are needed to elucidate the effects of these four peptides on subfertility due to PCOS.

Adropin, orexin, salusin, preptin, and adrenomedullin in PCOS

The relationship between adropin, orexin, salusin, adrenomedullin (ADM), preptin and PCOS have been comprehensively investigated by our team (143-145). We detected high levels of adropin and orexin in PCOS. We also showed negative correlation between orexin, LH, and free testosterone levels supporting orexin's role in PCOS (144,145). Likewise, plasma salusin α, β, ADM, and preptin levels were found to higher in PCOS women than controls. Serum levels of salusin β were correlated positively with LH, FSH, and total testosterone levels (146). Notwithstanding, the effects of these peptides on subfertility due to PCOS have not been investigated yet.

Treatment strategies of PCOS with newer peptide analogs or antagonists: future perspective

Subfertility in PCOS is not exclusively due to defective folliculogenesis but also might be resulted from high or low levels of peripheral or central peptides. Different types of peptides affect the functions of GnRH neurons, gonadotroph cells as well ovary and endometrium (16). Since underlying molecular causes of PCOS are largely unknown specific treatment options for subfertility due to PCOS are lacking. Exercise, weight loss, and insulin sensitizers improve reproductive performance (147). Likewise, transplantation of brown adipose tissue improves anovulation, hyperandrogenism, insulin resistance, and reproductive outcome in a rat model of PCOS (148). These results indicate a close relationship between adipose tissue and PCOS. Hence, regulating of adipose tissue functions with new peptide analogs or antagonists could be a promising therapeutic option for treating PCOS-related subfertility (148).

In order to design and develop new drugs derived from peptides we need to understand anatomical locations and functions of peptides regulating reproductive events. For example, the projections of kisspeptin neurons are associated with those of GnRH neurons. Because GnRH secretory neurons are outside the blood-brain barrier, kisspeptin analogs or antagonists could be used to induce or inhibit GnRH secretion (149). This offers opportunities for manipulating the arcuate neurons and pituitary gonadotrophs in PCOS subjects. Together, in view of the exclusive stimulation of LH secretion by kisspeptin, the development of kisspeptin antagonists could pave the way for new treatment strategies to treat subfertility due to PCOS (150).

The discovery of close link between leptin and PCOS allowed for treating some reproductive dysfunctions via leptin agonists or antagonists. In accordance with this, improved menstruation has been reported following leptin administration in women with hypogonadotrophic hypogonadism (61). Because leptin stimulates GnRH neurons blocking of leptin effect on arcuate nucleus makes this peptide an ideal candidate for replacing a GnRH agonists (13). As an example, by inhibiting leptin receptors, a superactive human leptin antagonist reverses the actions of leptin in ovarian follicles and blocks ovarian cyst formation (15). This approach may be used to restore defective follicle development in PCOS. Because interaction between leptin and Kiss1-expressing neurons can affect reproductive events agonist or antagonist forms of leptin and kisspeptins could provide a novel therapeutic approach to treating subfertility due to PCOS.

The close relationship between metformin and leptin is also worth investigating in subfertility due to PCOS. Irfan et al. reported that circulating leptin levels were significantly reduced in PCOS subjects who had received 3-6 months of metformin therapy (151). In addition to improve insulin resistance metformin may regulate circulating leptin and restore subfertility due to PCOS.

The antagonistic effect of ghrelin and GnIH on GnRH release makes these two peptides ideal replacement agents instead of GnRH antagonists. If we can achieve that both peptides may be used at ovarian stimulation protocols to prevent a premature LH surge. Since ghrelin acts as a survival factor for developing follicles, it could be used to treat women suffering from premature ovarian aging. Therefore, normalization of circulating ghrelin and GnIH might allow both restoration of functionally affected hypothalamus-gonad axis and then subfertility due to PCOS.

Another candidate peripheral peptide for treating PCOS-related subfertility is adiponectin. By binding its receptors in granulose cells adiponectin induces release of insulin-like growth factor I and sex steroids (41). Because brown adipose tissue transplantation enhances adiponectin secretion and exogenous adiponectin ameliorates DHEA-induced PCOS in rats it may be used to improve ovulatory dysfunction in PCOS (148).

Conclusions

Although ovulatory dysfunction, insulin resistance, hiperandrogenemia, and obesity are the obvious causes of subfertility, available data suggest that dysregulated peptide release also contributes to emerge of metabolic components of PCOS (1,73,146). During the reproductive period, differentiation and maturation of follicles are regulated by signals from central and peripheral reproductive tissues. Although possible association between peptides, ovary, and endometrium are not clear, some evidence indicates that either high or low levels of peptides may adversely affect fertility outcome in PCOS.

Without detailed knowledge about the etiology of PCOS-related subfertility, management of PCOS subjects is restricted to conventional treatment agents. Since peptides are essential factors in reproductive events they are potential candidates for designing drugs to treat subjects suffering from subfertility. Hence, understanding of neuroendocrine peptides and their role in human reproduction provides new opportunities for producing synthetic peptide drugs with multiple biological functions. Such approaches have gained significant attention in recent years because the need for subfertility treatment in PCOS has increased. Both agonist and antagonist forms of peptides are being developed as modulators of the HPG axis. These new drugs offer additional and alternative therapeutic options in PCOS-related subfertility. It is expected that drugs derived from peptides can be used to improve intra- and/or inter-follicular communication and failed implantation in subfertility due to PCOS.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

1. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. Reprod Biol Endocrinol 2016; 14:38.

2. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 2004; 89:2745-9.

3. MarchWA, MooreVM, Willson KJ, Phillips DI, NormanRJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod 2010; 25:544-51.

4. Carmina E, Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab 1999; 84:1897-9.

5. Lindholm A, Blomquist C, Bixo M, Dahlbom I, Hansson T, Sundström Poromaa I, et al. No difference in markers of adipose tissue inflammation between overweight women with polycystic ovary syndrome and weight-matched controls. Hum Reprod 2011; 26:1478-85.

6. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89:2548-56.

7. Kim SH, Chung JH, Song SW, Jung WS, Lee YA, Kim HN. Relationship between deep subcutaneous abdominal adipose tissue and metabolic syndrome: a case control study. Diabetol Metab Syndr 2016; 8:10.

8. Sahin SB, Durakoglugil T, Ayaz T, Sahin OZ, Durakoglugil E, Sumer F, et al. Evaluation of para- and perirenal fat thickness and its association with metabolic disorders in polycystic ovary syndrome. Endocr Pract 2015; 21:878-86.

9. Apparao KB, Lovely LP, Gui Y, Lininger RA, Lessey BA. Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome. Biol Reprod 2002; 66:297-304.

10. Celik O, Aydin S, Celik N, Yilmaz M. Peptides: Basic determinants of reproductive functions. Peptides 2015; 72:34-43.

11. Cermik D, Selam B, Taylor HS. Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome. J Clin Endocrinol Metab 2003; 88:238-43.

12. Suikkari AM, Ruutiainen K, Erkkola R, Seppala M. Low levels of low molecular weight insulin-like growth factor-binding protein in patients with polycystic ovarian disease. Hum Reprod 1989; 4:136-9.

13. Burcelin R, Thorens B, Glauser M, Gaillard RC, Pralong FP. Gonadotropin-releasing hormone secretion from hypothalamic neurons: stimulation by insulin and potentiation by leptin. Endocrinology 2003; 144:4484-91.

14. Celik O, Celik N, Aydin S, Aygun BK, Haberal ET, Kuloglu T, et al. Ghrelin action on GnRH neurons and pituitary gonadotropes might be mediated by GnIH-GPR147 system. Horm Mol Biol Clin Investig 2016; 25:121-8.

15. Gregoraszczuk EL, Rak-Mardyła A. Supraphysiological leptin levels shift the profile of steroidogenesis in porcine ovarian follicles toward progesterone and testosterone secretion through increased expressions of CYP11A1 and 17b-HSD: a tissue culture approach. Reproduction 2013; 145:311-7.

16. Kawwass JF, Summer R, Kallen CB. Direct effects of leptin and adiponectin on peripheral reproductive tissues: a critical review. Mol Hum Reprod 2014; 21:617-32.

17. Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum Reprod Update. 2014; 20:485-500.

18. Okon MA, Laird SM, Tuckerman EM, Li TC. Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. Fertil Steril 1998; 69:682-90.

19. Thornton JW. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci USA 2001; 98:5671-6.

20. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum Reprod Update 2010; 17:17-33.

21. Palin MF, Bordignon VV, Murphy BD. Adiponectin and the control of female reproductive functions. Vitam Horm 2014; 90:239-87.

22. Tabandeh MR, Golestani N, Kafi M, Hosseini A, Saeb M, Sarkoohi P. Gene expression pattern of adiponectin and adiponectin receptors in dominant and atretic follicles and oocytes screened based on brilliant cresyl blue staining. Anim Reprod Sci 2015; 131:30-40.

23. Carmina E, Orio F, Palomba S, Cascella T, Longo RA, Colao AM, et al. Evidence for altered adipocyte function in polycystic ovary syndrome. Eur J Endocrinol 2005; 152:389-94.

24. Mantzoros CS, Dunaif A, Flier JS. Leptin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metab 1997; 82:1687-91.

25. Moran LJ, Noakes M, Clifton PM, Wittert GA, Tomlinson L, Galletly C, et al. Ghrelin and measures of satiety are altered in polycystic ovary syndrome but not differentially affected by diet composition. J Clin Endocrinol Metab 2004; 89:3337-44.

26. Panidis D, Kourtis A, Farmakiotis D, Mouslech T, Rousso D, Koliakos G. Serum adiponectin levels in women with polycystic ovary syndrome. Human Reproduction 2003; 18:1790-6.
27. Acet M, Celik N, Acet T, Ilhan S, Yardim M, Aktun HL, et al. Serum and follicular fluid irisin levels in poor and high responder women undergoing IVF/ICSI. Eur Rev Med Pharmacol Sci 2016; 20:1940-6.
28. Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology 1985; 89:1070-7.
29. Bertoldo MJ, Faure M, Dupont J, Froment P. AMPK: a master energy regulator for gonadal function. Front Neurosci 2014; 9:235.
30. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. TrendsImmunol 2004; 25:4-7.
31. Mah PM, Wittert GA. Obesity and testicular function. Mol Cell Endocrinol 2015; 316:180-6.
32. Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. Arterioscler Thromb Vasc Biol 2005; 25:2062-8.
33. Tengstrand B, Carlstrom K, Hafstrom I. Gonadal hormones in men with rheumatoid arthritis--from onset through 2 years. J Rheumatol 2009; 36: 887-92.
34. Farooqi IS. Leptin and the onset of puberty: insights from rodent and human genetics. Semin Reprod Med 2002; 20:139-44.
35. Psilopanagioti A, Papadaki H, Kranioti EF, Alexandrides TK, Varakis JN. Expression of adiponectin and adiponectin receptors in human pituitary gland and brain. Neuroendocrinology 2009; 89:38-47.
36. Yan X, Yuan C, Zhao N, Cui Y, Liu J. Prenatal androgen excess enhances stimulation of the GNRH pulse in pubertal female rats. J Endocrinol 2014; 222:73-85.
37. Brown RE, Imran SA, Ur E, Wilkinson M. KiSS-1 mRNA in adipose tissue is regulated by sex hormones and food intake. Mol Cell Endocrinol 2008; 281:64-72.
38. Qi X, Salem M, Zhou W, Sato-Shimizu M, Ye G, Smitz J, et al. Neurokinin B Exerts Direct Effects on the Ovary to Stimulate Estradiol Production. Endocrinology 2016; 157:3355-65.
39. Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, et al. Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogondism. J Clin Endocrinol Metab 2005; 90:1849-55.
40. Chabrolle C, Tosca L, Crochet S, Tesseraud S, Dupont J. Expression of adiponectin and its receptors (AdipoR1 and AdipoR2) in chicken ovary: potential role in ovarian steroidogenesis. Domest Anim Endocrinol 2007; 33: 480-7.
41. Chabrolle C, Tosca L, Ramé C, Lecomte P, Royère D, Dupont J. Adiponectin increases insulin-like growth factor I-induced progesterone and estradiol secretion in human granulosa cells. Fertil Steril 2009; 92:1988-96.
42. Ersahin A, Acet M, Acet T, Yavuz Y. Disturbed endometrial NF-κB expression in women with recurrent implantation failure. Eur Rev Med Pharmacol Sci 2016; 20:5037-40.
43. Wickham EP 3rd, Tao T, Nestler JE, McGee EA. Activation of the LH receptor up regulates the type 2 adiponectin receptor in human granulosa cells. J AssistReprodGenet 2014; 30:963-8.
44. Wu L, Xu B, Fan W, Zhu X, Wang G, Zhang A. Adiponectin protects Leydig cells against proinflammatory cytokines by suppressing the nuclear factor-κB signaling pathway. Febs J 2015; 280:3920-7.
45. Hameed W, Yousaf I, Latif R, Aslam M. Effect of visfatin on testicular steroidogenesis in purified Leydig cells. J Ayub Med Coll Abbottabad 2014; 24:62-4.
46. Shen CJ, Tsai EM, Lee JN, Chen YL, Lee CH, Chan TF. The concentrations of visfatin in the follicular fluids of women undergoing controlled ovarian stimulation are correlated to the number of oocytes retrieved. Fertil Steril 2013; 93:1844-50.
47. Ballinger AB, Savage MO, Sanderson IR. Delayed puberty associated with inflammatory bowel disease. Pediatr Res 2003; 53:205-10.
48. Bozaoglu K, Bolton K, McMillan J, Zimmet P, Jowett J, Collier G, et al. Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinology 2007; 148:4687-94.
49. Chu Y, Cui Q, Feng G, Song Z, Jiang X. The expression of resistin in adipose tissues of patients with polycystic ovary syndrome and insulin resistance. J Huazhong Univ Sci Technolog Med Sci 2009; 29:642-5.
50. Ersahin A, Acet M, Ersahin SS, Acet T, Yardim M, Kenanoglu O, et al. Follicular Fluid Cerebellin and Betatrophin Regulate Metabolic Functions of Growing Follicles in PCOS. Clin Exp Reprod Med 2017; 44:33-9.
51. Strowski MZ, Kaczmarek P, Mergler S, Wiedenmann B, Domin D, Szwajkowski P, et al. Insulinostaticactivity of cerebellin--evidencefrom in vivoand in vitrostudies in rats. Regul Pept 2009; 157:19-24.
52. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocrine Reviews 2000; 21:697-738.
53. Coleman DL. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 1973; 9:294-8.
54. Friedman JM, Leibel RL, Siegel DS, Walsh J, Bahary N. Molecular mapping of the mouse ob mutation. Genomics 1991; 11:1054-62.
55. Moschos S, Chan JL, Mantzoros CS. Leptin and reproduction: a review. Fertil Steril 2002; 77:433-44.
56. Chou K, Perry CM. Metreleptin: first global approval. Drugs 2013; 73:989-97.
57. Abir R, Ao A, Jin S, Barnett M, Raanani H, Ben-Haroush A, et al. Leptin and its receptors in human fetal and adult ovaries. Fertil Steril 2005; 84:1779-82.
58. Moro C, Galitzky J, Sengenes C, Crampes F, Lafontan M, Berlan M. Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. J Pharmacol Exp Ther 2004; 308:984-92.
59. Ojeda SR, Lomniczi A, Mastronardi C, Heger S, Roth C, Parent AS, et al. Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach? Endocrinology 2006; 147:1166-74.
60. Mallinson RJ, Williams NI, Olmsted MP, Scheid JL, Riddle ES, De Souza MJ. A case report of recovery of menstrual function following a nutritional intervention in two exercising women with amenorrhea of varying duration. J Int Soc Sports Nutr 2014; 10:34.
61. von Schnurbein J, Moss A, Nagel SA, Muehleder H, Debatin KM, Farooqi IS, et al. Leptin substitution results in the induction of menstrual cycles in an adolescent with leptin deficiency and hypogonadotropic hypogonadism. Horm Res Paediatr 2014; 77:127-33.
62. Zheng SH, Du DF, Li XL. Leptin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. Reprod Sci 2016; 24:656-70.
63. Lian Y, Zhao F, Wang W. Central leptin resistance and hypothalamic inflammation are involved in letrozole-induced polycystic ovary syndrome rats. Biochem Biophys Res Commun 2016; 476:306-12.
64. Jacobs MB, Bazzano LA, Pridjian G, Harville EW. Childhood adiposity and fertility difficulties: the Bogalusa Heart Study. Pediatr Obes 2017; 12:477-84.
65. Celik O, Hascalik S, Ozerol E, Hascalik M, Yologlu S. Cerebrospinal fluid leptin levels in preeclampsia: relation to maternal serum leptin levels. Acta Obstet Gynecol Scand 2004; 83:519-23.

66. Garruti G, de Palo R, Rotelli MT, Nocera S, Totaro I, Nardelli C, et al. Association between follicular fluid leptin and serum insulin levels in nonoverweight women with polycystic ovary syndrome. Biomed Res Int 2014; 2014:980429.
67. De Placido G, Alviggi C, Clarizia R, Mollo A, Alviggi E, Strina I, et al. Intra-follicular leptin concentration as a predictive factor for in vitro oocyte fertilization in assisted reproductive techniques. J Endocrinol Invest 2006; 29:719-26.
68. Li MG, Ding GL, Chen XJ, Lu XP, Dong LJ, Dong MY, et al. Association of serum and follicular fluid leptin concentrations with granulosa cell phosphorylated signal transducer and activator of transcription 3 expression in fertile patients with polycystic ovarian syndrome. J Clin Endocrinol Metab 2007; 92:4771-6.
69. Blüher S, Mantzoros CS. Leptin in reproduction. Curr Opin Endocrinol Diabetes Obes 2007; 14:458-64.
70. Toulis KA, Goulis DG, Farmakiotis D, Georgopoulos NA, Katsikis I, Tarlatzis BC, et al. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. Hum Reprod Update 2009; 15:297-307.
71. Wickham EP, Cheang KI, Clore JN, Baillargeon JP, Nestler JE. Total and high-molecular weight adiponectin in women with the polycystic ovary syndrome. Metabolism 2011; 60:366-72.