Emerging roles of APLN and APELA in the physiology and pathology of the female reproductive system

Xueying Wang[1,2,*], Xiaofei Liu[1,2,*], Zifan Song[1], Xin Shen[1], Siying Lu[1], Yan Ling[3] and Haibin Kuang[1,4]

[1] Department of Physiology, Basic Medical College, Nanchang University, Nanchang, China
[2] Department of Clinical Medicine, School of Queen Mary, Nanchang University, Nanchang, China
[3] Department of Obstetrics and Gynecology, Jiangxi provincial People's Hospital affiliated Nanchang University, Nanchang, China
[4] Jiangxi Provincial Key Laboratory of Reproductive Physiology and Pathology, Medical Experimental Teaching Center of Nanchang University, Nanchang, China
[*] These authors contributed equally to this work.

ABSTRACT

APLN, APELA and their common receptor APLNR (composing the apelinergic axis) have been described in various species with extensive body distribution and multiple physiological functions. Recent studies have witnessed emerging intracellular cascades triggered by APLN and APELA which play crucial roles in female reproductive organs, including hypothalamus-pituitary-gonadal axis, ovary, oviduct, uterus and placenta. However, a comprehensive summary of APLN and APELA roles in physiology and pathology of female reproductive system has not been reported to date. In this review, we aim to concentrate on the general characteristics of APLN and APELA, as well as their specific physiological roles in female reproductive system. Meanwhile, the pathological contexts of apelinergic axis dysregulation in the obstetrics and gynecology are also summarized here, suggesting its potential prospect as a diagnostic biomarker and/or therapeutic intervention in the polycystic ovary syndrome, ovarian cancer, preeclampsia and gestational diabetes mellitus.

INTRODUCTION

Apelin receptor (APLNR, also known as APJ, APJR, AGTRL1 and HG11) was firstly identified as a class A G protein-coupled receptor in 1993. It consists of 380 amino acids, which has a sequence sharing 31% homology with that of the angiotensin type 1 receptor (*O'Dowd et al., 1993*). Nevertheless, APLNR cannot actually bind to angiotensin II and remains as an "orphan receptor" until its endogenous ligand apelin (APLN, also named APEL and XNPEP2) was later extracted from bovine stomach (*Tatemoto et al., 1998*). APLN is generally existed in functional isoforms which are cleaved and modified from the C-terminus of a 77-amino acid pre-pro-peptide encoded by *APLN* gene, with different affinities for APLNR and prevalent distribution (*Chapman, Dupré & Rainey, 2014*). Both

APLN and its receptor APLNR levels are universally high at multiple organs like brain, retina, heart, stomach, liver, kidney and blood vessels in many species (*Kawamata et al., 2001*; *Zeng et al., 2007*; *Kasai et al., 2008*; *Qian et al., 2011*; *Krist et al., 2013*; *Lv et al., 2017*). Recent years, apelin receptor early endogenous ligand (APELA, also named ELABELA, Toddler and Ende) was identified as a new endogenous ligand for APLNR in both Chng and Pauli's labs independently (*Chng et al., 2013*; *Pauli et al., 2014*). Similar to APLN, this 54-amino acid polypeptide is also processed into several isoforms. APELA is highly enriched in the early stage of embryo and confirmed to play a vital role in embryogenesis and angiogenesis (*Norris et al., 2017*). APLNR and its two ligands compose the apelinergic axis, which is well delineated in systemic physiological processes like cardiogenesis, angiogenesis, fluid homeostasis, vasodilation and energy metabolism.

More recently, several studies have been investigating the possible intervention of apelinergic axis in female reproductive system based on its precise regulation of steroidogenesis, angiogenesis and vasodilation, before moving onto the dysregulation of this system which hypothetically causes fertility disorders and pregnancy complications like polycystic ovary syndrome (PCOS), ovarian cancer, gestational diabetes mellitus (GDM) and preeclampsia (PE) (summarized in Table 1). This review summarizes and evaluates the current role of apelinergic system in female reproductive system at both physiological and pathological profiles (Fig. 1), as well as providing the direction for future research.

SURVEY METHODOLOGY

Recently published articles and reports (within 15 years) were conducted from PubMed, Google Scholar and Queen Mary Library databases. Based on the keywords 'APLN', 'APELA' and 'female reproduction', articles extracted were summarized to identify the physiological and pathological roles of apelinergic axis in female reproductive system. This study was approved by Jiangxi Provincial Key Laboratory of Reproductive Physiology and Pathology, Medical Experimental Teaching Center of Nanchang University.

APLN AND APELA, ENDOGENOUS LIGANDS OF APLNR

Characteristics of APLN

Human *APLN* gene is located on chromosome Xq25-26.1 which encodes a pre-propeptide of 77 amino acids. After cleavage of the 22-amino acid secretory sequence at N terminus by endopeptidases, the propeptide is subsequently processed into three active fragments at several dibasic residues (Arg-Lys and Arg-Arg), including APLN-36, APLN-17 and APLN-13. APLN-13 undergoes post-transcriptional cyclization at the N-terminal glutamine, generating pyroglutamate-APLN-13 (Pyr1-APLN-13) (*Tatemoto et al., 1998*). The potency and efficacy of APLN differ from different isoforms. For instance, APLN-36, APLN-13 and Pyr1-APLN-13 are preponderantly contributed in human cardiovascular regulation (*Maguire et al., 2009*), whereas APLN-17 plays crucial role in APLNR internalization (*El Messari et al., 2004*). To date, APLN is abundantly distributed in female reproductive system such as ovary, oviduct, uterus and placenta. Emphatically, APLN is identified as one

Wang et al. (2020), *PeerJ*, DOI 10.7717/peerj.10245

3/25

Table 1 Summary of studies about the expressional changes of APLN and APELA in the polycystic ovary syndrome (PCOS), Ovarian cancer (OvCa), preeclampsia (PE) and gestational diabetes mellitus (GDM).

Authors	Year	Disease type	Species	Samples	Molecule	No. of cases	No. of controls	Analyzed expression	Significance	Notes
Cekmez et al.	2011	PCOS	human	plasma	APLN	48	37	protein	higher in patients ($p < 0.001$)	positive with HOMA-IR
Altinkaya et al.	2014	PCOS	human	plasma	APLN	45	45	protein	lower in patients	positive with HOMA-IR
Olszanecka-Glinianowicz et al.	2015	PCOS	human	plasma	APLN	87	67	protein	lower in patients	negative with HOMA-IR ($p < 0.001$)
Sun et al.	2015	PCOS	human	plasma	APLN	63	40	protein	higher in patients ($p < 0.05$)	positive with HOMA-IR
Roche et al.	2016	PCOS	human	tissue	APLN and APLNR	65	60	mRNA and protein	higher in patients ($p < 0.05$)	
Bongrani et al.	2019	PCOS	human	plasma and tissue	APLN and APLNR	23	27(+28)	mRNA and protein	higher in patients ($p < 0.01$)	
Yi et al.	2017	OvCa	human	tissue	APELA	NA	NA	mRNA and protein	higher in patients	
Neelakantan et al.	2019	OvCa	human	tissue	APLNR	NA	NA	mRNA and protein	higher in patients ($p < 0.05$)	
Panaitescu et al.	2018	EOPE	human	plasma	APELA	56	59	protein	no difference	
Pritchard et al.	2018	EOPE	human	plasma	APELA and APLNR	32	32	mRNA	both no difference	
Villie et al.	2019	EOPE	human	plasma	APELA	12	14	protein	no difference	
Wang et al.	2019	EOPE	human	placenta	APELA	30	30	mRNA and protein	lower in patients ($p < 0.0001$)	
Zhou et al.	2019	EOPE	human	placenta	APELA and APLNR	6	11	mRNA and protein	APELA no difference; APLNR both lower in patients ($p < 0.05$)	
Zhou et al.	2019	EOPE	human	plasma	APELA	15	15	protein	no difference	
Para et al.	2020	EOPE	human	plasma	APELA	54	56	protein	no difference	
Panaitescu et al.	2018	LOPE	human	plasma	APELA and APLNR	57	60	protein	APELA higher in patients ($p = 0.01$); APLNR no difference	
Zhou et al.	2019	LOPE	human	placenta	APELA and APLNR	14	11	mRNA and protein	APELA both lower in patients ($p < 0.01$); APLNR protein lower in patients ($p < 0.01$)	
Zhou et al.	2019	LOPE	human	plasma	APELA	22	15	protein	lower in patients ($p < 0.01$)	
Para et al.	2020	LOPE	human	plasma	APELA	52	52	protein	higher in patients ($p < 0.001$)	
Cobellis et al.	2007	PE	human	placenta	APLN and APLNR	15	15	protein	APLN and APLNR expression both higher ($p < 0.05$)	
Inuzuka et al.	2013	PE	human	placenta	APLN	NA	NA	mRNA and protein	mRNA lower in patients ($p < 0.05$)	
Yamaleyeva et al.	2015	PE	human	placenta	APLN and APLNR	20	22	mRNA and protein	APLN lower in patients (only protein $p < 0.05$), APLNR no difference (both mRNA and protein)	

Table 1 (*continued*)

Authors	Year	Disease type	Species	Samples	Molecule	No. of cases	No. of controls	Analyzed expression	Significance	Notes
Van Mieghem et al.	2016	PE	human	plasma	APLN	8	10	mRNA	no difference	used antihypertensive treatment
Sattar Taha, Zahraei & Al-Hakeim	2020	PE	human	plasma	APLN	60	30	protein	lower in patients ($p < 0.01$)	
Aydin	2010	GDM	human	breast milk	APLN	10	10	protein	lower in patients	
Telejko et al.	2010	GDM	human	plasma	APLN and APLNR	101	101	mRNA	no difference	
Aslan et al.	2012	GDM	human	plasma and cord blood	APLN	30	30	protein	higher in patients ($p = 0.001$)	
Boyadzhieva et al.	2013	GDM	human	plasma	APLN	127	109	protein	lower in patients ($p = 0.009$)	
Oncul et al.	2013	GDM	human	plasma and cord blood	APLN	24	21	protein	no difference	
Akinci et al.,	2014	GDM	human	plasma	APLN	141	49	protein	lower in patients ($p < 0.001$)	
Caglayan	2016	GDM	human	plasma	APLN	20	20	protein	higher in patients ($p < 0.001$)	

Notes.

For each study, the authors, year, disease type, species, samples, molecule, sample size (No. of cases and controls), analyzed expression and significance were listed. There would be a significance when $p < 0.05$, and p values were listed in the table (if given). The table was ordered by diseases, molecules and year of publication.

PCOS, polycystic ovary syndrome; OvCa, ovarian cancer; PE, preeclampsia; GDM, gestational diabetes mellitus; NA, not available.
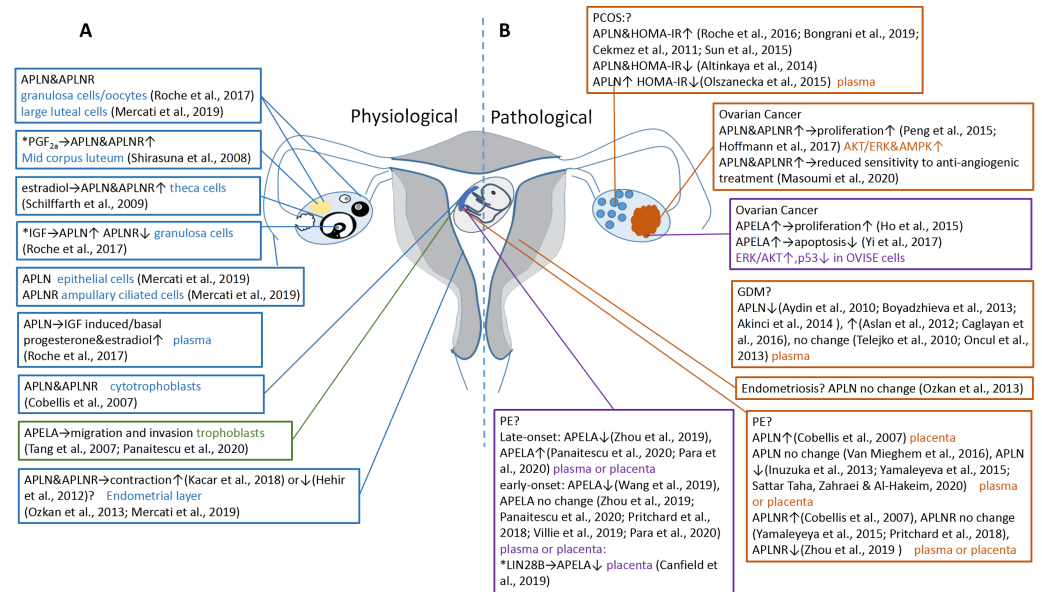
Figure 1 **Expression and function of APLN and APELA in reproduction system.** (A) In physiological conditions, APLN (in blue textbox) and APELA (in green textbox) play diverse roles at the different parts of the ovary, uterus and placenta. (B) In pathological conditions, aberrant expression of APLN (in orange textbox) and APELA (in purple textbox) lead to female reproductive disorders such as polycystic ovary syndrome (PCOS), ovarian cancer, preeclampsia (PE), gestational diabetes mellitus (GDM) and endometriosis. * indicating potential apelinergic stimulating factors. ? indicating still unclear or controversy about the functions or contributions of apelinergic molecules in these diseases. ↑ and ↓ in the textbox means increase and decrease respectively, both indicate statistically significant changes. → means resulting.

Full-size 🖼 DOI: 10.7717/peerj.10245/fig-1

type of adipokines secreted by white adipose tissue, which plays a role with other adipokines in regulating the secretion of gonadotropin releasing hormone (GnRH), gonadotropins and steroids through hypothalamo-pituitary-gonadal (HPG) axis (*Bertrand, Valet & Castan-Laurell, 2015*; *Yang et al., 2019*).

APLN dependent signaling pathway

APLN/APLNR activates different types of G protein and further stimulates three important signaling pathways, which are phosphorylation of phosphoinositide 3-kinase/protein kinase B (PI3K/Akt), reduction of cyclic adenosine monophosphate (cAMP) and activation of phospholipase C-β (PLC-β), respectively (Fig. 2A) (*Chapman, Dupré & Rainey, 2014*). There are two types of pertussis toxin-sensitive Gα protein (Gαi/o, Gαq/11) at the downstream of APLNR, mediating different signaling transduction (*Masri et al., 2002*). Gαi/o activates PI3K/Akt dependent manner which is crucial for cell survival and nitric oxide (NO) induced vasodilation (*Liu et al., 2010*). Akt phosphorylates Bcl-2-associated death promoter (Bad, a BH3-only protein) and shifts it to an inert form, which inhibits the binding of Bad and Bcl-2. Bcl-2 plays an anti-apoptotic role by disturbing the aggregation of Bak and Bax (BH123 proteins) in the mitochondrial outer membrane, and thereby attenuating the release of cytochrome c and activation of caspase-3 (*Liu et al., 2019*).
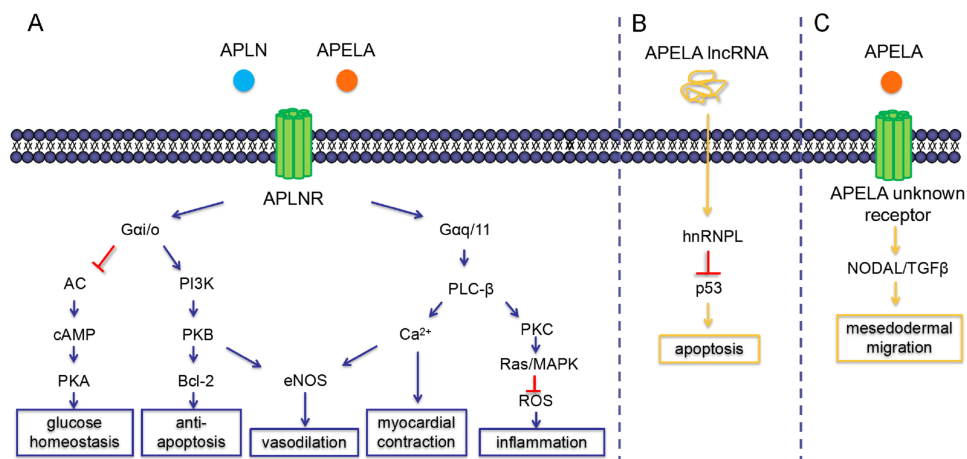
Figure 2 **Intracellular signaling pathways and physiological functions of APLN and APELA.** (A) Both APLN (in blue) and APELA (in orange) can classically activate Gαi/o and Gαq/11 mediated intracellular transduction via binding to their common receptor APLNR. (B) Non-coding APELA binds to hnRNPL and promotes p53-mediated cell apoptosis. (C) APELA also stimulates PI3K-independent NODAL/TGFβ signal through alternative receptors in hESCs. AC, adenylate cyclase; eNOS, endothelial nitric oxide synthase; lncRNA, long non-coding RNA; hnRNPL, heterogeneous nuclear ribonucleoprotein L.

Full-size 🖼 DOI: 10.7717/peerj.10245/fig-2

Moreover, endothelial nitric oxide synthase (eNOS) can also be activated by Akt through phosphorylation, triggering the release of NO for vasodilation (Fig. 2A) (*Yang et al., 2014*). Additionally, Gα-i/o inhibits adenylate cyclase (AC), following with the reduction of 3′, 5′-cAMP and protein kinase A (PKA), which could potentially regulate the glucose homeostasis (Fig. 2A) (*Masri et al., 2002*). Gαq/11 activates PLC-β hydrolyze phosphatidylinositol 4, 5-bisphosphate into second messengers diacylglycerol and inositol trisphosphate, which increases the release of calcium (Ca^{2+}) from intracellular store and activates protein kinase C (PKC) (*Carpéné et al., 2007*). Amplified intracellular Ca^{2+} not only mediates positive inotropic effect in cardiac smooth muscle, but also stimulates NO release in periphery via activating eNOS by calmodulin (*Dai, Ramirez-Correa & Gao, 2006*). PKC in turn activates Ras/MAPK system, which plays a crucial role in cell proliferation (*Szokodi et al., 2002*). Furthermore, MAPK halts the expression of pro-oxidant enzymes and subsequently attenuates the release of reactive oxygen species (ROS), which suppresses lipid metabolism and inflammatory reaction (Fig. 2A) (*Than et al., 2014*).

Characteristics of APELA

Apela gene, located on chromosome 4 of *Homo sapiens* (*Fagerberg et al., 2014*) (chromosome 8 in *Mus musculus* (*Yue et al., 2014*) and chromosome 1 in *Danio rerio* (*Ulitsky et al., 2011*), was originally annotated to be transcribed exclusively into a non-coding RNA in zebrafish embryo (*Chng et al., 2013*), while recently it was confirmed to encode a 54-amino acid precursor which further undergoes proteolysis and generates four mature isoforms: APELA-32, APELA-22, APELA-21 and APELA-11. The shortest isoform is conserved across vertebra species (*Huang et al., 2017*). Compared with APLN, APELA as the second discovered endogenous ligand of APLNR is also ubiquitously detected in

placenta, heart, kidney, prostate and mammalian plasma but not as widely as APLN (*Wang et al., 2015*). In addition, studies have reported that APELA is highly expressed in human embryonic stem cells (hESCs) where the APLNR is absent, indicating the existence of an alternative APLNR-independent transduction (*Ho et al., 2015*). A recent report has suggested that an orphan G protein-coupled receptor 25 (GPR25), associated with blood pressure regulation and autoimmune disease, could be activated by both APLN and APELA in non-vertebrates, which is similar as APLN in decreasing the intracellular cAMP level. However, the accurate role of this putative receptor in vertebrate remains to be determined (*Zhang et al., 2018*).

APELA dependent signaling pathway

Similar to APLN, APELA binds to APLNR, subsequently activating Gαi/o and Gα-q/11 mediated signaling cascades, including PI3K/Akt, PKC and PKC-independent Ras/MAPK pathways (Fig. 2A) (*Perjés et al., 2016*; *Zhang et al., 2018*). Nevertheless, it also plays non-redundant role for its specific properties during embryo development. In mouse ESCs, *Apela* acts as a long non-coding RNA that binds to heterogeneous nuclear ribonucleoprotein L (hnRNPL) at the 3′ UTR, which negatively regulates the interaction between p53 and hnRNPL, and promotes p53-mediated DNA damage induced apoptosis (Fig. 2B) (*Li et al., 2015*). In hESCs, APELA acts as a paracrine secreted hormone that binds to an alternative unknown receptor (non-APLNR) and activates PI3K/AKT signaling for cell survival and self-renewal. This process resembles other fibroblast growth factor like exogenous insulin and endogenous insulin-like growth factors (IGFs) in PI3K-mediated cell proliferation. However, APELA-pulsed hESCs is non-redundant as it also implicates in mesendodermal linage commitment through a PI3K-independent manner (*Ho et al., 2015*). During zebrafish gastrulation, a proper level of APELA acting as a mitogen, indirectly mediates the internalization of ventrolateral mesendodermal cells. This process is presumably achieved via activating NODAL/TGFβ signaling pathway (Fig. 2C), whereas its specific mechanism remains unknown (*Pauli et al., 2014*).

ROLES OF APLN AND APELA IN HPG AXIS

Endocrine function of female reproduction initiates from hypothalamic GnRH neurons, which mainly receipt projections from arcuate, paraventricular, supraoptic and medial preoptic nuclei of hypothalamus. These neurons secrete GnRH in a pulsatile manner that favours the secretion of lutenizing hormone (LH) and follicle stimulating hormone (FSH) from gonadotroph cells in the anterior pituitary (*Jin & Yang, 2014*). APLN and its receptor are intensively detected in the same nucleic group of the hypothalamus (*Pope et al., 2012*), indicating an essential behavior of them in reproductive regulation. It was reported in both intracerebroventricular and intraperitoneal infusion that APLN-13 suppressed the secretion of FSH and LH in frontal hypophysis in rats, but it cannot cause a disturbance at the GnRH level (*Taheri et al., 2002*; *Tekin et al., 2017*). The structural and functional similarities between APLN and GnRH (*Cho et al., 2007*) were reasonably suspected that APLN could be a competitive inhibitor in the adenohypophysis for GnRH receptors. In addition, the central action of APELA in hypothalamus was also demonstrated that

it exerted as an anorexigenic hormone via binding to APLNR and activating arginine vasopressin and corticotropin releasing hormone neurons in the paraventricular nuclei (*Santoso et al., 2015*). Whether it has an effect on reproductive-dependent hormone or not remains to be illuminated.

ROLES OF APLN AND APELA IN UTERINE APPENDAGES

Distribution and role of APLN in the ovarian follicle

Under the normal physiological states, APLN has been identified as a steroidogenic regulator in ovaries of various species including bovine, rat, porcine, sheep and human (*Roche et al., 2016*; *Roche et al., 2017*; *Shuang et al., 2016*; *Rak et al., 2017*; *Mercati et al., 2019*). In cultured bovine follicles, it was firstly reported that *APLN* mRNA was not found in granulosa cells (GCs), whereas *APLNR* mRNA was detected and significantly stimulated by estradiol and progesterone in GCs of estrogen-inactive follicles. In interstitial theca cells, both *APLN* and its receptor mRNA were obviously expressed (*Shimizu et al., 2009*). Two years later, another group cultured bovine ovarian follicles at the similar condition, and found that estradiol over 5 ng/ml (evaluation for follicular maturation) stimulated the expression of APLN and APLNR in theca cells. However, it had no significant effect on the expression of APLN and APLNR in GCs (*Schilffarth et al., 2009*). Recent research confirmed that the expression level of APLN and APLNR were up-regulated in both GCs and oocytes, but remained constant in theca cells (*Schilffarth et al., 2009*; *Shimizu et al., 2009*; *Roche et al., 2017*). In vitro, APLN from GCs of inactive follicles, in response to IGF1 but not to FSH, markedly increased the progesterone production (*Roche et al., 2017*). In porcine follicles, elevated APLN and APLNR were detected following the follicular growth. In turn, APLN significantly increased the secretion of basal steroid hormone (progesterone and estradiol) through the activation of steroidogenic enzyme (3βHSD and CYP19A1) via AMPKα stimulation, whereas it also decreased the IGF1- and FSH-induced steroid secretion (*Rak et al., 2017*).

Role of APLN in corpus luteum (CL)

As a potent angiogenic factor, apelinergic axis also plays a role in the transient luteal stage after ovulation. It has been mentioned that this system exclusively exists in the bovine smooth muscle of intraluteal arterioles, with ligands elevated from early to late CL and followed by a significant decrease at regressed CL, while receptors increased from early to mid CL and remained constant till regressed CL (*Shirasuna et al., 2008*). Paradoxically, another study showed that APLNR also decreased significantly after mid CL (*Schilffarth et al., 2009*). Luteolytic factor prostaglandin F2α stimulates APLN and APLNR mRNA expression particularly at the periphery of mid CL (*Shirasuna et al., 2008*). In ewes, both APLN and APLNR proteins were observed in large luteal cells, and the highest level of APLN mRNA was detected in the luteal phase of the ovarian cycle compared to ewes in the anestrous one (*Mercati et al., 2019*). In porcine cultured CL, APLN stimulates 3βHSD activity, which converts inert 5-ene-3 βHSD to the active 4-ene-3-oxo steroid, therefore it has a pivotal role in progesterone biosynthesis, suggesting an auto/paracrine pattern of the APLN/APLNR system in the ovary (*Rózycka et al., 2018*). In human, this system is found

in the whole ovary through different developmental stages, including luteinized human GCs, theca, oocytes and corona cumulus complex. In cultured luteinized human GCs and follicular fluid, IGF1 exclusively stimulates APLNR expression whereas LH and FSH cannot show the same effect. Conversely, recombinant human APLN-13 and -17 stimulates the secretion of both basal and IGF-induced progesterone and estradiol in a dose-dependent manner, and this process is significantly accelerated in response to IGF1 (*Roche et al., 2016*). This hormone regulation is in agreement to those discovered in bovine that demonstrated APLN could stimulate steroidogenesis and it is speculatively implemented via 3β HSD activation and Akt and MAPK3/1 signaling (*Roche et al., 2016*).

Regulation of APLN in PCOS

PCOS is a common gynecological endocrinopathy characterized by over-expressed LH triggered hyperandrogenism, chronic oligo/anovulation and polycystic ovaries morphology, with clinical manifestations described as "hirsutism, acne, irregular menstruation and subfertility" (*Teede, Deeks & Moran, 2010*; *Teede et al., 2018*). Despite of the positive correlation between PCOS and complications such as visceral obesity, insulin resistance and type 2 diabetes (*Farrell & Antoni, 2010*), the definite aetiology of PCOS at the molecular level still need to be elucidated. It is known that adipokines are bridges to link the energy metabolism and reproductive system, thus they are probably implicated in this process. Hypothetically, APLN controls several aspects of ovarian function in PCOS, underpinned by its role in steroid hormone regulation and insulin resistance. Firstly, the concentration of APLN and its receptors were detected to be significantly increased in PCOS patients with a positive correlation between follicle count and APLN levels (*Bongrani et al., 2019*). This process could be explained by a steroid hormone disturbance effect of APLN in HPG axis. Moreover, as mentioned above, the secretion of APLN in atretic follicles is notably increased in response to IGF1 and insulin, and subsequently stimulates steroidogenesis in GCs (*Boucher et al., 2005*; *Roche et al., 2016*). It indicates a possible implication of insulin in APLN synthesis via activating PI3K/Akt and MAPK3/1 signaling pathways (*Boucher et al., 2005*). Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Body Mass Index (BMI) as hall markers of PCOS have been confirmed to be associated with adipocytokines, even if there is still an inconsistency among different researches. In normal cases, studies have revealed either positive or negative correlations of APLN with HOMA-IR and BMI (*Cekmez et al., 2011*; *Olszanecka-Glinianowicz et al., 2015*). In PCOS cases, several groups have shown an enhanced level of serum APLN positively correlated with HOMA-IR and BMI (*Sun et al., 2015*; *Roche et al., 2016*; *Bongrani et al., 2019*), while one research reported a decreased serum APLN level which was positively associated with HOMA-IR and BMI (*Altinkaya et al., 2014*). These discrepant findings among published literature may be attributed to the differences in research design, different stages of PCOS, sample size, genetic characteristics of patients and APLN evaluation methodology.

Roles of APLN and APELA in ovarian cancer

Previous studies demonstrated that the level of APLN expression was significantly increased in ovarian cancer cells. In MCF-7 cells, the APLN-APLNR system was involved in regulating

the proliferation and metastasis via phosphorylating ERK1/2 pathway (*Peng et al., 2015*). Secretion and expression of APLN as a mitogenic factor was also detected in OVCAR3 cell line which regulates the proliferation progress in a dose-dependent manner (*Hoffmann, Fiedor & Ptak, 2017*). In SKOV3 cell line, over-expressed APLN and its receptor reduced the sensitivity of anti-angiogenic therapeutic regimen (*Masoumi et al., 2020*). Recently, an elevated APELA level was documented in various histotypes of ovarian cancers, especially in ovarian clear cell carcinoma (OCCC) (*Yi et al., 2017*). It is speculated that APELA was involved in multiple pathways in tumorigenesis. For instances, it accelerates cell mitosis and migration through activating ERK and PI3K/AKT cascades (*Ho et al., 2015*). In addition, it was also reported that APELA might negatively regulate p53 in OCCC cell lines, causing non-apoptotic cell growth through an APLNR-independent pathway (*Yi et al., 2017*). However, another study showed that increased APLNR expression was significantly correlated with decreased median overall survival by 14.7 months in patients with high-grade serous ovarian cancer, and APLNR expression was both necessary and sufficient to increase prometastatic phenotypes of ovarian cancer cells including the proliferation, cell adhesion, migration and invasion in vitro (*Neelakantan et al., 2019*).

Distribution of APLN and APLNR in the oviduct

The expression of apelinergic system in the ovary has been widely discussed. However, currently only one study mentioned its expression in the sheep oviduct. This study showed that APLN was detected in the epithelial cell coat of ampullary ciliated cells, which facilitated the transport of oocytes and spermatozoa through the oviductal tract. APLNR was expressed exclusively in the ampullary secretory cells, suggesting the fertilization and implantation roles of this system during the luteal stage (*Mercati et al., 2019*). In ewe oviduct, the mRNA level of both APLN and APLNR were detected higher in estrus when compared with those in anestrus. As the function of oviduct is to provide place for embryogenesis and transport of early embryo, the disruption of normal oviduct function may cause infertility, which is considering as a serious concern recently, and attracting more expected studies (*Mercati et al., 2019*).

ROLE OF APLN IN UTERUS

Distribution and function of APLN and APLNR in uterus

Recently, it has been witnessed that APLN and its receptor also display potential behaviors in uterus among species such as rat, mouse, ewe and human. The expression of APLNR mRNA in uterus was firstly detected through a nonspecific rat tissue RT-PCR screen (*Hosoya et al., 2000*), then its ligand APLN was described to be elevated during the secretory phase in the glandular cells of endometrial layer whereas it remained at a low level in the stromal cells (*Kawamata et al., 2001*; *Ozkan et al., 2013*; *Mercati et al., 2019*). It is evidently deduced that the apelinergic system is stimulated by elevated steroid hormones during the uterine secretory phase also known as the luteal phase of ovarian cycle. APLN subsequently plays a spatio-temporal role in spiral arterioles maturation and interstitial edema in endometrium where angiogenesis is taking place. An in vitro study showed that APLN played a vasodilation role in suppressing both spontaneous and oxytocin-induced

contraction in human myometrial fibers (*Hehir & Morrison, 2012*). However, serum APLN was also reported to exert a positive inotropic effect in rat myometrial layer via PKC-mediated intracellular Ca $^{2+}$ amplication (*Kacar et al., 2018*). These opposite results may be explained by the intracellular balance between vascular dilation and smooth muscle contraction mechanisms of apelinergic system, as well as the impacts of species diversity and reagent concentrations.

Role of APLN in endometriosis

Endometriosis is defined as an estrogen-dependent invasion of endometrial tissue from uterus to uterine adnexa (*Bulun et al., 2019*). It is a very common cause for chronic pain in the pelvis and could even lead to infertility in moderate and significant patients (Chaljub, Medlock & Services, 2018). Current explanations of endometriosis pathogenesis are endometrial implantation, coelomic metaplasia and induction theories which are all in agreement with the impacts of steroid hormone dysregulation and inflammatory response. Similar expression pattern of APLN was seen in both eutopic and ectopic endometrium during the menstrual cycle indicated that the ectopic endometrial lesion could share some characteristics with eutopic cellular processes in endometrium regeneration (*Mercati et al., 2019*). Additionally, the angiogenesis and vasodilation effects of APLN could potentially be one of the causes in triggering the symptoms of endometriosis, whereas more studies are expected to confirm this point.

ROLES OF APLN AND APELA DURING PREGNANCY

Role of APLN and APELA in embryonic development

APLNR was reported to be expressed in the angioblast of frog embryo, which would contribute to the formation of aortic arch vessels and posterior cardinal veins. APLN was detected either within or adjacent to the endothelial cells expressed by APLNR, functioning as an angiogenic agent for nascent blood vessels, especially the intersegmental vessels formation. It also showed the chemotactic ability of APLN to induce the migration of endothelial cells (*Cox et al., 2006*). Moreover, APLN was proved possessing an anti-apoptotic role in osteoblastic cell line of humans and mice (*Tang et al., 2007*; *Xie et al., 2007*). It releases Bcl-2 molecule from Bad via activating PI3K/Akt pathway, which subsequently attenuates the activation of downstream apoptotic factors, such as cytochrome c and caspase-3, resulting in the inhibition of osteoblastic cell apoptosis.

Additionally, APELA has also been revealed to hold a key role in cardiogenesis, angiogenesis and bone formation during the embryonic development. In APELA knockout mice, the hearts are developed poorly or not developed at all, suggesting the essential role in heart morphogenesis (*Chng et al., 2013*). It triggers the endothelial precursor (angioblasts) to migrate towards midline and coalesce underneath the notochord, and form the first axial vessels (*Pauli et al., 2014*; *Helker et al., 2015*). Consistently, APELA-APLNR axis is involved in early placental development and angiogenesis (*Ho et al., 2015*; *Ho et al., 2017*). In mouse placenta, APELA is robustly expressed in syncytiotrophoblasts from early-to-mid gestation, which favors the sprout of new formed blood vessels (*Ho et al., 2017*). It is also associated with skeletal formation through inhibiting the expression of

Sox32, which can bind to Pou5f3 and Nanog molecules as a transcription factor in dorsal endoderm during gastrulation, and inhibit the formation of Pou5f3-Nanog complex. APELA-APLNR pathway can reduce Sox32 expression and allow Pou5f3-Nanog complexes formation, subsequently activating bone morphogenetic protein signaling for sclerotome fate determination (*Perez-Camps et al., 2016*).

Distribution and function of APLN and APELA in the placenta

Apelinergic system has been hypothesized as a key factor in placental angiogenesis. APLN was strongly expressed in the cytoplasm of human cytotrophoblasts during the first two trimester of pregnancy, and then decreased at the third trimester. Subtle signals were also detected in the syncytiotrophoblasts during the first trimester, but it disappeared completely in the third trimester (*Cobellis et al., 2007*). The expression of APLNR in the placenta was later than that of APLN. In the first trimester, it was relatively low and exclusively in the cytotrophoblasts. However, in the third trimester, APLNR was expressed intensely not only in cytotrophoblasts but also in syncytiotrophoblasts, smooth muscle cells and endothelial cells inside of the placental villi (*Cobellis et al., 2007*). This change suggests a potential chemoarractant and vasculogenic role of APLN in the invasion process of interstitial and endovascular extravillous trophoblasts. In mouse, APELA was detected initially in the trophoblasts and then increased robustly after the allantoic fusion. At the mid-gestation, it was expressed restrictedly in syncytiotrophoblasts, where APLNR was wildly existed in adjacent endothelial cells, indicating a paracrine function of this system to favor the placental angiogenic sprouting (*Ho et al., 2017*). However, in human placenta, APELA was expressed in both cytotrophoblasts and syncytiotrophoblasts synchronously during the whole pregnancy (*Ho et al., 2017*) and its speculated role remains to be illuminated.

Regulation of APELA and APLN in PE

The basic pathological changes of hypertensive disorders in pregnancy are currently recognized as insufficient spiral arteries recasting and inflammation mediated endothelial damage triggered by the intricate network of signaling cascades. APELA as mentioned above plays a crucial role in placental angiogenesis via activating PI3K/AKT/mTOR pathway (*Ho et al., 2017*), and whether its reduction could lead to PE is now being widely studied. It was firstly discovered that APELA knockout pregnant mice exhibited a hypertensive symptom accompanied with proteinuria and glomerular endotheliosis, which were manifested as preeclampsia-like symptoms (*Ho et al., 2017*). Scientists therefore started investigating the change of APELA in PE patients and wanted to know whether the APELA could act as a biomarker (*Zhou et al., 2019*). In the late-onset PE (LOPE), two studies measured a significant increased concentration of APELA in the placenta and serum (*Panaitescu et al., 2020*; *Para et al., 2020*), while one study measured significant decrease (*Zhou et al., 2019*). And for early-onset PE (EOPE), only one study observed decrease in both APELA mRNA and protein (*Wang et al., 2019*), while other studies report no significant change of APELA level on either protein or mRNA (*Pritchard et al., 2018*; *Villie et al., 2019*; *Zhou et al., 2019*; *Panaitescu et al., 2020*; *Para et al., 2020*). Furthermore, it was found that hypoxia significantly decreased the expression of LIN28B, LIN28A and APELA, and the

downregulation of LIN28B and APELA may play a role in PE by reducing trophoblast invasion and syncytialization (*Canfield et al., 2019*).

There are also contradictions about the expression level of APLN in PE patients. Initially, a clinical study found an increased APLN protein level in the placental samples of PE patients, indicating a speculated correlation between APLN and PE (*Cobellis et al., 2007*). This study was further proved by an experiment which showed intravenous injection of APLN in male mice could lead to the downregulation of blood pressure, suggesting that APLN might act as a vasodilator in PE (*Lee et al., 2000*). However, case studies also found either decreased (*Inuzuka et al., 2013*; *Yamaleyeva et al., 2015*; *Sattar Taha, Zahraei & Al-Hakeim, 2020*) or no significantly changed (*Van Mieghem et al., 2016*) APLN level in PE patients compared with normotensive pregnancies.

Not only its ligands, the expression of APLNR is also rather conflicting. It has long been thought that APLNR level increases when the patient suffer from PE (*Cobellis et al., 2007*), but two other studies suggest APLNR level remains unchanged when PE occurs (*Yamaleyeva et al., 2015*; *Pritchard et al., 2018*). However, one recent study found a significant decrease both in APLNR mRNA expression and in situ expression between PE patients and normal control, and this significance can be found when controls compared to both EOPE and LOPE groups (*Zhou et al., 2019*).

Altogether, the different expression of apelinergic system could be explained by confounding factors like BMI and mean maternal age mismatches between the cases and controls. Moreover, the balance between vasorelaxant and myocardial contractile effects of apelinergic system, as well as the crosslink of apelinergic axis with intricate inflammatory and endothelial factors in PE should also be taken into consideration. Further investigations should focus on the specific molecular mechanisms of APLN and APELA in the hypertensive disorders of pregnancy.

Regulation of APLN and APELA in GDM

APLN as one of the adipose tissue-derived hormones has been identified to play a role in blood glucose metabolism (*Antushevich & Wójcik, 2018*). It has been described that insulin may up-regulate the expression of APLN through PKC and PI3K signaling pathways in both murine and human adipocytes (*Boucher et al., 2005*). Raised apelin levels were found in both insulin-resistant mice and type 2 diabetes mellitus patients (*Xu, Tsao & Yue, 2011*), which supported the speculation that insulin can stimulate APLN secretion. Nevertheless, the correlation of APLN levels with GDM has not yet reached an agreement in clinical researches. Three studies reported a decrease of serum APLN level in GDM patients (*Aydin, 2010*; *Boyadzhieva et al., 2013*; *Akinci et al., 2014*) while two other groups revealed an increase (*Aslan et al., 2012*; *Kiyak Caglayan et al., 2016*). In contrast, there were also no significant association reports between normal control and GDM patients (*Telejko et al., 2010*; *Oncul et al., 2013*). Thus, the correlation of APLN with the pathophysiology of GDM remains to be elucidated. In addition, factors like BMI, HOMA-IR and birth weight have been shown not correlated with serum APLN level according to these studies (*Aslan et al., 2012*; *Oncul et al., 2013*), but these confounding factors varied a lot among different groups during pregnancy, which was probably one of the cases in the controversy. APELA

had a positive correlation with fasting plasma glucose levels in healthy pregnant women during the second trimester, while decreased APELA circulating level was observed in GDM patients at the same time. In the third trimester, circulating APELA level decreased significantly in both GDM and healthy groups. This study suggested that APELA could be a physiological demand in glucose metabolism, and further contributions should focus on dynamic levels monitoring and mechanism analysis (*Guo et al., 2020*).

CONCLUSION

This review presents a landscape of the novel APLN/APELA-APLNR system in the female reproductive field (Table 1 and Fig. 1). Intricate signaling pathways and crosslinks of APLN and APELA imply their multifunctional roles in different organs like ovary, uterus and placenta, during specific developmental stages. APLN as an adipokine appears to have specific effects in steriodogenesis and metabolic regulation in GCs and CL of the ovary. Insulin and IGF1-induced APLN secretion possibly plays a role in glucose regulation in GDM patients. In addition, APLN may sustain a balance between the vasodilative and myocontractile effects in the uterus which could be correlated with hypertensive disorders during the pregnancy. Similarly, APELA as a novel ligand of APLNR also has a potential role in PE, based on the angiogenic effect of spiral arterioles. APELA is essential for fetal and placental development through stimulating the invasion of extravillous trophoblasts. This process is potentially achieved through a chemo-attractant mechanism in placental angiogenic sprouting. Moreover, there is a hyperplasia effect of APELA which could be one of the causes in ovarian tumorigenesis.

All the data suggest that there should be additional studies to further investigate the precise roles of this axis in female reproductive system especially at the pathological profile. In the future, it will be important to clarify the crosslink and interaction between APLN and other adipokines in sex hormone regulation and energy metabolism. Specific expression and biological effects of APELA in ovary and uterus are also needed in prospect. It may also be crucial to identify the balance of smooth muscle contraction and vasodilation in apelinergic system at a molecular hierarchy. Collectively, the apelinergic axis is still a novel project for further investigation in both physiological and pathological aspects, and probably brings better therapeutic or prophylactic intervention towards female reproductive disorders.

List of Abbreviations

AC	adenylate cyclase
APELA	apelin receptor early endogenous ligand
APLN	apelin
APLNR	apelin receptor
Bad	Bcl-2-associated death promoter
BMI	body mass index
Ca^{2+}	calcium
cAMP	cyclic adenosine monophosphate
CL	corpus luteum
eNOS	endothelial nitric oxide synthase

EOPE	early-onset preeclampsia
FSH	follicle stimulating hormone
GDM	gestational diabetes mellitus
GnRH	gonadotropin releasing hormone
GPR25	G protein-coupled receptor 25
GC	granulosa cell
hESC	human embryonic stem cell
hnRNPL	heterogeneous nuclear ribonucleoprotein L
HOMA-IR	homeostatic model assessment for insulin resistance
HPG axis	hypothalamo-pituitary-gonadal axis
IGF	insulin-like growth factor
LH	lutenizing hormone
LOPE	late-onset preeclampsia
NO	nitric oxide
OCCC	ovarian clear cell carcinoma
PCOS	polycystic ovary syndrome
PE	preeclampsia
PI3K/Akt	phosphoinositide 3-kinase/protein kinase B
PKA	protein kinase A
PKC	protein kinase C
PLC-β	phospholipase C-β
ROS	reactive oxygen species.

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Xueying Wang and Xiaofei Liu conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Zifan Song, Xin Shen and Siying Lu performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Yan Ling and Haibin Kuang conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

This is a literature review without any raw data.

REFERENCES

Akinci B, Celtik A, Tunali S, Genc S, Yuksel F, Secil M, Ozcan MA, Bayraktar F. 2014. Circulating apelin levels are associated with cardiometabolic risk factors in women with previous gestational diabetes. *Archives of Gynecology and Obstetrics* **289**:787–793 DOI 10.1007/s00404-013-3070-y.

Altinkaya SO, Nergiz S, Kucuk M, Yuksel H. 2014. Apelin levels in relation with hormonal and metabolic profile in patients with polycystic ovary syndrome. *European Journal of Obstetrics and Gynecology and Reproductive Biology* **176**:168–172 DOI 10.1016/j.ejogrb.2014.06.004.

Antushevich H, Wójcik M. 2018. Review: apelin in disease. *Clinica Chimica Acta* **483**:241–248 DOI 10.1016/j.cca.2018.05.012.

Aslan M, Celik O, Celik N, Turkcuoglu I, Yilmaz E, Karaer A, Simsek Y, Celik E, Aydin S. 2012. Cord blood nesfatin-1 and apelin-36 levels in gestational diabetes mellitus. *Endocrine* **41**:424–429 DOI 10.1007/s12020-011-9577-8.

Aydin S. 2010. The presence of the peptides apelin, ghrelin and nesfatin-1 in the human breast milk, and the lowering of their levels in patients with gestational diabetes mellitus. *Peptides* **31**:2236–2240 DOI 10.1016/j.peptides.2010.08.021.

Bertrand C, Valet P, Castan-Laurell I. 2015. Apelin and energy metabolism. *Frontiers in Physiology* **6**:Article 115 DOI 10.3389/fphys.2015.00115.

Bongrani A, Mellouk N, Rame C, Cornuau M, Guérif F, Froment P, Dupont J. 2019. Ovarian expression of adipokines in polycystic ovary syndrome: a role for chemerin, omentin, and apelin in follicular growth arrest and ovulatory dysfunction? *International Journal of Molecular Sciences* **20**:1–22 DOI 10.3390/ijms20153778.

Boucher J, Masri B, Daviaud D, Gesta S, Guigné C, Mazzucotelli A, Castan-Laurell I, Tack I, Knibiehler B, Carpéné C, Audigier Y, Saulnier-Blache JS, Valet . 2005. Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology* **146**:1764–1771 DOI 10.1210/en.2004-1427.

Boyadzhieva M, Atanasova I, Zacharieva S, Kedikova S. 2013. Adipocytokines during pregnancy and postpartum in women with gestational diabetes and healthy controls. *Journal of Endocrinological Investigation* **36**:944–949 DOI 10.3275/8968.

Bulun SE, Yilmaz BD, Sison C, Miyazaki K, Bernardi L, Liu S, Kohlmeier A, Yin P, Milad M, Wei J. 2019. Endometriosis. *Endocrine Reviews* **40**:1048–1079 DOI 10.1210/er.2018-00242.

Canfield J, Arlier S, Mong EF, Lockhart J, VanWye J, Guzeloglu-Kayisli O, Schatz F, Magness RR, Lockwood CJ, Tsibris JCM, Kayisli UA, Totary-Jain H. 2019. Decreased LIN28B in preeclampsia impairs human trophoblast differentiation and migration. *FASEB Journal* **33**:2759–2769 DOI 10.1096/fj.201801163R.

Carpéné C, Dray C, Attané C, Valet P, Portillo MP, Churruca I, Milagro FI, Castan-Laurell I. 2007. Expanding role for the apelin/APJ system in physiopathology. *Journal of Physiology and Biochemistry* **63**:359–374 DOI 10.1007/BF03165767.

Cekmez F, Cekmez Y, Ö Pirgon, Canpolat FE, Aydinöz S, Ipcioglu OM, Karademir F. 2011. Evaluation of new adipocytokines and insulin resistance in adolescents with polycystic ovary syndrome. *European Cytokine Network* **22**:32–37 DOI 10.1684/ecn.2011.0279.

Chapman NA, Dupré DJ, Rainey JK. 2014. The apelin receptor: physiology, pathology, cell signalling, and ligand modulation of a peptide-activated class A GPCR. *Biochemistry and Cell Biology* **92**:431–440 DOI 10.1139/bcb-2014-0072.

Chng SC, Ho L, Tian J, Reversade B. 2013. ELABELA: a hormone essential for heart development signals via the apelin receptor. *Developmental Cell* **27**:672–680 DOI 10.1016/j.devcel.2013.11.002.

Cho HJ, Acharjee S, Moon MJ, Oh DY, Vaudry H, Kwon HB, Seong JY. 2007. Molecular evolution of neuropeptide receptors with regard to maintaining high affinity to their authentic ligands. *General and Comparative Endocrinology* **153**:98–107 DOI 10.1016/j.ygcen.2006.12.013.

Cobellis L, De Falco M, Mastrogiacomo A, Giraldi D, Dattilo D, Scaffa C, Colacurci N, De Luca A. 2007. Modulation of apelin and APJ receptor in normal and preeclampsia-complicated placentas. *Histology and Histopathology* **22**:1–8 DOI 10.14670/HH-22.1.

Cox CM, D'Agostino SL, Miller MK, Heimark RL, Krieg PA. 2006. Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. *Developmental Biology* **296**:177–189 DOI 10.1016/j.ydbio.2006.04.452.

Dai T, Ramirez-Correa G, Gao WD. 2006. Apelin increases contractility in failing cardiac muscle. *European Journal of Pharmacology* **553**:222–228 DOI 10.1016/j.ejphar.2006.09.034.

El Messari S, Iturrioz X, Fassot C, De Mota N, Roesch D, Llorens-Cortes C. 2004. Functional dissociation of apelin receptor signaling and endocytosis: implications for the effects of apelin on arterial blood pressure. *Journal of Neurochemistry* **90**:1290–1301 DOI 10.1111/j.1471-4159.2004.02591.x.

Fagerberg L, Hallstrom BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, Asplund A, Sjostedt E, Lundberg E, Szigyarto CAK, Skogs M, Ottosson Takanen J, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, Von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F, Uhlen M. 2014. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Molecular and Cellular Proteomics* **13**:397–406 DOI 10.1074/mcp.M113.035600.

Farrell K, Antoni MH. 2010. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. *Fertility and Sterility* **94**:1565–1574 DOI 10.1016/j.fertnstert.2010.03.081.

Guo YY, Li T, Liu H, Tang L, Li YC, Hu HT, Su YF, Lin Y, Wang YY, Li C, Huang HF, Jin L, Liu XM. 2020. Circulating levels of Elabela and Apelin in the second and third trimesters of pregnancies with gestational diabetes mellitus. *Gynecological Endocrinology* **36**:890–894 DOI 10.1080/09513590.2020.1739264.

Hehir MP, Morrison JJ. 2012. The adipokine apelin and human uterine contractility. *American Journal of Obstetrics and Gynecology* **206**:359.e1–359.e5 DOI 10.1016/j.ajog.2012.01.032.

Helker CSM, Schuermann A, Pollmann C, Chng SC, Kiefer F, Reversade B, Herzog W. 2015. The hormonal peptide Elabela guides angiob lasts to the midline during vasculogenesis. *eLife* **4**:1–13 DOI 10.7554/eLife.06726.

Ho L, Dijk M Van, Tan S, Chye J, Messerschmidt DM, Chng SC, Ong S, Yi LK, Boussata S, Goh GH, Afink GB, Lim CY, Dunn NR, Solter D, Knowles BB, Reversade B. 2017. ELABELA deficiency promotes preeclampsia and cardiovascular malformations in mice. *Science* **357**:707–713 DOI 10.1126/science.aam6607.

Ho L, Tan SJCSYX, Wee S, Wu Y, Tan SJCSYX, Ramakrishna NB, Chng SC, Nama S, Szczerbinska I, Chan YS, Avery S, Tsuneyoshi N, Ng HH, Gunaratne J, Dunn NR, Reversade B. 2015. ELABELA is an endogenous growth factor that sustains hESC Self-Renewal via the PI3K/AKT pathway. *Cell Stem Cell* **17**:435–447 DOI 10.1016/j.stem.2015.08.010.

Hoffmann M, Fiedor E, Ptak A. 2017. Bisphenol A and its derivatives tetrabromobisphenol A and tetrachlorobisphenol A induce apelin expression and secretion in ovarian cancer cells through a peroxisome proliferator-activated receptor gamma-dependent mechanism. *Toxicology Letters* **269**:15–22 DOI 10.1016/j.toxlet.2017.01.006.

Hosoya M, Kawamata Y, Fukusumi S, Fujii R, Habata Y, Hinumat S, Kitada C, Honda S, Kurokawa T, Onda H, Nishimura O, Fujino M. 2000. Molecular and functional characteristics of APJ: tissue distribution of mRNA and interaction with the endogenous ligand apelin. *Journal of Biological Chemistry* **275**:21061–21067 DOI 10.1074/jbc.M908417199.

Huang SK, Shin K, Sarker M, Rainey JK. 2017. Apela exhibits isoform- and headgroup-dependent modulation of micelle binding, peptide conformation and dynamics. *Biochimica et Biophysica Acta—Biomembranes* **1859**:767–778 DOI 10.1016/j.bbamem.2017.01.028.

Inuzuka H, Nishizawa H, Inagaki A, Suzuki M, Ota S, Miyamura H, Miyazaki J, Sekiya T, Kurahashi H, Udagawa Y. 2013. Decreased expression of apelin in placentas from severe pre-eclampsia patients. *Hypertension in Pregnancy* **32**:410–421 DOI 10.3109/10641955.2013.813535.

Jin JM, Yang WX. 2014. Molecular regulation of hypothalamus-pituitary-gonads axis in males. *Gene* **551**:15–25 DOI 10.1016/j.gene.2014.08.048.

Kacar E, Ercan Z, Serhatlioglu I, Sumer A, Kelestimur H, Kutlu S. 2018. The effects of apelin on myometrium contractions in pregnant rats. *Cellular and Molecular Biology* **64**:74–79 DOI 10.14715/cmb/2018.64.11.13.

Kasai A, Shintani N, Kato H, Matsuda S, Gomi F, Haba R, Hashimoto H, Kakuda M, Tano Y, Baba A. 2008. Retardation of retinal vascular development in apelin-deficient mice. *Arteriosclerosis, Thrombosis, and Vascular Biology* **28**:1717–1722 DOI 10.1161/ATVBAHA.108.163402.

Kawamata Y, Habata Y, Fukusumi S, Hosoya M, Fujii R, Hinuma S, Nishizawa N, Kitada C, Onda H, Nishimura O, Fujino M. 2001. Molecular properties of apelin: Tissue distribution and receptor binding. *Biochimica et Biophysica Acta- Molecular Cell Research* **1538**:162–171 DOI 10.1016/S0167-4889(00)00143-9.

Kiyak Caglayan E, Engin-Ustun Y, Sari N, Gocmen AY, Polat MF. 2016. The effects of prolonged fasting on the levels of adiponectin, leptin, apelin, and omentin in pregnant women. *Journal of Obstetrics and Gynaecology* **36**:555–558 DOI 10.3109/01443615.2015.1103716.

Krist J, Wieder K, Klöting N, Oberbach A, Kralisch S, Wiesner T, Schön MR, Gärtner D, Dietrich A, Shang E, Lohmann T, Dreßler M, Fasshauer M, Stumvoll M, Blüher M. 2013. Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity. *Obesity Facts* **6**:57–69 DOI 10.1159/000348667.

Lee DL, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, Osmond DH, George SR, O'Dowd BF. 2000. Characterization of apelin, the ligand for the APJ receptor. *Journal of Neurochemistry* **74**:34–41 DOI 10.1046/j.1471-4159.2000.0740034.x.

Li M, Gou H, Tripathi BK, Huang J, Jiang S, Dubois W, Waybright T, Lei M, Shi J, Zhou M. 2015. An Apela RNA-containing negative feedback loop regulates p53-mediated apoptosis in embryonic stem cells. *Cell Stem Cell* **16**:669–683 DOI 10.1016/j.stem.2015.04.002.

Liu C, Su T, Li F, Li L, Qin X, Pan W, Feng F, Chen F, Liao D, Chen L. 2010. PI3K/Akt signaling transduction pathway is involved in rat vascular smooth muscle cell proliferation induced by apelin-13. *Acta Biochimica et Biophysica Sinica* **42**:396–402 DOI 10.1093/abbs/gmq035.

Liu Y, Zhang T, Wang Y, Wu P, Li Y, Wang C, Xu S, Shi H. 2019. Apelin-13 attenuates early brain injury following subarachnoid hemorrhage via suppressing neuronal apoptosis through the GLP-1R/PI3K/Akt signaling. *Biochemical and Biophysical Research Communications* **513**:105–111 DOI 10.1016/j.bbrc.2019.03.151.

Lv SY, Cui B, Chen WD, Wang YD. 2017. Apelin/APJ system: a key therapeutic target for liver disease. *Oncotarget* **8**:112145–112151 DOI 10.18632/oncotarget.22841.

Maguire JJ, Kleinz MJ, Pitkin SL, Davenport AP. 2009. [Pyr1]apelin-13 identified as the predominant apelin isoform in the human heart: vasoactive mechanisms and inotropic action in disease. *Hypertension* **54**:598–604 DOI 10.1161/HYPERTENSIONAHA.109.134619.

Masoumi J, Jafarzadeh A, Khorramdelazad H, Abbasloui M, Abdolalizadeh J, Jamali N. 2020. Role of Apelin/APJ axis in cancer development and progression. *Advances in Medical Sciences* **65**:202–213 DOI 10.1016/j.advms.2020.02.002.

Masri B, Knibiehler B, Audigier Y, Lahlou H, Mazarguil H. 2002. Apelin (65-77) activates extracellular signal-regulated kinases via a PTX-sensitive G protein. *Biochemical and Biophysical Research Communications* **290**:539–545 DOI 10.1006/bbrc.2001.6230.

Mercati F, Scocco P, Maranesi M, Acuti G, Petrucci L, Cocci P, Renzi A, De Felice E, Dall'Aglio C. 2019. Apelin system detection in the reproductive apparatus of ewes grazing on semi-natural pasture. *Theriogenology* **139**:156–166 DOI 10.1016/j.theriogenology.2019.08.012.

Neelakantan D, Dogra S, Devapatla B, Jaiprasart P, Mukashyaka MC, Janknecht R, Dwivedi SKD, Bhattacharya R, Husain S, Ding K, Woo S. 2019. Multifunctional APJ pathway promotes ovarian cancer progression and metastasis. *Molecular Cancer Research* **17**:1378–1390 DOI 10.1158/1541-7786.MCR-18-0989.

Norris ML, Pauli A, Gagnon JA, Lord ND, Rogers KW, Mosimann C, Zon LI, Schier AF. 2017. Toddler signaling regulates mesodermal cell migration downstream of nodal signaling. *eLife* **6**:1–18 DOI 10.7554/eLife.22626.

O'Dowd BF, Heiber M, Chan A, Heng HHQ, Tsui LC, Kennedy JL, Shi X, Petronis A, George SR, Nguyen T. 1993. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* **136**:355–360 DOI 10.1016/0378-1119(93)90495-O.

Olszanecka-Glinianowicz M, Madej P, Owczarek A, Chudek J, Skałba P. 2015. Circulating anti-Müllerian hormone levels in relation to nutritional status and selected adipokines levels in polycystic ovary syndrome. *Clinical Endocrinology* **83**:98–104 DOI 10.1111/cen.12687.

Oncul M, Tuten A, Erman H, Gelisgen R, Benian A, Uzun H. 2013. Maternal and cord blood apelin, resistin and visfatin levels in gestational diabetes mellitus. *Minerva Medica* **104**:527–535 DOI 10.1186/1471-2296-14-145.

Ozkan ZS, Cilgin H, Simsek M, Cobanoglu B, Ilhan N. 2013. Investigation of apelin expression in endometriosis. *Journal of Reproduction and Infertility* **14**:50–55.

Panaitescu B, Romero R, Gomez-Lopez N, Pacora P, Erez O, Vadillo-Ortega F, Yeo L, Hassan SS, Hsu CD. 2020. ELABELA plasma concentrations are increased in women with late-onset preeclampsia. *Journal of Maternal-Fetal and Neonatal Medicine* **33**:5–15 DOI 10.1080/14767058.2018.1484089.

Para R, Romero R, Gomez-Lopez N, Tarca AL, Panaitescu B, Done B, Hsu R, Pacora P, Hsu CD. 2020. Maternal circulating concentrations of soluble Fas and Elabela in early- and late-onset preeclampsia. *Journal of Maternal-Fetal and Neonatal Medicine* **0**:1–14 DOI 10.1080/14767058.2020.1716720.

Pauli A, Norris ML, Valen E, Chew GL, Gagnon JA, Zimmerman S, Mitchell A, Ma J, Dubrulle J, Reyon D, Tsai SQ, Joung JK, Saghatelian A, Schier AF. 2014. Toddler: an embryonic signal that promotes cell movement via apelin receptors. *Science* **343**:Article 1248636 DOI 10.1126/science.1248636.

Peng X, Li F, Wang P, Jia S, Sun L, Huo H. 2015. Apelin-13 induces MCF-7 cell proliferation and invasion via phosphorylation of ERK1/2. *International Journal of Molecular Medicine* **36**:733–738 DOI 10.3892/ijmm.2015.2265.

Perez-Camps M, Tian J, Chng SC, Sem KP, Sudhaharan T, Teh C, Wachsmuth M, Korzh V, Ahmed S, Reversade B. 2016. Quantitative imaging reveals real-time Pou5f3-nanog complexes driving dorsoventral mesendoderm patterning in zebrafish. *eLife* **5**:1–21 DOI 10.7554/eLife.11475.

Perjés Á, Kilpiö T, Ulvila J, Magga J, Alakoski T, Szabó Z, Vainio L, Halmetoja E, Vuolteenaho O, Petäjä-Repo U, Szokodi I, Kerkelä R. 2016. Characterization of apela, a novel endogenous ligand of apelin receptor, in the adult heart. *Basic Research in Cardiology* **111**:1–12 DOI 10.1007/s00395-015-0521-6.

Pope GR, Roberts EM, Lolait SJ, Carroll AM. 2012. Central and peripheral apelin receptor distribution in the mouse: species differences with rat. *Peptides* **33**:139–148 DOI 10.1016/j.peptides.2011.12.005.

Pritchard N, Kaitu'u-Lino TJ, Gong S, Dopierala J, Smith GCS, Charnock-Jones DS, Tong S. 2018. ELABELA/APELA levels are not decreased in the maternal circulation or placenta among women with preeclampsia. *American Journal of Pathology* **188**:1749–1753 DOI 10.1016/j.ajpath.2018.04.008.

Qian J, Lu Q, Tao Y, Jiang YR. 2011. Vitreous and plasma concentrations of apelin and vascular endothelial growth factor after intravitreal bevacizumab in eyes with proliferative diabetic retinopathy. *Retina* **31**:161–168 DOI 10.1097/IAE.0b013e3181e46ad8.

Rak A, Drwal E, Rame C, Knapczyk-Stwora K, Słomczyńska M, Dupont J, Gregoraszczuk EL. 2017. Expression of apelin and apelin receptor (APJ) in porcine ovarian follicles and in vitro effect of apelin on steroidogenesis and proliferation through APJ activation and different signaling pathways. *Theriogenology* **96**:126–135 DOI 10.1016/j.theriogenology.2017.04.014.

Roche J, Rame C, Reverchon M, Mellouk N, Cornuau M, Guerif F, Froment P, Dupont J. 2016. Apelin (APLN) and Apelin Receptor (APLNR) in human ovary: expression, signaling, and regulation of steroidogenesis in primary human luteinized granulosa cells. *Biology of Reproduction* **95**:104–104 DOI 10.1095/biolreprod.116.141754.

Roche J, Ramé C, Reverchon M, Mellouk N, Rak A, Froment P, Dupont J. 2017. Apelin (APLN) regulates progesterone secretion and oocyte maturation in bovine ovarian cells. *Reproduction* **153**:589–603 DOI 10.1530/REP-16-0677.

Rózycka M, Kurowska P, Grzesiak M, Kotula-Balak M, Tworzydło W, Rame C, Gregoraszczuk E, Dupont J, Rak A. 2018. Apelin and apelin receptor at different stages of corpus luteum development and effect of apelin on progesterone secretion and 3β-hydroxysteroid dehydrogenase (3β-HSD) in pigs. *Animal Reproduction Science* **192**:251–260 DOI 10.1016/j.anireprosci.2018.03.021.

Santoso P, Maejima Y, Kumamoto K, Takenoshita S, Shimomura K. 2015. Central action of ELABELA reduces food intake and activates arginine vasopressin and corticotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. *NeuroReport* **26**:820–826 DOI 10.1097/WNR.0000000000000431.

Sattar Taha A, Zahraei Z, Al-Hakeim HK. 2020. Serum apelin and galectin-3 in preeclampsia in Iraq. *Hypertension in Pregnancy* **00**:1–8 DOI 10.1080/10641955.2020.1777300.

Schilffarth S, Antoni B, Schams D, Meyer HHD, Berisha B. 2009. The expression of apelin and its receptor APJ during different physiological stages in the bovine ovary. *International Journal of Biological Sciences* **5**:344–350 DOI 10.7150/ijbs.5.344.

Shimizu T, Kosaka N, Murayama C, Tetsuka M, Miyamoto A. 2009. Apelin and APJ receptor expression in granulosa and theca cells during different stages of follicular development in the bovine ovary: involvement of apoptosis and hormonal regulation. *Animal Reproduction Science* **116**:28–37 DOI 10.1016/j.anireprosci.2009.01.009.

Shirasuna K, Shimizu T, Sayama K, Asahi T, Sasaki M, Berisha B, Schams D, Miyamoto A. 2008. Expression and localization of apelin and its receptor APJ in the bovine corpus luteum during the estrous cycle and prostaglandin F2α-induced luteolysis. *Reproduction* **135**:519–525 DOI 10.1530/REP-07-0409.

Shuang L, Jidong W, Hongjuan P, Zhenwei Y. 2016. Effects of apelin on proliferation and apoptosis in rat ovarian granulosa cells. *Clinical and Experimental Obstetrics and Gynecology* **43**:409–413 DOI 10.12891/ceog2133.2016.

Sun X, Wu X, Zhou Y, Yu X, Zhang W. 2015. Evaluation of apelin and insulin resistance in patients with PCOS and therapeutic effect of drospirenone-ethinylestradiol plus metformin. *Medical Science Monitor* **21**:2547–2552 DOI 10.12659/MSM.894926.

Szokodi I, Tavi P, Földes G, Voutilainen-Myllylä S, Ilves M, Tokola H, Pikkarainen S, Piuhola J, Rysä J, Tóth M, Ruskoaho H. 2002. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circulation Research* **91**:434–440 DOI 10.1161/01.RES.0000033522.37861.69.

Taheri S, Murphy K, Cohen M, Sujkovic E, Kennedy A, Dhillo W, Dakin C, Sajedi A, Ghatei M, Bloom S. 2002. The effects of centrally administered apelin-13 on food intake, water intake and pituitary hormone release in rats. *Biochemical and Biophysical Research Communications* **291**:1208–1212 DOI 10.1006/bbrc.2002.6575.

Tang SY, Xie H, Yuan LQ, Luo XH, Huang J, Cui RR, Zhou H De, Wu XP, Liao EY. 2007. Apelin stimulates proliferation and suppresses apoptosis of mouse osteoblastic cell line MC3T3-E1 via JNK and PI3-K/Akt signaling pathways. *Peptides* **28**:708–718 DOI 10.1016/j.peptides.2006.10.005.

Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H, Fujino M. 1998. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochemical and Biophysical Research Communications* **251**:471–476 DOI 10.1006/bbrc.1998.9489.

Teede H, Deeks A, Moran L. 2010. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Medicine* **8**:1–10 DOI 10.1186/1741-7015-8-41.

Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Cert Pub Health G, Piltonen T, Norman RJ. 2018. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome on behalf of the International PCOS. *Fertility and Sterility* **110**:364–379 DOI 10.1016/j.fertnstert.2018.05.004.

Tekin S, Erden Y, Sandal S, Etem Onalan E, Ozyalin F, Ozen H, Yilmaz B. 2017. Effects of apelin on reproductive functions: relationship with feeding behavior and energy metabolism. *Archives of Physiology and Biochemistry* **123**:9–15 DOI 10.1080/13813455.2016.1211709.

Telejko B, Kuzmicki M, Wawrusiewicz-Kurylonek N, Szamatowicz J, Nikolajuk A, Zonenberg A, Zwierz-Gugala D, Jelski W, Laudański P, Wilczynski J, Kretowski A, Gorska M. 2010. Plasma apelin levels and apelin/APJ mRNA expression in patients with gestational diabetes mellitus. *Diabetes Research and Clinical Practice* **87**:176–183 DOI 10.1016/j.diabres.2009.10.018.

Than A, Zhang X, Leow MKS, Poh CL, Chong SK, Chen P. 2014. Apelin attenuates Oxidative stress in human Adipocytes. *Journal of Biological Chemistry* **289**:3763–3774 DOI 10.1074/jbc.M113.526210.

Ulitsky I, Shkumatava A, Jan CH, Sive H, Bartel DP. 2011. Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. *Cell* **147**:1537–1550 DOI 10.1016/j.cell.2011.11.055.

Van Mieghem T, Doherty A, Baczyk D, Drewlo S, Baud D, Carvalho J, Kingdom J. 2016. Apelin in normal pregnancy and pregnancies complicated by placental insufficiency. *Reproductive Sciences* **23**:1037–1043 DOI 10.1177/1933719116630422.

Villie P, Lefevre G, Arrestier R, Rousseau A, Berkane N, Hertig A. 2019. ELA-BELA concentration is not decreased in maternal plasma before the onset of preeclampsia. *American Journal of Obstetrics and Gynecology* **220**:284–285 DOI 10.1016/j.ajog.2018.12.020.

Wang Z, Yu D, Wang M, Wang Q, Kouznetsova J, Yang R, Qian K, Wu W, Shuldiner A, Sztalryd C, Zou M, Zheng W, Gong DW. 2015. Elabela-apelin receptor signaling pathway is functional in mammalian systems. *Scientific Reports* **5**:1–8 DOI 10.1038/srep08170.

Wang L, Zhang Y, Qu H, Xu F, Hu H, Zhang Q, Ye Y. 2019. Reduced ELABELA expression attenuates trophoblast invasion through the PI3K/AKT/mTOR pathway in early onset preeclampsia. *Placenta* **87**:38–45 DOI 10.1016/j.placenta.2019.08.077.

Xie H, Yuan LQ, Luo XH, Huang J, Cui RR, Guo LJ, Zhou H De, Wu XP, Liao EY. 2007. Apelin suppresses apoptosis of human osteoblasts. *Apoptosis* **12**:247–254 DOI 10.1007/s10495-006-0489-7.

Xu S, Tsao PS, Yue P. 2011. Apelin and insulin resistance: another arrow for the quiver? *Journal of Diabetes* **3**:225–231 DOI 10.1111/j.1753-0407.2011.00132.x.

Yamaleyeva LM, Chappell MC, Bridget Brosnihan K, Anton L, Caudell DL, Shi S, McGee C, Pirro N, Gallagher PE, Taylor RN, Merrill DC, Mertz HL. 2015. Downregulation of apelin in the human placental chorionic villi from preeclamptic pregnancies. *American Journal of Physiology—Endocrinology and Metabolism* **309**:852–860 DOI 10.1152/ajpendo.00272.2015.

Yang N, Li T, Cheng J, Tuo Q, Shen J. 2019. Role of apelin/APJ system in hypothalamic-pituitary axis. *Clinica Chimica Acta* **499**:149–153 DOI 10.1016/j.cca.2019.09.011.

Yang X, Zhu W, Zhang P, Chen K, Zhao L, Li J, Wei M, Liu M. 2014. Apelin-13 stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in myocardial microvascular endothelial cells. *Molecular Medicine Reports* **9**:1590–1596 DOI 10.3892/mmr.2014.1984.

Yi Y, Tsai SH, Cheng JC, Wang EY, Anglesio MS, Cochrane DR, Fuller M, Gibb EA, Wei W, Huntsman DG, Karsan A, Hoodless PA. 2017. APELA promotes tumour growth and cell migration in ovarian cancer in a p53-dependent manner. *Gynecologic Oncology* **147**:663–671 DOI 10.1016/j.ygyno.2017.10.016.

Yue F, Cheng Y, Breschi A, Vierstra J, Wu W, Ryba T, Sandstrom R, Ma Z, Davis C, Pope BD, Shen Y, Pervouchine DD, Djebali S, Thurman RE, Kaul R, Rynes E, Kirilusha A, Marinov GK, Williams BA, Trout D, Amrhein H, Fisher-Aylor K, Antoshechkin I, DeSalvo G, See LH, Fastuca M, Drenkow J, Zaleski C, Dobin A, Prieto P, Lagarde J, Bussotti G, Tanzer A, Denas O, Li K, Bender MA, Zhang M, Byron R, Groudine MT, McCleary D, Pham L, Ye Z, Kuan S, Edsall L, Wu YC, Rasmussen MD, Bansal MS, Kellis M, Keller CA, Morrissey CS, Mishra T, Jain D, Dogan N, Harris RS, Cayting P, Kawli T, Boyle AP, Euskirchen G, Kundaje A, Lin S, Lin Y, Jansen C, Malladi VS, Cline MS, Erickson DT, Kirkup VM, Learned K, Sloan CA, Rosenbloom KR, De Sousa BL, Beal K, Pignatelli M, Flicek P, Lian J, Kahveci T, Lee D, Kent WJ, Santos MR, Herrero J, Notredame C, Johnson A, Vong S, Lee K, Bates D, Neri F, Diegel M, Canfield T, Sabo PJ, Wilken MS, Reh TA, Giste E, Shafer A, Kutyavin T, Haugen E, Dunn D, Reynolds AP, Neph S, Humbert R, Hansen RS, De Bruijn M, Selleri L, Rudensky A, Josefowicz S, Samstein R, Eichler EE, Orkin SH, Levasseur D, Papayannopoulou T, Chang KH, Skoultchi A, Gosh S, Disteche C, Treuting P, Wang Y, Weiss MJ, Blobel GA, Cao X, Zhong S, Wang T, Good PJ, Lowdon RF, Adams LB, Zhou XQ, Pazin MJ, Feingold EA, Wold B, Taylor J, Mortazavi A, Weissman SM, Stamatoyannopoulos JA, Snyder MP, Guigo R, Gingeras TR, Gilbert DM, Hardison RC, Beer MA, Ren B. 2014. A comparative encyclopedia of DNA elements in the mouse genome. *Nature* **515**:355–364 DOI 10.1038/nature13992.

Zeng XXI, Wilm TP, Sepich DS, Solnica-Krezel L. 2007. Apelin and its receptor control heart field formation during zebrafish gastrulation. *Developmental Cell* **12**:391–402 DOI 10.1016/j.devcel.2007.01.011.

Zhang J, Wan Y, Fang C, Chen J, Ouyang W, Li J, Wang Y. 2018. The orphan G protein-coupled receptor 25 (GPR25) is activated by Apelin and Apela in non-mammalian vertebrates. *Biochemical and Biophysical Research Communications* **501**:408–414 DOI 10.1016/j.bbrc.2018.04.229.

Zhou L, Sun H, Cheng R, Fan X, Lai XS, Deng C. 2019. ELABELA, as a potential diagnostic biomarker of preeclampsia, regulates abnormally shallow placentation via APJ. *American Journal of Physiology—Endocrinology and Metabolism* **316**:E773–E781 DOI 10.1152/ajpendo.00383.2018.