Published in final edited form as:

Front Biosci (Schol Ed). ; 3: 145–155.

Endothelins in regulating ovarian and oviductal function

Phillip J. Bridges¹, Jongki Cho^{1,2}, and CheMyong Ko¹

¹ Reproductive Sciences Graduate Program, Department of Clinical Sciences, University of Kentucky, Lexington KY 40536

² College, Chungnam National University, Daejeon, Republic of Korea, 305-764

³ Research Institute of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea, 305-764

Abstract

In the last 30 years, remarkable progress has been made in our understanding of the biological role of endothelins in the regulation of reproductive function and fertility. A peptide hormone identified for its ability to regulate blood pressure has now been shown as a potent mediator of several reproductive pathways. Ligand- and receptor-specific roles have been identified and/or postulated during follicular development and ovulation as well as in the function and regression of the corpus luteum. In this review we have attempted to organize endothelin-mediated ovarian processes in a process-specific manner, rather than compile a review of ligand- or isoform-specific actions. Further, we have included a discussion on “post-ovarian” or oviductal function, as well as the future directions that we believe will increase our understanding of endothelin biology as a whole.

Keywords

Endothelin; ovary; oviduct; ovulation; follicle; corpus luteum

2. INTRODUCTION

2.1 ENDOTHELINS

Endothelins were first identified in a hallmark paper by Yanigasawa et al. (1) as potent vasoactive peptides, regulating vascular tone and blood pressure. However, diversity in biological function became readily apparent with many other roles for these peptides now described. Three isoforms of endothelins (EDN1, 2 and 3) coded by three different genes (2) have been identified to date (3). These 21 amino acid peptides are derived by cleavage of the biologically inactive “big-endothelin” by the action of at least 2 endothelin converting enzymes (ECE1 and ECE2). The biological effects of endothelin production are then determined via activation of one of two G-protein coupled receptors, endothelin receptors A and B (EDNRA and EDNRB). EDNRA has a high affinity for both EDN1 and EDN2 (and a low affinity for EDN3) whereas EDNRB has a similar affinity for all the isoforms (4).

Genetic deletion of components of the endothelin system has highlighted the importance of these peptides rather than defining tissue specific effects. Endothelin-1 knockout mice die of respiratory failure at birth (5), EDN2 knockout mice exhibit growth retardation and juvenile

lethality (6), EDN3 knockout mice exhibit aganglionic megacolon and die young (7), EDNRA knockout mice are embryonic lethal (8) and EDNRB knockout mice die close to the age of puberty (9). Researchers have therefore relied heavily on the use of agonists and antagonists to study the function of these peptides, however conditional transgenic models are now becoming more readily available. As expected from their structure, the three purified peptide ligands are available for commercial purchase, albeit not always in a species-specific state. Similarly, a plethora of antagonists can be purchased, selective (or not) in their antagonism to either EDNRA or EDNRB. While several of the studies described in this review have utilized the “BQ compounds”, receptor-specific antagonists such as BQ123 (an EDNRA-antagonist) and BQ788 (an EDNRB-antagonist), there are many non-selective or dual-receptor antagonists that have been developed for therapeutic applications. Unfortunately, the unavailability of some of these has hampered their use to the reproductive biologist.

2.2 EXPRESSION

At the level of the whole body, low amounts of endothelins are universally present. Each isoform, however, shows a tissue type-dependent pattern of expression. Endothelin-1 is widely expressed in endothelial cells, vascular smooth muscles, the central nervous system (CNS), and reproductive tissues. Levels of EDN2 are high in the intestine and kidney, whereas EDN3 is mainly expressed in the brain (3). Endothelin converting enzymes are predominantly found in the same, or in close proximity to, those cells expressing each endothelin. Endothelin receptor subtypes are also distinctive in their localization, with co-localization to various tissues signifying the presence of autocrine and paracrine functions. At the level of the ovary, all the components of the endothelin system with the exception of EDN3 are expressed. The expression sites of EDN1, EDN2, EDNRA, EDNRB, ECE1 and ECE2 in the human, mouse, rat and bovine are summarized in Table 1. It should be noted though that throughout the different species, a high level of similarity is seen in their spatial localization. EDN1 expression is apparent in the granulosa cells, luteal cells and endothelial cells of the ovary, whereas EDN2 appears exclusively expressed in the granulosa cells. Endothelin receptor A is found in a variety of cells that include granulosa cells, theca cells, smooth muscle cells and luteal cells and EDNRB is found in the granulosa cells, luteal cells and endothelial cells. Furthermore, both the converting enzymes (ECE1 and ECE2) are expressed in the granulosa cells, luteal cells and theca cells.

3. OVARIAN FOLLICLE

Not long after the identification of endothelins by Yanagisawa et al. (1), a role for these peptides within the ovarian follicle emerged. The effects during follicular development appear to be mediated by EDN1, rather than EDN2 and EDN3, although a role(s) for these other isoforms during folliculogenesis should not be discounted. Overall, levels of EDN1 within the ovary are regulated with stage of follicular development. Kamada et al. (10) reported that the concentration of EDN1 in large porcine follicles was higher than that in small to medium sized follicles and Flores (11) expanded on this using in situ hybridization and immunohistochemistry, also utilizing tissues collected from swine. Those authors reported that EDN1 was absent in primordial and primary follicles and that less than 5% of secondary follicles expressed this isoform. EDN1 immunoreactivity was then reported to increase in the granulosa cells of follicles as the antrum developed, with EDN1 present in 2/3 of large antral follicles.

Continuing through follicular development and into the periovulatory period, Shimizu et al. (12) evaluated the expression of mRNA for EDN1 in follicular samples collected from cows after gonadotropin releasing hormone (GnRH) was administered to induce the preovulatory surge of luteinizing hormone (LH) and follicle stimulating hormone (FSH). In their study,

mRNA for EDN1 remained relatively stable after treatment with GnRH, while mRNA for both EDNRA and EDNRB decreased. Of course, the role for endothelins during the next process, rupture of the follicle or ovulation, has become a topic of intense interest that will be discussed later in this review. To turn to receptor binding, Iwai et al. (13) localized mRNA for EDNRB to the granulosa cell layer of healthy dominant follicles collected from rats by in situ hybridization. Although only low levels of mRNA for EDNRA were detected in their study, other studies using tissues collected from rodents indicate relatively constitutive expression of both isoforms (14,15). Overall, expression of endothelin receptors does not appear to be a limiting factor to their biological function within the ovary, however Usuki et al. (16) reported that FSH up-regulates the expression of both isoforms in granulosa cells collected from rats, suggesting some regulation of function at the level of receptor expression.

From a functional perspective, the role for endothelins within the follicle centers around the regulation of steroidogenesis, however the stimulation of granulosa cell growth and DNA synthesis by EDN1 have also been described (10). Endothelin-1 inhibits LH- (17), FSH- (18) and hCG- (10) stimulated progesterone secretion by granulosa cells, suggestive of a function for this peptide in the control of premature luteinization. Tedeschi et al. (19) demonstrated that this inhibition of progesterone secretion was due to the down-regulation of progesterone-forming enzymes (cholesterol side-chain cleavage and 3 beta-hydroxysteroid dehydrogenase (HSD)/isomerase) as well as the up-regulation of those involved in progesterone metabolism or degradation (20 alpha-HSD and 5 alpha-reductase). Several authors also examined signaling pathways in their studies. EDN1 inhibits cAMP accumulation by granulosa cells (17,18) and consistent with this, EDN1 also inhibited forskolin-stimulated progesterone accumulation by granulosa cells (20). Kamada et al. (21) took this line of research further, provided evidence that EDN1 may also act at sites distal to the generation of cAMP as a second messenger. The requirement for endothelins in the regulation of follicular steroidogenesis is, however, more than a simple steroidogenic brake as FSH has been shown to increase, while LH decrease, the specific binding for EDN1 to cultured rat granulosa cells (22).

It is worthy to note that non-progestogenic attributes have also been described for EDN1, including acting as an inhibitor in the synthesis of estradiol (23). However, the regulation of estradiol by EDN1 is not as clearly defined as for progesterone. Interestingly, using a perfusion system where preovulatory follicles were collected from cows and implanted with capillary dialysis membranes, Acosta et al. (24) observed the now expected inhibition of progesterone secretion by EDN1, however EDN1 stimulated the secretion of estradiol. These authors also reported the release of EDN1 from the theca layer of these follicles and an interaction of endothelins with prostaglandins (PGE₂) and cytokines. Given this, it appears that the local regulation of follicular function by these peptides must be considered inclusive to both steroids, the theca layer and with respect to the cross-talk that occurs between these follicular compartments.

3.1 OVULATION

The ovulatory process, ending with rupture of the follicular wall and expulsion of the oocyte, is a central event in the reproductive cycle. Perhaps due to human curiosity concerning the core process of egg production, the mechanism governing ovulation has challenged researchers for decades. Up to the middle of the 1960s, it had been generally accepted that increased intrafollicular pressure was a driving force of rupture (25). However, the consensus was challenged by reports that there was no significant increase in pressure before ovulation and that artificially increasing intrafollicular pressure by the injection of saline into preovulatory follicles of rabbits did not induce rupture (26,27). These reports steered researchers to the view that decreasing tensile strength of the follicular wall was a

main biochemical event required for ovulation, thus emphasizing the importance of proteolytic enzymes during this cascade. Since then, numerous studies have demonstrated the involvement of matrix metalloproteinases (MMPs) and the plasminogen activators (PAs) in the degradation of the collagenous tissues, which is present in the basement membrane, theca externa, and tunica albuginea of the follicular wall (28). Subsequent findings showed that both ovarian cells and leukocytes are involved in the tissue degradation process (28–30). In addition to the increased intrafollicular pressure and proteolytic activity, in the early 1980s, Talbot and her colleagues indicated that follicular contraction might be associated with follicle rupture (31–33). This idea was supported by the identification of the presence of smooth muscle cells in the follicular wall and evidence of their contraction in the gonadotropin-stimulated hamster ovary using electron microscopy (32). Interestingly, not knowing a direct trigger of the contraction, a neuronal regulation was suggested as a controlling mechanism of the follicular constriction.

We recently sought to identify an endogenous molecule that was produced immediately prior to ovulation and which induced follicular constriction. A genome-wide gene expression profiling approach was employed and we gave attention to vasoconstrictors that were expressed prior to and/or at the time of ovulation. This approach led us to the identification of a potent vasoconstrictor, EDN2. In striking contrast to most of the components that are expressed constitutively in multiple cell types, the pattern of EDN2 expression is quite unique; mRNA for EDN2 is dramatically increased within hours of ovulation and specifically in granulosa cells of the periovulatory follicle. At all other times, follicular expression of mRNA for EDN2 appears to be maintained at a basal level. The physiological properties of this ligand and unique expression pattern made EDN2 a strong candidate as an endogenous molecule that could induce follicular constriction at the time of ovulation. We therefore examined whether this peptide induced contraction of ovarian tissue. For this purpose, strips of ovarian tissue were dissected from the ovaries of gonadotropin-primed rats and the isometric contraction response recorded after treatment with the peptide ligands (14). Using this approach we found that EDN2 induces a rapid and sustained contraction of ovarian strips and that the addition of tezosentan, a dual endothelin receptor antagonist, decreased the contractile force (14). When administered *in vivo*, treatment with tesozentan resulted in a marked decrease or delay in ovulation.

In addition to EDN2, several other vasoconstrictive molecules have been identified in the preovulatory follicle, including prostaglandins (PGs), pituitary adenylate cyclase activating peptide (PACAP) and vasoactive intestinal peptide (VIP) (34,35). Their pattern of ovarian expression dramatically increases after stimulation by the pre-ovulatory surge of gonadotropins or by injection of hCG. However, it is unlikely that these molecules play a direct role in inducing the contraction involved in follicular rupture because the time of peak expression of the relevant genes regulating their production is temporally distant from the time of follicle rupture. For example, the peak in the expression of mRNA and/or protein for PACAP and VIP are 6 to 9 hours from the actual time of follicular rupture. In addition, their contraction-inducing abilities are weaker than those of EDN2 (unpublished data). With respect to prostaglandins, studies in domestic ruminants indicate that both vasoconstrictive and vasodilatory prostaglandins and their receptor subtypes are present at ovulation (36,37). This, along with the temporal regulation observed in the expression of mRNA for the specific receptor subtypes throughout the entire periovulatory period, would suggest that prostaglandins are not acting as the primary contractile signal at the time of follicle rupture.

In regard to the mechanism that EDN2 utilizes in inducing follicular constriction, it is likely that EDN2 released from the granulosa cells travels across the follicular wall to stimulate the contraction of smooth muscle cells in the theca externa as well as in the ovarian interstitium.

In fact, the presence of smooth muscle cells in the ovary has been reported by multiple groups of researchers (38–45). As shown in Fig. 1, each follicle is surrounded by a layer of smooth muscle cells, with neighboring smooth muscle layers directly contacting or interweaving with each other. This forms a “sponge-like” smooth muscle network at the level of the ovary. The contraction of individual smooth muscle cells should then generate a synergic constriction force at the level of the ovary, leading to simultaneous follicle rupture and, thus, rupture of weakened follicles and release of their oocytes. This may also explain why dozens of oocytes are released within a narrow window of time when follicular development and ovulation are stimulated and synchronized by treatment with exogenous gonadotropins.

It should be noted that functionally, both EDNRA and EDNRB are present within the ovary and upon activation by ligand binding, these receptors often elicit opposite physiological responses in their target tissues. Activation of EDNRA leads to vasoconstriction and the contractile forces that we believe are required for ovulation, whereas EDNRB has other actions including that of vasodilation. With emphasis on the expression of EDNRB in granulosa cells and the theca interna, an EDNRB-mediated pathway to follicle rupture has also been proposed (46). This hypothesis speculates that EDN2 produced by granulosa cells acts on either granulosa cells or the endothelial cells of the blood vessels in the theca interna via EDNRB to induce nitric oxide signaling, local vasodilation and an increase in follicular pressure. In support of this hypothesis, Palanisamy et al. (46) reported that treatment with an EDNRB antagonist inhibited ovulation in gonadotropin-primed mice. In their same experiments, an EDNRA antagonist showed little activity in inhibiting ovulation. Interestingly, we were not able to block ovulation using these receptor-specific antagonists (47). These contrasting results may be caused by differences in the pharmacokinetics of the antagonists (slight differences in the time of treatment were noted between the two studies) and/or subtle differences in the animal models utilized. Further studies are therefore warranted, including the use of conditional gene targeting approaches, which should clearly characterize the role(s) of endothelin receptor signaling during ovulation.

With respect to the mechanism regulating EDN2 expression in granulosa cells, Palanisamy et al. (46) reported that the progesterone receptor (PR) and Kim et al. (48) that PPAR were the upstream transcription regulators of EDN2 gene expression. We have reported that hypoxia is a main inducer of EDN2 (49). In our studies, we first observed that granulosa cells cultured under hypoxic condition expressed significantly higher level of mRNA for EDN2. We then conducted a promoter analysis which revealed that when presumptive hypoxia-inducing factor binding sites were deleted from the promoter, its activity significantly decreased. Interestingly, in this same study we also demonstrated that neither RU-486 (a PR antagonist) nor indomethacin (a cyclooxygenase inhibitor) altered EDN2 gene expression, suggesting no involvement of those pathways in the regulation of EDN2 within the ovary. Meanwhile, the importance of hypoxia in the regulation of EDN2 has been reported by others. Using mice, Kim et al. (50) reported that injection of echinomycin, an inhibitor of hypoxia inducible factors, suppressed both EDN2 expression and ovulation. Consistent with this, Klipper et al. (51) reported that treatment with cobalt chloride, a hypoxia-mimicking agent, elevated EDN2 in bovine granulosa cell cultures. Intrafollicular oxygen concentrations are also believed to decrease with development of the preovulatory follicle (52). Overall, it should be not surprising to find consensus for hypoxia as an inducer of EDN2 expression.

Taken together, these findings have led us to formulate an ‘inclusive’ model of follicle rupture which incorporates the three main factors described above: intrafollicular pressure, degradation of collagenous tissue, and follicular constriction. Base upon this model, the preovulatory surge of gonadotropins induces the sequential activation of proteolytic

enzymes causing partial digestion of the extracellular matrix, including that of the follicular basement membrane. Concurrently, gonadotropin-induced ovarian inflammation brings more fluid into the follicular antrum, increasing follicular size, decreasing oxygen content and further weakening the structure of the follicular wall. While the integrity of the intrafollicular structure becomes loose due to the degradation of the extracellular matrix of the follicular wall, the overall follicular structure is maintained because of the balance between the pressure from intrafollicular fluid and the tensile force of theca externa. Finally, as a final trigger for ovulation, EDN2 causes a rapid and sustained follicular constriction via EDNRA at the time when the follicle is mature and ready to release its oocyte, breaking the balance and therefore resulting in rupture of the follicular wall and ovulation.

4. CORPUS LUTEUM

An ovulating follicle then goes through a complete transformation to become the endocrine organ known as the corpus luteum (CL). This transformation includes the formation of new vasculature or angiogenesis, leading to the formation of an extensive capillary network within the CL. This is required to support these endocrine cells, supplying oxygen and precursors of steroid production, as well as releasing progesterone in preparation for potential implantation and the maintenance of pregnancy (53–55). VEGF (vascular endothelial growth factor) plays a central role in angiogenesis. In the ovary, luteinizing granulosa cells secrete VEGF proteins that in turn activate two VEGF receptors (VEGFR-1 and -2) that are localized to luteal endothelial cells and steroidogenic cells (56,57). It is well established that hypoxia is a key factor in inducing VEGF in the luteinizing granulosa cells. Interestingly, Klipper et al. (51) reported that EDN2 directly induces VEGF in granulosa cells of the bovine ovary. It is also noteworthy that we have shown that the HIF-1 α binding site in the proximal promoter region of the EDN2 gene is required for the induction of EDN2 expression by hypoxia (49). Taken together, it is very likely that the well-known hypoxia effect on VEGF expression and angiogenesis, and therefore CL formation is achieved by inducing EDN2 via activating HIF-1 α . In the meantime, the EDNRA is believed to mediate EDN2 action as this receptor has been shown to be responsible for endothelin's action in a variety of angiogenic pathologies (56,58,59).

Endothelin-1 is a CL-peptide (60) that can bind locally to this transient endocrine organ (61) and it is now well established that luteal-derived EDN1 plays a role during luteolysis. However, the precise role(s) for this peptide during luteal regression remains controversial, in part due to the variety of techniques and experimental models that have been utilized to address this paradigm. Within the confines of a normal estrous cycle, luteal and systemic concentrations of EDN1 have been well characterized in the cow. Ohtani et al. (62) reported increasing plasma concentrations of EDN1 during the final days of the estrous cycle, concomitant with decreasing progesterone as the corpus luteum regressed. Interestingly, peripheral concentrations of EDN1 continued to rise well after the demise of the CL with concentrations of this peptide peaking around the time of estrus. In a later study by Shirasuna et al. (63), microdialysis (MDS) lines were implanted into the corpora lutea of naturally cycling cows and the local release of several hormones of interest, including EDN1, determined during spontaneous luteolysis. These authors observed a pulsatile pattern in the local release of EDN1 by the regressing CL, truly suggestive of a regulated, physiological role. From the timing and pattern of release, it is therefore tempting to conclude that EDN1 acts in a luteolytic manner, in concert with PGF2 α to ensure the demise of this steroidogenic tissue, however an alternate hypothesis has been proposed. With EDN1 reported to stimulate in vitro secretion of the luteotropic prostaglandin, PGE, but not PGF2 α , by slices of luteal tissue collected from cows (64), a protective role for this endothelin on luteal function has been postulated and must be considered.

Weems et al. (65) went on to install catheters into naturally cycling ewes and infused EDN1 into either the uterine lumen or ovarian pedicle adjacent to the luteal-bearing ovary and their results again indicated a luteotropic, not luteolytic, role for this peptide. In ewes infused with vehicle, the expected spontaneous luteal regression was observed. However, by Day 18 of the cycle, luteal weights remained 2.5 to 3-fold greater and progesterone secretion remained above 1 ng/ml (the benchmark of luteal function) in animals that had been infused with this peptide. That being said, several experiments utilizing receptor-specific antagonists and/or models of induced luteal regression have produced a contrasting result. Doerr et al. (66) utilized osmotic minipumps filled with several of the commercially available receptor-specific antagonists to directly infuse the CL during PGF2 α -induced luteolysis. Infusion of the EDNRA-specific antagonist BQ610 maintained serum concentrations of progesterone, suggesting a luteotropic role of the antagonist and hence, luteolytic role of the endogenous peptide. Consistent with this, intrauterine infusion of BQ610 on Days 16 to 19 of the estrous cycle appeared luteotropic in a study from Connecticut, delaying luteal regression in cattle for ~24 h (67). Unfortunately, a compilation of all the literature describing the pharmacological manipulation of luteal function in vivo will not answer the most pertinent question of whether EDN1 is luteolytic or luteotropic. With a thorough analysis of the process of luteal regression beyond the scope of this paper, and with several comprehensive reviews readily available (68–70), the authors urge readers to follow this line of inquiry with an open mind and an understanding of the physiology behind spontaneous versus PGF2 α -induced luteal regression and the pharmacokinetics of the various compounds used for experimental purposes.

5. OVIDUCT

Like the ovary, the oviduct has presented itself as a tissue with function(s) mediated by this family of peptide hormones. Also consistent with the ovary is spatial and temporal regulation in the expression of functional isoforms of endothelins and their receptors, with luminal epithelial cells consistently reported as the dominant site of endothelin production (71–73). Cyclic regulation of endothelin gene expression is reported to occur during the estrous cycle in cattle (74), a result consistent with our recent findings in mice (75) and the oviduct ipsilateral to the ovulating ovary appears to produce more peptide than the contralateral side (76). From a ligand perspective, the focus of oviductal endothelins has centered around EDN1 (73,74) with estradiol, tumor necrosis factor- α (TNF α) and angiotensin II all shown to stimulate this isoform (77,78). Reinhart et al. (72) did report an inhibitory effect of estradiol on the synthesis of this peptide in vitro though. Overall, the role of endothelins in the regulation of oviductal function is well established, however the role(s) attributed to this family of hormones should not be considered specific to EDN1. We have postulated a role for ovarian-produced EDN2 (79) and most recently, oviductal produced EDN3 (75), which is described in more detail below.

Studies on the localization of endothelin receptor subtypes within the oviduct show some inconsistency in the literature, however the presence of both receptor subtypes within this tissue has now become relatively well documented. Sakamoto et al. (73) noted dominant localization of EDNRB to the tubal epithelium of the human oviduct, with some stromal expression apparent, which is consistent with the pattern of expression that we observed in the mouse (75). However, they identified EDNRA to be located more specifically to the muscle layer of the oviduct, in contrast to our immunohistochemical findings which pointed again to the luminal epithelium as a major site of action for EDNRA. We did still detect some stromal expression of this isoform though. From a functional viewpoint, the majority of reports on endothelins within the oviduct focus on the ability of these peptides to affect smooth muscle tone, modulating oviductal contractility and transport of the gametes (71,74,79–81). There is a strong physiological basis for the regulation of oviductal tone by

these peptides, with receptor subtype-specific contractile responses reported (80) as well as the temporal regulation of contractile forces over the course of an estrous cycle (74).

Given the notable role of endothelins in the regulation of vascular tone and hence blood pressure, a role in contractility within the oviduct is intuitive. However it is still interesting to note, given the indication of both epithelial and stromal receptor localization, that the concurrent (82) regulation of epithelial function by these peptides is not as well defined. One focus of our research has shifted in that direction, and we have now proposed an independent, non-contractile role for endothelins within the oviduct that we believe is acting concomitant to this established role in contraction. Using mice, we reported a gonadotropin-induced increase in mRNA for EDN3 within the luminal epithelial cells of the oviduct (75) and hypothesized that this isoform was regulating the epithelial cell secretions that are known to affect fertilization and early embryonic development (83,84). With EDN3 perhaps the least recognized isoform, at least from the viewpoint of a reproductive biologist, this was an exciting discovery for us. We also observed the specific induction of mRNA for EDN3 within the isthmus of the oviduct around the time of ovulation, which is again consistent with the cyclic regulation of secretions by this tissue (85,86). In this report, we also provide evidence for a dramatic requirement for endothelins during early embryonic development. We treated mice with the dual-receptor antagonist tezosentan around the time of breeding and observed a marked decrease in the number of 2-cell embryos that we could collect from within their oviducts.

6. FUTURE STUDIES

Over the last 20 years, EDN1 has been the major subject of study within the ovary, leading to recognition of the importance of these peptides in folliculogenesis, steroidogenesis and luteal function. Excitement to the study of endothelins in female reproduction was added by the identification of EDN2 as one of the final triggers of follicle rupture and as a critical regulator of luteal formation. Further excitement is brewing as the third endothelin, EDN3, has been identified as a major endothelin in the oviduct. In the next few years, it is expected that endothelin research in the ovary and oviduct will lead to a better understanding of mechanisms involved, with an emphasis on the receptor-type specific endothelin regulated pathways. Conditional gene targeting approaches are anticipated to be used heavily and a significant effort made to translate the findings to clinical applications.

Acknowledgments

This work was supported by National Institutes of Health Grants RO1HD052694 and P20 RR15592 (to C.K.).

This is an, un-copied, author manuscript that has been accepted for publication in the *Frontiers in Bioscience*. Cite this article as appearing in the *Journal of Frontiers in Bioscience*. Full citation can be found by searching the *Frontiers in Bioscience* (<http://bioscience.org/search/authors/htm/search.htm>) following publication and at PubMed (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=pubmed>) following indexing. This article may not be duplicated or reproduced, other than for personal use or within the rule of "Fair Use of Copyrighted Materials" (section 107, Title 17, U.S. Code) without permission of the copyright holder, the *Frontiers in Bioscience*. From the time of acceptance following peer review, the full final copy edited article of this manuscript will be made available at <http://www.bioscience.org/>. The *Frontiers in Bioscience* disclaims any responsibility or liability for errors or omissions in this version of the un-copied manuscript or in any version derived from it by the National Institutes of Health or other parties.

References

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411–5. [PubMed: 2451132]

2. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci U S A* 1989;86:2863–7. [PubMed: 2649896]
3. Masaki T. Historical review: Endothelin. *Trends Pharmacol Sci* 2004;25:219–24. [PubMed: 15063086]
4. Masaki T, Ninomiya H, Sakamoto A, Okamoto Y. Structural basis of the function of endothelin receptor. *Mol Cell Biochem* 1999;190:153–6. [PubMed: 10098982]
5. Kurihara Y, Kurihara H, Suzuki H, Kodama T, Maemura K, Nagai R, Oda H, Kuwaki T, Cao WH, Kamada N, et al. Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. *Nature* 1994;368:703–10. [PubMed: 8152482]
6. Chang, I.; Bramall, AN.; Baynash, AG.; McInnes, RR.; Stewart, DJ.; Yanagisawa, M. Genetic Study of ET-2 function in mice. ET-11: APS International Conference on Endothelin; Montreal Canada. 2009. p. 4.3
7. Baynash AG, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE, Yanagisawa M. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell* 1994;79:1277–85. [PubMed: 8001160]
8. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, Kumada M, Hammer RE, Yanagisawa M. Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. *Development* 1998;125:813–24. [PubMed: 9449664]
9. Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, Yanagisawa M. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* 1994;79:1267–76. [PubMed: 8001159]
10. Kamada S, Kubota T, Hirata Y, Imai T, Ohta K, Taguchi M, Marumo F, Aso T. Endothelin-1 is an autocrine/paracrine regulator of porcine granulosa cells. *J Endocrinol Invest* 1993;16:425–31. [PubMed: 8370918]
11. Flores JA. Gene Expression of Endothelin-1 in the Porcine Ovary: Follicular Development. *Biol Reprod* 2000;63:1377–82. [PubMed: 11058541]
12. Shimizu T, Berisha B, Schams D, Miyamoto A. Changes in the messenger RNA expressions of the endothelin-1 and angiotensin systems in mature follicles of the superovulated bovine ovary. *J Reprod Dev* 2007;53:655–62. [PubMed: 17380043]
13. Iwai M, Hori S, Shigemoto R, Kanzaki H, Mori T, Nakanishi S. Localization of endothelin receptor messenger ribonucleic acid in the rat ovary and fallopian tube by in situ hybridization. *Biol Reprod* 1993;49:675–80. [PubMed: 8218631]
14. Ko C, Gieske MC, Al-Alem L, Hahn Y, Su W, Gong MC, Iglarz M, Koo Y. Endothelin-2 in ovarian follicle rupture. *Endocrinology* 2006;147:1770–9. [PubMed: 16410304]
15. Palanisamy GS, Cheon Y-P, Kim J, Kannan A, Li Q, Sato M, Mantena SR, Sitruk-Ware RL, Bagchi MK, Bagchi IC. A Novel Pathway Involving Progesterone Receptor, Endothelin-2, and Endothelin Receptor B Controls Ovulation in Mice. *Mol Endocrinol* 2006;20:2784–95. [PubMed: 16887885]
16. Usuki S, Kobayashi S, Sugimoto M, Kotani E, Otani S, Kubo T, Ishii T, Murakami K, Miyazaki H. Effects of follicle-stimulating hormone on endothelin receptors in cultured rat ovarian granulosa cells. *J Cardiovasc Pharmacol* 1998;31(Suppl 1):S225–9. [PubMed: 9595444]
17. Iwai M, Hasegawa M, Taii S, Sagawa N, Nakao K, Imura H, Nakanishi S, Mori T. Endothelins inhibit luteinization of cultured porcine granulosa cells. *Endocrinology* 1991;129:1909–14. [PubMed: 1655389]
18. Flores JA, Quyyumi S, Leong DA, Veldhuis JD. Actions of endothelin-1 on swine ovarian (granulosa) cells. *Endocrinology* 1992;131:1350–8. [PubMed: 1324159]
19. Tedeschi C, Hazum E, Kokia E, Ricciarelli E, Adashi EY, Payne DW. Endothelin-1 as a luteinization inhibitor: inhibition of rat granulosa cell progesterone accumulation via selective modulation of key steroidogenic steps affecting both progesterone formation and degradation. *Endocrinology* 1992;131:2476–8. [PubMed: 1425445]
20. Tedeschi C, Lohman C, Hazum E, Ittoop O, Ben-Shlomo I, Resnick CE, Payne DW, Adashi EY. Rat ovarian granulosa cell as a site of endothelin reception and action: attenuation of

- gonadotropin-stimulated steroidogenesis via perturbation of the A-kinase signaling pathway. *Biol Reprod* 1994;51:1058–65. [PubMed: 10.1095/biolreprod51.5.1058]
21. Kamada S, Kubota T, Hirata Y, Taguchi M, Eguchi S, Marumo F, Aso T. Direct effect of endothelin-1 on the granulosa cells of the porcine ovary. *J Endocrinol* 1992;134:59–66. [PubMed: 1323639]
 22. Otani H, Yamoto M, Fujinaga H, Nakano R. Presence and localization of endothelin receptor in the rat ovary and its regulation by pituitary gonadotropins. *Eur J Endocrinol* 1996;135:449–54. [PubMed: 8921828]
 23. Calogero AE, Burrello N, Ossino AM. Endothelin (ET)-1 and ET-3 inhibit estrogen and cAMP production by rat granulosa cells in vitro. *J Endocrinol* 1998;157:209–15. [PubMed: 9659283]
 24. Acosta TJ, Miyamoto A, Ozawa T, Wijayagunawardane MP, Sato K. Local release of steroid hormones, prostaglandin E₂, and endothelin-1 from bovine mature follicles In vitro: effects of luteinizing hormone, endothelin-1, and cytokines. *Biol Reprod* 1998;59:437–43. [PubMed: 9687319]
 25. Komar CM, Matousek M, Mitsube K, Mikuni M, Brannstrom M, Curry TE Jr. Effects of genistein on the periovulatory expression of messenger ribonucleic acid for matrix metalloproteinases and tissue inhibitors of metalloproteinases in the rat ovary. *Reproduction* 2001;121:259–65. [PubMed: 11226050]
 26. Espey LL, Lipner H. Measurements of Intrafollicular Pressures in the Rabbit Ovary. *Am J Physiol* 1963;205:1067–72. [PubMed: 14084958]
 27. Rondell P. Follicular Pressure and Distensibility in Ovulation. *Am J Physiol* 1964;207:590–4. [PubMed: 14220028]
 28. Curry TE Jr, Osteen KG. The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. *Endocr Rev* 2003;24:428–65. [PubMed: 12920150]
 29. Richards JS, Russell DL, Ochsner S, Espey LL. Ovulation: new dimensions and new regulators of the inflammatory-like response. *Annu Rev Physiol* 2002;64:69–92. [PubMed: 11826264]
 30. Brannstrom M, Enskog A. Leukocyte networks and ovulation. *J Reprod Immunol* 2002;57:47–60. [PubMed: 12385833]
 31. Martin GG, Talbot P. The role of follicular smooth muscle cells in hamster ovulation. *J Exp Zool* 1981;216:469–82. [PubMed: 7276896]
 32. Schroeder PC, Talbot P. Intrafollicular pressure decreases in hamster preovulatory follicles during smooth muscle cell contraction in vitro. *J Exp Zool* 1982;224:417–26. [PubMed: 7153733]
 33. Talbot P, Chacon RS. In vitro ovulation of hamster oocytes depends on contraction of follicular smooth muscle cells. *J Exp Zool* 1982;224:409–15. [PubMed: 7153732]
 34. Ko C, Park-Sarge OK. Progesterone receptor activation mediates LH-induced type-I pituitary adenylate cyclase activating polypeptide receptor (PAC(1)) gene expression in rat granulosa cells. *Biochem Biophys Res Commun* 2000;277:270–9. [PubMed: 11027674]
 35. Ko, CY-HIaO-KP-S. Role of Progesterone Receptor Activation in Pituitary Adenylate Cyclase Activating Polypeptide Gene Expression in Rat Ovary. *Endocrinology* 1999;140:5185–94. [PubMed: 10537148]
 36. Bridges PJ, Fortune JE. Regulation, action and transport of prostaglandins during the periovulatory period in cattle. *Mol Cell Endocrinol* 2007;263:1–9. [PubMed: 17064845]
 37. Bridges PJ, Komar CM, Fortune JE. Gonadotropin-induced expression of messenger ribonucleic acid for cyclooxygenase-2 and production of prostaglandins E and F₂α in bovine preovulatory follicles are regulated by the progesterone receptor. *Endocrinology* 2006;147:4713–22. [PubMed: 16825323]
 38. O'Shea JD. An electron microscope study of smooth muscle, and its innervation, in the ovary of the rat. *J Anat* 1970;106:196.
 39. Osvaldo-Decima L. Smooth muscle in the ovary of the rat and monkey. *J Ultrastruct Res* 1970;30:218–37. [PubMed: 4983666]
 40. Burden HW. The distribution of smooth muscle in the cat ovary with a note on its adrenergic innervation. *J Morphol* 1973;140:467–75. [PubMed: 4722569]

41. Doss DN, Boulos IK, Fredens K. The presence of smooth muscle cells in the rat ovary. *Histochemistry* 1984;81:205–6. [PubMed: 6208173]
42. Paranko J, Pelliniemi LJ. Differentiation of smooth muscle cells in the fetal rat testis and ovary: localization of alkaline phosphatase, smooth muscle myosin, F-actin, and desmin. *Cell Tissue Res* 1992;268:521–30. [PubMed: 1628308]
43. Van Nassauw L, Harisson F, Callebaut M. Immunolocalization of smooth muscle-like cells in the quail ovary. *Eur J Morphol* 1992;30:275–88. [PubMed: 1284832]
44. Miyamoto K, Morishita Y, Yamazaki M, Minamino N, Kangawa K, Matsuo H, Mizutani T, Yamada K, Minegishi T. Isolation and characterization of vascular smooth muscle cell growth promoting factor from bovine ovarian follicular fluid and its cDNA cloning from bovine and human ovary. *Arch Biochem Biophys* 2001;390:93–100. [PubMed: 11368520]
45. Madekurozwa MC, Oke BO, Akinloye AK. The immunohistochemical localization of desmin and smooth muscle actin in the ovary of the African giant rat (*Cricetomys gambianus*) during the oestrous cycle. *Anat Histol Embryol* 2010;39:81–6. [PubMed: 19958343]
46. Palanisamy GS, Cheon YP, Kim J, Kannan A, Li Q, Sato M, Mantena SR, Sitruk-Ware RL, Bagchi MK, Bagchi IC. A novel pathway involving progesterone receptor, endothelin-2, and endothelin receptor B controls ovulation in mice. *Mol Endocrinol* 2006;20:2784–95. [PubMed: 16887885]
47. Bridges PJ, Jo M, Al Alem L, Na G, Su W, Gong MC, Jeoung M, Ko C. Production and binding of endothelin-2 (EDN2) in the rat ovary: Endothelin receptor subtype A (EDNRA) mediated contraction. *Reprod Fertil Dev* 2010;22:1–8.
48. Kim J, Sato M, Li Q, Lydon JP, Demayo FJ, Bagchi IC, Bagchi MK. Peroxisome proliferator-activated receptor gamma is a target of progesterone regulation in the preovulatory follicles and controls ovulation in mice. *Mol Cell Biol* 2008;28:1770–82. [PubMed: 18172011]
49. Na G, Bridges PJ, Koo Y, Ko C. Role of hypoxia in the regulation of periovulatory EDN2 expression in the mouse. *Can J Physiol Pharmacol* 2008;86:310–9. [PubMed: 18516093]
50. Kim J, Bagchi IC, Bagchi MK. Signaling by hypoxia-inducible factors is critical for ovulation in mice. *Endocrinology* 2009;150:3392–400. [PubMed: 19325003]
51. Klipper E, Levit A, Mastich Y, Berisha B, Schams D, Meidan R. Induction of Endothelin-2 Expression by Luteinizing Hormone and Hypoxia: Possible Role in Bovine Corpus Luteum Formation. *Endocrinology*. 2010
52. Redding GP, Bronlund JE, Hart AL. Theoretical investigation into the dissolved oxygen levels in follicular fluid of the developing human follicle using mathematical modelling. *Reprod Fertil Dev* 2008;20:408–17. [PubMed: 18402761]
53. Chabbert-Buffet N, Ouzounian S, Kairis AP, Bouchard P. Contraceptive applications of progesterone receptor modulators. *Eur J Contracept Reprod Health Care* 2008;13:222–30. [PubMed: 18821461]
54. Bazer FW, Burghardt RC, Johnson GA, Spencer TE, Wu G. Interferons and progesterone for establishment and maintenance of pregnancy: interactions among novel cell signaling pathways. *Reprod Biol* 2008;8:179–211. [PubMed: 19092983]
55. Bazer FW, Slayden OD. Progesterone-induced gene expression in uterine epithelia: a myth perpetuated by conventional wisdom. *Biol Reprod* 2008;79:1008–9. [PubMed: 18953018]
56. Goligorsky MS, Budzikowski AS, Tsukahara H, Noiri E. Co-operation between endothelin and nitric oxide in promoting endothelial cell migration and angiogenesis. *Clin Exp Pharmacol Physiol* 1999;26:269–71. [PubMed: 10081626]
57. Pauli SA, Tang H, Wang J, Bohlen P, Posser R, Hartman T, Sauer MV, Kitajewski J, Zimmermann RC. The vascular endothelial growth factor (VEGF)/VEGF receptor 2 pathway is critical for blood vessel survival in corpora lutea of pregnancy in the rodent. *Endocrinology* 2005;146:1301–11. [PubMed: 15591152]
58. Grimshaw MJ. Endothelins and hypoxia-inducible factor in cancer. *Endocr Relat Cancer* 2007;14:233–44. [PubMed: 17639040]
59. Smollich M, Wulfig P. The endothelin axis: a novel target for pharmacotherapy of female malignancies. *Curr Vasc Pharmacol* 2007;5:239–48. [PubMed: 17627567]
60. Usuki S, Suzuki N, Matsumoto H, Yanagisawa M, Masaki T. Endothelin-1 in luteal tissue. *Mol Cell Endocrinol* 1991;80:147–51. [PubMed: 1955074]

61. Flores JA, Winters TA, Knight JW, Veldhuis JD. Nature of endothelin binding in the porcine ovary. *Endocrinology* 1995;136:5014–9. [PubMed: 7588236]
62. Ohtani M, Kobayashi S, Miyamoto A, Hayashi K, Fukui Y. Real-time relationships between intraluteal and plasma concentrations of endothelin, oxytocin, and progesterone during prostaglandin F2alpha-induced luteolysis in the cow. *Biol Reprod* 1998;58:103–8. [PubMed: 9472929]
63. Shirasuna K, Shimizu T, Hayashi KG, Nagai K, Matsui M, Miyamoto A. Positive association, in local release, of luteal oxytocin with endothelin 1 and prostaglandin F2alpha during spontaneous luteolysis in the cow: a possible intermediary role for luteolytic cascade within the corpus luteum. *Biol Reprod* 2007;76:965–70. [PubMed: 17287495]
64. Weems YS, Randel RD, Tatman S, Lewis AW, Neuendorff DA, Weems CW. Effects of estrous synchronization on response to nitric oxide donors, nitric oxide synthase inhibitors, and endothelin-1 in vitro. *Prostaglandins Other Lipid Mediat* 2004;74:45–59. [PubMed: 15560115]
65. Weems YS, Johnson D, Uchima T, Raney A, Lennon E, Goto K, Bowers G, Saldana J, Pang J, Randel RD, Weems CW. Is endothelin-1 luteolytic or antiluteolytic in ewes? *Prostaglandins Other Lipid Mediat* 2009;90:63–8. [PubMed: 19723588]
66. Doerr MD, Goravanhally MP, Rhinehart JD, Inskeep EK, Flores JA. Effects of endothelin receptor type-A and type-B antagonists on prostaglandin F2alpha-induced luteolysis of the sheep corpus luteum. *Biol Reprod* 2008;78:688–96. [PubMed: 18077801]
67. Keator CS, Schreiber DT, Hoagland TA, McCracken JA, Milvae RA. Intrauterine infusion of BQ-610, an endothelin type A receptor antagonist, delays luteolysis in dairy heifers. *Domest Anim Endocrinol* 2008;34:411–8. [PubMed: 18258406]
68. Schams D, Berisha B. Regulation of corpus luteum function in cattle--an overview. *Reprod Domest Anim* 2004;39:241–51. [PubMed: 15225277]
69. Meidan R, Milvae RA, Weiss S, Levy N, Friedman A. Intraovarian regulation of luteolysis. *J Reprod Fertil Suppl* 1999;54:217–28. [PubMed: 10692857]
70. Meidan R, Levy N, Kisliouk T, Podlovny L, Rusiansky M, Klipper E. The yin and yang of corpus luteum-derived endothelial cells: balancing life and death. *Domest Anim Endocrinol* 2005;29:318–28. [PubMed: 15927442]
71. Rosselli M, Imthurn B, Macas E, Keller PJ. Endothelin production by bovine oviduct epithelial cells. *J Reprod Fertil* 1994;101:27–30. [PubMed: 8064689]
72. Reinhart KC, Dubey RK, Cometti B, Keller PJ, Rosselli M. Differential effects of natural and environmental estrogens on endothelin synthesis in bovine oviduct cells. *Biol Reprod* 2003;68:1430–6. [PubMed: 12606437]
73. Sakamoto M, Sakamoto S, Kubota T, Aso T, Azuma H. Localization and role of endothelin-1 and endothelin receptors in the human Fallopian tube. *Mol Hum Reprod* 2001;7:1057–63. [PubMed: 11675472]
74. Priyadarsana M, Wijayagunawardane B, Miyamoto A. Endothelin-1 system in the bovine oviduct: a regulator of local contraction and gamete transport. *J Cardiovasc Pharmacol* 2004;44(Suppl 1):S248–51. [PubMed: 15838292]
75. Jeoung M, Lee S, Hawng H, Cheon Y, Jeong YK, Gye MC, Iglarz M, Bridges PJ. Identification of a novel role for endothelins within the oviduct. *Endocrinology*. 2010 In Press.
76. Wijayagunawardane MP, Miyamoto A, Cerbito WA, Acosta TJ, Takagi M, Sato K. Local distributions of oviductal estradiol, progesterone, prostaglandins, oxytocin and endothelin-1 in the cyclic cow. *Theriogenology* 1998;49:607–18. [PubMed: 10732039]
77. Wijayagunawardane MP, Miyamoto A, Sato K. Prostaglandin E2, prostaglandin F2 alpha and endothelin-1 production by cow oviductal epithelial cell monolayers: effect of progesterone, estradiol 17 beta, oxytocin and luteinizing hormone. *Theriogenology* 1999;52:791–801. [PubMed: 10735120]
78. Wijayagunawardane MP, Miyamoto A, Taquahashi Y, Acosta TJ, Nishimura M, Sato K. Angiotensin II and atrial natriuretic peptide in the cow oviductal contraction in vitro: direct effect and local secretion of prostaglandins, endothelin-1, and angiotensin II. *Biol Reprod* 2001;65:799–804. [PubMed: 11514344]

79. Al-Alem L, Bridges PJ, Su W, Gong MC, Iglarz M, Ko C. Endothelin-2 induces oviductal contraction via endothelin receptor subtype A in rats. *J Endocrin* 2007;193:383–91.
80. Jankovic SM, Jankovic SV, Lukic G, Radonjic V, Cupara S, Stefanovic S. Contractile effects of endothelins on isolated ampullar segment of human oviduct in luteal phase of menstrual cycle. *Pharmacol Res*. 2008
81. Wijayagunawardane MP, Miyamoto A, Taquahashi Y, Gabler C, Acosta TJ, Nishimura M, Killian G, Sato K. In vitro regulation of local secretion and contraction of the bovine oviduct: stimulation by luteinizing hormone, endothelin-1 and prostaglandins, and inhibition by oxytocin. *J Endocrinol* 2001;168:117–30. [PubMed: 11139776]
82. Shirasuna K, Watanabe S, Yamamoto D, Hayashi M, Nagai K, Miyamoto A. Bovine endothelial cells interact with fully-luteinized, but not luteinizing, granulosa cells in the mRNA expression of endothelin-1 system in response to prostaglandin F(2alpha). *Reprod Domest Anim* 2007;42:637–42. [PubMed: 17976072]
83. Kito S, Hayao T, Noguchi-Kawasaki Y, Ohta Y, Hideki U, Tateno S. Improved in vitro fertilization and development by use of modified human tubal fluid and applicability of pronucleate embryos for cryopreservation by rapid freezing in inbred mice. *Comp Med* 2004;54:564–70. [PubMed: 15575371]
84. Munuce MJ, Serravalle A, Caille AM, Zumoffen C, Botti G, Cabada M, Ghersevich S. Human tubal secretion can modify the affinity of human spermatozoa for the zona pellucida. *Fertil Steril* 2009;91:407–13. [PubMed: 18295213]
85. Bareither ML, Verhage HG. Control of the secretory cell cycle in cat oviduct by estradiol and progesterone. *Am J Anat* 1981;162:107–18. [PubMed: 7304467]
86. Shirley B, Reeder RL. Cyclic changes in the ampulla of the rat oviduct. *J Exp Zool* 1996;276:164–73. [PubMed: 8900079]
87. Karam H, Valdenaire O, Belair MF, Prigent-Sassy C, Rakotosalama A, Clozel M, Itskovitz J, Bruneval P. The endothelin system in human and monkey ovaries: in situ gene expression of the different components. *Cell Tissue Res* 1999;295:101–9. [PubMed: 10048935]
88. Apa R, Miceli F, de Feo D, Pierro E, Ayala G, Mancuso S, Napolitano M, Lanzzone A. Endothelin-1: expression and role in human corpus luteum. *Am J Reprod Immunol* 1998;40:370–6. [PubMed: 9870082]
89. Matsumoto S, Yorio T, Magnino PE, DeSantis L, Pang IH. Endothelin-induced changes of second messengers in cultured human ciliary muscle cells. *Invest Ophthalmol Vis Sci* 1996;37:1058–66. [PubMed: 8631621]
90. Mancina R, Barni T, Calogero AE, Filippi S, Amerini S, Peri A, Susini T, Vannelli GB, Burrello N, Forti G, Maggi M. Identification, characterization, and biological activity of endothelin receptors in human ovary. *J Clin Endocrinol Metab* 1997;82:4122–9. [PubMed: 9398725]
91. Yoshioka S, Fujiwara H, Yamada S, Tatsumi K, Nakayama T, Higuchi T, Inoue T, Maeda M, Fujii S. Endothelin-converting enzyme-1 is expressed on human ovarian follicles and corpora lutea of menstrual cycle and early pregnancy. *J Clin Endocrinol Metab* 1998;83:3943–50. [PubMed: 9814473]
92. Korth P, Bohle RM, Corvol P, Pinet F. Cellular distribution of endothelin-converting enzyme-1 in human tissues. *J Histochem Cytochem* 1999;47:447–62. [PubMed: 10082746]
93. Kawamura K, Ye Y, Liang CG, Kawamura N, Gelpke MS, Rauch R, Tanaka T, Hsueh AJ. Paracrine regulation of the resumption of oocyte meiosis by endothelin-1. *Dev Biol* 2009;327:62–70. [PubMed: 19111534]
94. Bridges PJ, Jo M, Al Alem L, Na G, Su W, Gong MC, Jeoung M, Ko C. Production and binding of endothelin-2 (EDN2) in the rat ovary: Endothelin receptor subtype A (EDNRA) mediated contraction. *Reprod Fertil Dev* 2010;22:1–8.
95. Magazine HI, Andersen TT, Goligorsky MS, Malik AB. Evaluation of endothelin receptor populations using endothelin-1 biotinylated at lysine-9 sidechain. *Biochem Biophys Res Commun* 1991;181:1245–50. [PubMed: 1662495]
96. Levy N, Gordin M, Mamluk R, Yanagisawa M, Smith MF, Hampton JH, Meidan R. Distinct cellular localization and regulation of endothelin-1 and endothelin-converting enzyme-1

- expression in the bovine corpus luteum: implications for luteolysis. *Endocrinology* 2001;142:5254–60. [PubMed: 11713223]
97. Girsh E, Wang W, Mamluk R, Arditi F, Friedman A, Milvae RA, Meidan R. Regulation of endothelin-1 expression in the bovine corpus luteum: elevation by prostaglandin F 2 alpha. *Endocrinology* 1996;137:5191–6. [PubMed: 8940334]
98. Levy N, Gordin M, Smith MF, Bolden-Tiller OU, Meidan R. Hormonal regulation and cell-specific expression of endothelin-converting enzyme 1 isoforms in bovine ovarian endothelial and steroidogenic cells. *Biol Reprod* 2003;68:1361–8. [PubMed: 12606425]

Figure 1.

The ovarian smooth muscle network. (Top panels) A section through a 5-week old mouse ovary. (Bottom panels) A section through a 47-years old woman's ovary. The sections were stained with an antibody for α -SMA (reddish staining) followed by a counter staining with Mayer's hematoxylin (blue). The high magnification images (400X) were taken in the boxed areas. CA, corpus albicans; FSM, follicular smooth muscle.

Figure 2. Endothelin types responsible for ovarian and oviductal function. Different stages of follicles, oocytes and CL are shown. The biological target of endothelin actions and responsible endothelin subtypes are listed.

Table 1

Ovarian expression of endothelins, their receptors and converting enzymes

Species	Types	Localization	References
Human	EDN1	GC, EC, LC	(87-89)
	EDN2		
	EDNRA	SC, GC, TC	(87,90)
	EDNRB	LC	(87,91,92)
	ECE-1	GC, LC, TC	(91,92)
	ECE-2		
Mouse	EDN1	GC	(46,93)
	EDN2	GC	(46,49)
	EDNRA	GC,	(46,93)
	EDNRB	GC, EC	(46)
	ECE-1		(94)
	ECE-2		(94)
Rat	EDN1	GC	(14)
	EDN2	GC	(14)
	EDNRA	SC, GC	(95,13)
	EDNRB	GC	(13)
	ECE-1	GC	(14)
	ECE-2	GC	(14)
Bovine	EDN1	GC, LC, EC	(96,97)
	EDN2	LC	(51)
	EDNRA	LC	(97)
	EDNRB		
	ECE-1	GC, TC, LC, EC	(70,96,98)
	ECE-2		

GC, granulosa cells; TC, theca cells; LC, luteal cells; EC, endothelial cells; SC, smooth muscle cells