

Pigment Epithelium - Derived Factor (PEDF) as an Early Marker of Reproductive Aging in Female - A Crosssectional Study

Abirami Girija

Department of Zoology, Gurudas College, University of Calcutta, 1/1, Suren Sarkar Rd, Jewish Graveyard, Phool Bagan, Narkeldanga, Kolkata, West Bengal 700054, India
abirami.gir@gmail.com

Abstract: *Reproductive aging is a biological phenomenon that is seen in women, noted by a progressive decline in ovarian function and a decrease in both the quantity and quality of oocytes by changes at the molecular level. Studies have shown that the pigment epithelium - derived factor (PEDF) plays a crucial role in maintaining ovarian angiogenic, and inflammatory and oxidative balance. Thus, an exploration of the involvement of PEDF with ovarian aging is necessary to gain a deeper knowledge of the mechanism which depicts the decreasing ovarian function and the deterioration of oocyte quantity and quality during the mid - 30s to 40s of a woman's life. The evaluation of PEDF in reproductive aging holds promise for advancing our knowledge of the factors and possible signal cascade that change ovarian health which eventually leads to ovarian aging. It may also provide valuable insights into potential therapeutic strategies or interventions that could help to solve female fertility - related problems due to the global trend in late - age childbearing. In this review we are debating whether PEDF could be a potential candidate for an early marker to detect reproductive aging; also, we relate oxidative stress (OS) with reproductive aging and the possible role of PEDF in it.*

Keywords: Reproductive aging, Cancer, Ovulation, Oxidative stress, AKT, Estrogen

1. Introduction

Reproductive aging is a biological process, commonly seen in women. The process begins in women in their mid to late 30s and continues till menopause when females stop ovulating [1]. Reproductive aging is characterized by a gradual decrease in ovarian function, eventually leading to the disturbance of the hormonal feedback loop controlling female reproductive events. In general, the number of growing follicles is decreased as a result of key secretory products like Estrogens, Follicular Stimulation Hormone (FSH), and Luteinizing Hormone (LH) production being disturbed, thus narrowing the fertility window until it is finally shut down [2, 3]. This long process continues for several years and can be categorized into three phases [4]. The first phase is an initial stage of ovarian aging where the hormonal feedback loop tries to compensate for the effect of declining growing follicle numbers accompanied by an increased level of FSH and a decreased level of Anti - Müllerian Hormone (AMH), thus indicating reduced numbers of egg and ovulation rate. In the second phase, the ongoing follicle loss leads to irregular cycles, abnormal and irregular follicle development, and a level of Estradiol followed by a dramatic increase in FSH [5, 6, 7]. This phase is also known as progressive ovarian aging. In the third and final menstrual period, only a reduced level of ovarian hormone secretion can be detected [4].

Reproductive aging and reduced fertility rates are related to physiological and molecular changes. This biological phenomenon develops along the canonical molecular pathway and can impact aging processes in the organism at the level of cells, tissues, organs, and systems. The changes due to reproductive aging are detected both in oocytes and granulosa cells (GC), which maintain a vital bi - directional communication for the ovulation of good - quality oocytes.

Research has indicated that reproductively - aged (RA) patients often experience excessive aneuploidy in their oocytes. Aneuploidy refers to errors in the number of chromosomes, which can have significant implications for reproductive health [8]. In addition to aneuploidy, mitochondrial dysfunction is also observed in these patients. This dysfunction is primarily caused by an over - production of reactive oxygen species (ROS) [9]. The impaired mitochondrial function can hinder the proper maturation of oocytes [10, 11]. This is evidenced by a decrease in germinal vesicle (GV) breakdown and first polar - body extrusion (PBE) [12, 13]. These are important markers of oocyte maturation. When these processes are disrupted, it can negatively impact the overall quality of the oocytes. Furthermore, studies represent that the primary granulosa cells (pGCs) of reproductively - aged women (≥ 35 years) and mice bear significant changes in mRNA, proteome, and transcriptome profiles. For instance, Ye *et al.* identified 371 differentially expressed genes between ovarian secondary follicles of reproductively - aged mice (32 weeks old) and young mice (9 weeks old) [15]. These genes are associated with various biological processes related to reproductive aging, such as immune response, DNA transcription and replication, and apoptosis. In summary, the research suggests reproductively aged individuals often exhibit excessive aneuploidy in their oocytes and mitochondrial dysfunction. These factors can lead to compromised oocyte maturation and changes in gene expression profiles, highlighting the biological processes associated with reproductive aging [14]. PEDF, which is encoded by the SERPINF1 gene, is a glycoprotein that is secreted by cells. It belongs to the non - inhibitory members of the serine protease inhibitors (serpin) superfamily [16]. PEDF was first purified from the conditioned media of human retinal pigment epithelium [17]. Initially, PEDF was identified as a neurotrophic agent with the ability to induce the differentiation of Y79

retinoblastoma tumor cells into non - proliferative, mature neurons [18]. Later in 1999, it is discovered that PEDF possesses not only neurotrophic properties but also significant anti - angiogenic effects [19]. This finding expanded the understanding of PEDF's functions. PEDF was recognized as an anti - angiogenic factor regulating vascularity in ocular compartments [20]. Research indicated that PEDF plays a role in the development of angiogenic eye diseases like proliferative diabetic retinopathy and others [21]. PEDF's anti - angiogenic activity is noteworthy due to its selectivity and reversibility. It specifically targets newly forming blood vessels while leaving existing ones untouched. Moreover, it does not interfere with the transient but essential process of creating new blood vessels during times of injury [20]. Later research shows that PEDF also has anti - inflammatory, and anti - oxidative qualities [25, 26].

The 50 - kDa PEDF glycoprotein is also secreted and expressed from the female reproductive system [22]. Previous investigations focused on elucidating the role of PEDF in the ovary, revealing that GCs in both rodents and humans are responsible for producing and secreting PEDF in a manner influenced by hormonal signals. Notably, the expression and secretion of PEDF in GCs exhibit an inverse relationship with the up - regulation of vascular endothelial growth factor (VEGF). Hormones such as Estradiol, LH, and progesterone exert down - regulatory effects on PEDF expression and secretion by GCs [23, 24].

Moreover, earlier research proposed that low endogenous levels of PEDF in the ovary may be associated with various pathologies, including ovarian hyper stimulation syndrome (OHSS) [24, 27], and polycystic ovary syndrome (PCOS) [28]. However, little is known about PEDF regarding reproductive aging. Many efforts are invested to reduce and overcome the fertility problems of patients in their 40s and prioritizing this fact, this review is focused on the potential role of PEDF in ovarian aging, and probable involvement in OS and the onset of reproductive aging.

2. POST - translational modification of PEDF

PEDF is a versatile protein expressed by most cells in the body [29]. It is primarily secreted as a soluble monomeric glycoprotein. During its synthesis, PEDF undergoes N - glycosylation at the Asn285 site, which adds sugar molecules to the protein structure. PEDF is a phosphoprotein, meaning it undergoes phosphorylation, a process where phosphate groups are added to specific amino acids in the protein. The phosphorylation sites of PEDF play a crucial role in determining its function as either neurotrophic or anti - angiogenic [30, 31]. The neurotrophic activity of PEDF is mediated by protein kinase A (PKA), which phosphorylates PEDF on Ser 227, an amino acid residue. On the other hand, the anti - angiogenic activity of PEDF is mediated by casein kinase 2 (CK2), which phosphorylates PEDF on Ser 24 and Ser 114. Interestingly, the most potent anti - angiogenic and neurotrophic activities of PEDF are observed when PKA phosphorylation precedes CK2 phosphorylation [31]. In addition to its neurotrophic and anti - angiogenic properties, PEDF has been found to exhibit anti - inflammatory effects [32] and acts as an anti -

oxidative factor [33]. These additional functions contribute to the diverse roles that PEDF plays in cellular processes and its potential therapeutic applications.

3. PEDF Receptor

The diverse function of PEDF has caused researchers to investigate the receptor/s of PEDF. The result shows that there are multiple receptors of PEDF involved in various functioning of PEDF [31]. The palatine - like phospholipase domain - containing 2 (PNPLA - 2 or PEDF - RN) is involved in the neurotrophic activity of PEDF. Laminin receptor (LF or PEDF - RA) is believed to involve in angiogenic and pro - apoptotic activity [25]. On the other hand, Lipoprotein receptor - related protein - 5 (LRP - 5) also known as Wnt co - receptor has been suggested as a potential receptor of PEDF [34] and also cell surface F1FO - ATP synthase is identified as a mediator of PEDF for its angiogenic activity [35].

4. PEDF relation with VEGF

Emerging evidence suggests that the interplay between PEDF and VEGF is crucial for maintaining a balanced angiogenic environment under normal physiological conditions. PEDF has been shown to counteract the pro - angiogenic effects of VEGF. Specifically, in certain situations such as OS conditions, the level of PEDF can decrease while VEGF remains unaffected. This disruption in the balance between PEDF and VEGF provides a selective advantage to the pro - angiogenic activity of VEGF [36]. Consequently, this imbalance can contribute to the development of choroidal neo - vascularization, a characteristic feature of age - related macular degeneration (AMD). Studies have demonstrated that treatment with recombinant PEDF (rPEDF) can restore the angiogenic balance between PEDF and VEGF. By administering rPEDF, the disrupted equilibrium can be rectified, leading to a reduction in AMD symptoms [37]. The delicate interplay between PEDF and VEGF underscores the importance of maintaining their balance for proper angiogenic regulation. Understanding this relationship provides insights into the pathogenesis of AMD and highlights the potential therapeutic applications of restoring the PEDF - VEGF equilibrium.

Another experiment shows an increase in PEDF mRNA levels in the GCs of rheumatoid arthritis (RA) patients, coupled with a decrease in intracellular PEDF protein levels, which suggests potential flaws in the translational mechanisms or alterations in protein stability within the GCs of RA patients [38]. Aging is commonly associated with a general reduction in protein synthesis [39]. In somatic - aged cells of various tissues, the altered transcriptome is often attributed to impaired transcription, translation, and protein regulation within the cells [40]. Under stressful conditions, including cellular aging, the integrated stress response (ISR) is activated. The ISR is an intracellular signaling network that maintains protein balance in response to environmental changes [39, 41, 42]. In the case of RA GCs, the elevated PEDF mRNA levels may be a compensatory response to the decrease in protein levels. This up - regulation of transcription could be a feedback mechanism to

counterbalance the decline in PEDF protein levels. Excessive transcription of PEDF mRNA may occur in an attempt to restore PEDF protein levels in the GCs of RA patients. On the other hand, it has been also observed that there is a decrease in the level of PEDF protein in the follicular fluid (FF) of RA patients compared to young individuals. Interestingly, the ratio of FF to GC PEDF protein levels remained similar between the two age groups, again indicating that the reduction in PEDF protein observed in RA patients is not due to altered secretion but rather reflects a decrease in its production or stability within the GCs.

Consistent with previous studies [43, 44], an elevated concentration of VEGF protein has been found in the FF of RA patients. This implies that the increase in VEGF is a gene - specific mechanism rather than a general property of aging. The concurrent decrease in PEDF and increase in VEGF protein levels further intensify the inverted relationship between PEDF and VEGF, as demonstrated in various models [36, 45, 46]. As a result, the calculated PEDF/VEGF protein ratio was significantly decreased in RA patients and inversely correlated with patient age. This suggests an impaired angiogenic balance at the onset of ovarian aging in RA patients. These findings highlight the potential of measuring PEDF protein levels as an informative and reliable method for detecting the initiation of reproductive aging in women.

5. PEDF can be associated with age - related endometrial cancer

Throughout a woman's fertile life, the human endometrium undergoes significant cyclic morphological and biochemical changes during each menstrual cycle. These modifications involve regeneration, breakdown, and shedding of the endometrial tissue, and are tightly regulated by the ovarian steroids estradiol (E2) and progesterone (P4), and their respective receptors [47]. Mice lacking Estrogen receptor alpha (ER α) failed to develop uterine angiogenesis thus suggesting the pro - angiogenic activity of E2 in the uterus [48]. On the other hand, the role of P4 in endometrial angiogenesis is not as well - established. Studies have shown that P4 administration combined with low doses of E2 can inhibit the proliferation of endothelial cells. However, high doses of E2 and P4 administration can cause a significant increase in the proliferation of endometrial endothelial cells [49]. The spatiotemporal expression of VEGF can be a result of the E2 and P4 effects on the endometrium. VEGF has many isoforms like VEGF 165, VEGF 121, VEGF 145, and VEGF 189, all regulated by hormones. The dominant isoform seen in the human endometrium and ovary is VEGF 165, and its expression is altered dynamically during the menstrual cycle [50].

The dynamic expression patterns of PEDF in the endometrium of both humans and rodents are seen during the menstrual and oestrus cycles respectively. This expression pattern was found to be reciprocally related to the expression of VEGF, another important factor involved in uterine physiology. PEDF levels decrease with age, suggesting that this decrease could contribute to the higher incidence of cancer seen in older individuals. Further

research shows that the hormones E2 and P4 play a role in modulating PEDF expression. E2 was found to decrease PEDF levels, while P4, when administered after E2 treatment, increased PEDF expression. Further investigations revealed the presence of PEDF - RN in the human endometrium, primarily localized within the endometrial glands. Moreover, *in vitro* experiments demonstrated that rPEDF down - regulates VEGF expression [24].

6. PEDF and OS

Production of ROS and decrease in antioxidants is inversely related with age, thus suggesting OS increases with increasing age. PEDF is a good candidate for antioxidants and has been demonstrated in several models. Investigation into PEDF and its ability to fight OS shows that administration of PEDF blocks activation and aggregation of platelets which eventually leads to the inhibition of the formation of occlusive thrombosis [51]. It is assumed that PEDF fights OS by activating the AKT signaling pathway in GCs, thus promoting its anti - apoptotic action.

The serine/threonine kinase AKT plays a crucial role in regulating GC survival and apoptosis throughout folliculogenesis in the ovary. AKT is known to be activated by various cellular stimuli, including growth factors and cytokines. However, it is later discovered that treatment with sphingosine - 1 - phosphate can inhibit granulosa cell apoptosis induced by OS caused by hydrogen peroxide (H₂O₂) through the AKT signaling pathway [52]. Considering the anti - apoptotic properties of PEDF in granulosa cells, the researchers hypothesized that PEDF may activate AKT in granulosa cells. Their investigations supported this hypothesis, as they found that the administration of recombinant rPEDF to both a rat granulosa cell line and human primary granulosa cell culture resulted in AKT phosphorylation. Interestingly, PEDF induced more pronounced AKT phosphorylation in the human primary granulosa cell culture compared to the rat cell line, suggesting that the abundance of PEDF receptors may differ between the two. Additionally, the researchers observed that rPEDF counteracted the down - regulation of AKT induced by H₂O₂ in human primary granulosa cell culture. These findings led to the conclusion that PEDF acts as a novel intrinsic antioxidant in granulosa cells, exerting its pro - survival effect, at least in part, through the AKT signaling pathway, particularly under conditions of OS [25].

These discoveries contribute to our understanding of the molecular mechanisms involved in granulosa cell survival and apoptosis regulation. By uncovering the role of PEDF and its interaction with AKT, this research highlights the potential therapeutic implications for manipulating these pathways in the context of reproductive health and fertility - related disorders. Further investigations are needed to fully elucidate the complex interplay between PEDF, AKT, and other signaling molecules in granulosa cell function and ovarian physiology.

7. Conclusion

The ovarian aging process has gained significant attention due to the global trend of delaying childbearing. Several markers have been identified to predict the onset of ovarian aging, including antral follicle count, basal FSH, and AMH levels. However, new studies focusing on the late stages of folliculogenesis, such as oocyte maturation, GC transition during ovulation, and FF production, suggest that PEDF may be involved in the early onset of reproductive aging. The study proposes PEDF as a potential early marker for this process. Also, by uncovering the role of PEDF and its interaction with AKT, PEDF is highlighted as a potential therapeutic supplement candidate in assisted reproductive technology (ART) for individuals with reduced PEDF levels. However, the specific mechanism behind this process requires further research to be determined.

References

- [1] Nikolaou D, Templeton A. Early ovarian aging: a hypothesis. Detection and clinical relevance. *Hum Reprod.*2003 Jun; 18 (6): 1137 - 9. doi: 10.1093/humrep/deg245. PMID: 12773436.
- [2] McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev.*2000 Apr; 21 (2): 200 - 14. doi: 10.1210/edrv.21.2.0394. PMID: 10782364.
- [3] Shi L, Zhang J, Lai Z, Tian Y, Fang L, Wu M, Xiong J, Qin X, Luo A, Wang S. Long - Term Moderate OS Decreased Ovarian Reproductive Function by Reducing Follicle Quality and Progesterone Production. *PLoS One.*2016 Sep 27; 11 (9): e0162194. doi: 10.1371/journal.pone.0162194. PMID: 27676390; PMCID: PMC5038974.
- [4] Hall JE. Endocrinology of the Menopause. *Endocrinol Metab Clin North Am.*2015 Sep; 44 (3): 485 - 96. doi: 10.1016/j.ecl.2015.05.010. PMID: 26316238; PMCID: PMC6983294.
- [5] Traub ML, Santoro N. Reproductive aging and its consequences for general health. *Ann N Y Acad Sci.*2010 Aug; 1204: 179 - 87. doi: 10.1111/j.1749 - 6632.2010.05521. x. PMID: 20738289.
- [6] Nelson HD. Menopause. *Lancet.*2008 Mar 1; 371 (9614): 760 - 70. doi: 10.1016/S0140 - 6736 (08) 60346 - 3. PMID: 18313505.
- [7] Park SU, Walsh L, Berkowitz KM. Mechanisms of ovarian aging. *Reproduction.*2021 Jul 14; 162 (2): R19 - R33. doi: 10.1530/REP - 21 - 0022. PMID: 33999842; PMCID: PMC9354567.
- [8] Lagirand - Cantaloube J, Ciabrini C, Charrasse S, Ferrieres A, Castro A, Anahory T, Lorca T. Loss of Centromere Cohesion in Aneuploid Human Oocytes Correlates with Decreased Kinetochores Localization of the Sac Proteins Bub1 and Bubl1. *Sci Rep.*2017 Mar 13; 7: 44001. doi: 10.1038/srep44001. PMID: 28287092; PMCID: PMC5347135.
- [9] Rizzo A, Roscino MT, Binetti F, Sciorsci RL. Roles of reactive oxygen species in female reproduction. *Reprod Domest Anim.*2012 Apr; 47 (2): 344 - 52. doi: 10.1111/j.1439 - 0531.2011.01891. x. Epub 2011 Oct 25. PMID: 22022825.
- [10] May - Panloup P, Boucret L, Chao de la Barca JM, Desquiret - Dumas V, Ferré - L'Hotellier V, Morinière C, Descamps P, Procaccio V, Reynier P. Ovarian aging: the role of mitochondria in oocytes and follicles. *Hum Reprod Update.*2016 Nov; 22 (6): 725 - 743. doi: 10.1093/humupd/dmw028. Epub 2016 Aug 25. PMID: 27562289.
- [11] Van der Reest J, Nardini Cecchino G, Haigis MC, Kordowitzki P. Mitochondria: Their relevance during oocyte ageing. *Ageing Res Rev.*2021 Sep; 70: 101378. doi: 10.1016/j.arr.2021.101378. Epub 2021 Jun 4. PMID: 34091076.
- [12] Liu XM, Zhang YP, Ji SY, Li BT, Tian X, Li D, Tong C, Fan HY. Mitoguardin - 1 and - 2 promote maturation and the developmental potential of mouse oocytes by maintaining mitochondrial dynamics and functions. *Oncotarget.*2016 Jan 12; 7 (2): 1155 - 67. doi: 10.18632/oncotarget.6713. PMID: 26716412; PMCID: PMC4811450.
- [13] Mihalas BP, De Iuliis GN, Redgrove KA, McLaughlin EA, Nixon B. The lipid peroxidation product 4 - hydroxynonenal contributes to OS - mediated deterioration of the ageing oocyte. *Sci Rep.*2017 Jul 24; 7 (1): 6247. doi: 10.1038/s41598 - 017 - 06372 - z. PMID: 28740075; PMCID: PMC5524799.
- [14] Al - Edani T, Assou S, Ferrières A, Bringer Deutsch S, Gala A, Lecellier CH, Ait - Ahmed O, Hamamah S. Female aging alters expression of human cumulus cells genes that are essential for oocyte quality. *Biomed Res Int.*2014; 2014: 964614. doi: 10.1155/2014/964614. Epub 2014 Sep 3. PMID: 25276836; PMCID: PMC4168028.
- [15] Ye W, Shen W, Yan W, Zhou S, Cheng J, Pan G, Wu M, Ma L, Luo A, Wang S. What Changed on the Folliculogenesis in the Process of Mouse Ovarian Aging? *Biomed Res Int.*2019 Apr 1; 2019: 3842312. doi: 10.1155/2019/3842312. PMID: 31058188; PMCID: PMC6463680.
- [16] Becerra SP, Sagasti A, Spinella P, Notario V. Pigment epithelium - derived factor behaves like a noninhibitory serpin. Neurotrophic activity does not require the serpin reactive loop. *J Biol Chem.*1995 Oct 27; 270 (43): 25992 - 9. doi: 10.1074/jbc.270.43.25992. PMID: 7592790.
- [17] Tombran - Tink J, Chader GG, Johnson LV. PEDF: a pigment epithelium - derived factor with potent neuronal differentiative activity. *Exp Eye Res.*1991 Sep; 53 (3): 411 - 4. doi: 10.1016/0014 - 4835 (91) 90248 - d. PMID: 1936177.
- [18] Steele FR, Chader GJ, Johnson LV, Tombran - Tink J. Pigment epithelium - derived factor: neurotrophic activity and identification as a member of the serine protease inhibitor gene family. *Proc Natl Acad Sci U S A.*1993 Feb 15; 90 (4): 1526 - 30. doi: 10.1073/pnas.90.4.1526. PMID: 8434014; PMCID: PMC45907.
- [19] Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, Bouck NP. Pigment epithelium - derived factor: a potent inhibitor of angiogenesis. *Science.*1999 Jul 9; 285 (5425): 245 - 8. doi: 10.1126/science.285.5425.245. PMID: 10398599.
- [20] Bouck N. PEDF: anti - angiogenic guardian of ocular function. *Trends Mol Med.*2002 Jul; 8 (7): 330 - 4. doi: 10.1016/s1471 - 4914 (02) 02362 - 6. PMID: 12114112.

- [21] Volpert OV, Zaichuk T, Zhou W, Reiher F, Ferguson TA, Stuart PM, Amin M, Bouck NP. Inducer - stimulated Fas targets activated endothelium for destruction by anti - angiogenic thrombospondin - 1 and pigment epithelium - derived factor. *Nat Med.*2002 Apr; 8 (4): 349 - 57. doi: 10.1038/nm0402 - 349. PMID: 11927940.
- [22] Becerra SP, Dass CR, Yabe T, Crawford SE. Pigment epithelium - derived factor: chemistry, structure, biology, and applications. *J Biomed Biotechnol.*2012; 2012: 830975. doi: 10.1155/2012/830975. Epub 2012 Sep 5. PMID: 22988425; PMCID: PMC3441038.
- [23] Chuderland D, Ben - Ami I, Kaplan - Kraicer R, Grossman H, Ron - El R, Shalgi R. The role of pigment epithelium - derived factor in the pathophysiology and treatment of ovarian hyperstimulation syndrome in mice. *J Clin Endocrinol Metab.*2013 Feb; 98 (2): E258 - 66. doi: 10.1210/jc.2012 - 3037. Epub 2013 Jan 7. PMID: 23295464.
- [24] Chuderland D, Ben - Ami I, Kaplan - Kraicer R, Grossman H, Komsky A, Satchi - Fainaro R, Eldar - Boock A, Ron - El R, Shalgi R. Hormonal regulation of pigment epithelium - derived factor (PEDF) in granulosa cells. *Mol Hum Reprod.*2013 Feb; 19 (2): 72 - 81. doi: 10.1093/molehr/gas046. Epub 2012 Oct 16. PMID: 23075882.
- [25] Bar - Joseph H, Ben - Ami I, Ron - El R, Shalgi R, Chuderland D. Pigment epithelium - derived factor exerts antioxidative effects in granulosa cells. *Fertil Steril.*2014 Sep; 102 (3): 891 - 898. e3. doi: 10.1016/j.fertnstert.2014.06.012. Epub 2014 Jul 23. PMID: 25064413.
- [26] Nemerovsky L, Bar - Joseph H, Eldar - Boock A, Miller I, Ben - Ami I, Shalgi R. Pigment epithelium - derived factor negates OS in mouse oocytes. *FASEB J.*2021 Jun; 35 (6): e21637. doi: 10.1096/fj.202002443RR. PMID: 33948995.
- [27] Miller I, Chuderland D, Grossman H, Ron - El R, Ben - Ami I, Shalgi R. The Dual Role of PEDF in the Pathogenesis of OHSS: Negating Both Angiogenic and Inflammatory Pathways. *J Clin Endocrinol Metab.*2016 Dec; 101 (12): 4699 - 4709. doi: 10.1210/jc.2016 - 1744. Epub 2016 Sep 28. PMID: 27680872.
- [28] Miller I, Bar - Joseph H, Nemerovsky L, Ben - Ami I, Shalgi R. Pigment epithelium - derived factor (PEDF) negates hyperandrogenic PCOS features. *J Endocrinol.*2020 May; 245 (2): 291 - 300. doi: 10.1530/JOE - 19 - 0603. PMID: 32171180.
- [29] Becerra SP, Notario V. The effects of PEDF on cancer biology: mechanisms of action and therapeutic potential. *Nat Rev Cancer.*2013 Apr; 13 (4): 258 - 71. doi: 10.1038/nrc3484. Epub 2013 Mar 14. PMID: 23486238; PMCID: PMC3707632.
- [30] Maik - Rachline G, Shaltiel S, Seger R. Extracellular phosphorylation converts pigment epithelium - derived factor from a neurotrophic to an antiangiogenic factor. *Blood.*2005 Jan 15; 105 (2): 670 - 8. doi: 10.1182/blood - 2004 - 04 - 1569. Epub 2004 Sep 16. PMID: 15374885.
- [31] Maik - Rachline G & Seger R. Variable phosphorylation states of pigment - epithelium - derived factor differentially regulate its function. *Blood.*2006 107 2745-2752. (doi: 10.1182/blood - 2005 - 06 - 2547)
- [32] Wang JJ, Zhang SX, Mott R, Chen Y, Knapp RR, Cao W, Ma JX. Anti - inflammatory effects of pigment epithelium - derived factor in diabetic nephropathy. *Am J Physiol Renal Physiol.*2008 May; 294 (5): F1166 - 73. doi: 10.1152/ajprenal.00375.2007. Epub 2008 Mar 5. PMID: 18322021.
- [33] Yamagishi S, Nakamura K, Ueda S, Kato S, Imaizumi T. Pigment epithelium - derived factor (PEDF) blocks angiotensin II signaling in endothelial cells via suppression of NADPH oxidase: a novel anti - oxidative mechanism of PEDF. *Cell Tissue Res.*2005 Jun; 320 (3): 437 - 45. doi: 10.1007/s00441 - 005 - 1094 - 8. Epub 2005 Apr 22. PMID: 15846509.
- [34] Anne SL, Govek EE, Ayrault O, Kim JH, Zhu X, Murphy DA, Van Aelst L, Roussel MF, Hatten ME. WNT3 inhibits cerebellar granule neuron progenitor proliferation and medulloblastoma formation via MAPK activation. *PLoS One.*2013 Nov 26; 8 (11): e81769. doi: 10.1371/journal.pone.0081769. PMID: 24303070; PMCID: PMC3841149.
- [35] Notari L, Arakaki N, Mueller D, Meier S, Amaral J, Becerra SP. Pigment epithelium - derived factor binds to cell - surface F (1) - ATP synthase. *FEBS J.*2010 May; 277 (9): 2192 - 205. doi: 10.1111/j.1742 - 4658.2010.07641. x. PMID: 20412062; PMCID: PMC2929661.
- [36] Ohno - Matsui K, Morita I, Tombran - Tink J, Mrazek D, Onodera M, Uetama T, Hayano M, Murota SI, Mochizuki M. Novel mechanism for age - related macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF. *J Cell Physiol.*2001 Dec; 189 (3): 323 - 33. doi: 10.1002/jcp.10026. PMID: 11748590.
- [37] Popp N, Chu XK, Shen D, Tuo J, Chan CC. Evaluating Potential Therapies in a Mouse Model of Focal Retinal Degeneration with Age - related Macular Degeneration (AMD) - Like Lesions. *J Clin Exp Ophthalmol.*2013 Oct 1; 4 (5): 1000296. doi: 10.4172/2155 - 9570.1000296. PMID: 24432192; PMCID: PMC3890246.
- [38] Nemerovsky L, Bar - Joseph H, Eldar - Boock A, Tarabeih R, Elmechaly C, Ben - Ami I, Shalgi R. The Role of PEDF in Reproductive Aging of the Ovary. *Int J Mol Sci.*2022 Sep 8; 23 (18): 10359. doi: 10.3390/ijms231810359. PMID: 36142276; PMCID: PMC9499037.
- [39] Derisbourg MJ, Hartman MD, Denzel MS. Perspective: Modulating the integrated stress response to slow aging and ameliorate age - related pathology. *Nat Aging.*2021 Sep; 1 (9): 760 - 768. doi: 10.1038/s43587 - 021 - 00112 - 9. Epub 2021 Sep 13. PMID: 35146440; PMCID: PMC7612338.
- [40] Woodward K, Shirokikh NE. Translational control in cell ageing: an update. *Biochem Soc Trans.*2021 Dec 17; 49 (6): 2853 - 2869. doi: 10.1042/BST20210844. PMID: 34913471; PMCID: PMC8786278.
- [41] Llerena Cari E, Hagen - Lillevik S, Giornazi A, Post M, Komar AA, Appiah L, Bitler B, Polotsky AJ, Santoro N, Kieft J, Lai K, Johnson J. Integrated stress response control of granulosa cell translation and proliferation during normal ovarian follicle

- development. *Mol Hum Reprod.*2021 Aug 7; 27 (8): gaab050. doi: 10.1093/molehr/gaab050. PMID: 34314477; PMCID: PMC8660582.
- [42] Costa - Mattioli M, Walter P. The integrated stress response: From mechanism to disease. *Science.*2020 Apr 24; 368 (6489): eaat5314. doi: 10.1126/science.aat5314. PMID: 32327570; PMCID: PMC8997189.
- [43] Fujii EY, Nakayama M. The measurements of RAGE, VEGF, and AGEs in the plasma and follicular fluid of reproductive women: the influence of aging. *Fertil Steril.*2010 Jul; 94 (2): 694 - 700. doi: 10.1016/j.fertnstert.2009.03.029. Epub 2009 Apr 18. PMID: 19376511.
- [44] Wu WB, Chen HT, Lin JJ, Lai TH. VEGF Concentration in a Preovulatory Leading Follicle Relates to Ovarian Reserve and Oocyte Maturation during Ovarian Stimulation with GnRH Antagonist Protocol in *in vitro*Fertilization Cycle. *J Clin Med.*2021 Oct 28; 10 (21): 5032. doi: 10.3390/jcm10215032. PMID: 34768551; PMCID: PMC8584358.
- [45] Miller I, Chuderland D, Ron - El R, Shalgi R, Ben - Ami I. GnRH Agonist Triggering Modulates PEDF to VEGF Ratio Inversely to hCG in Granulosa Cells. *J Clin Endocrinol Metab.*2015 Nov; 100 (11): E1428 - 36. doi: 10.1210/jc.2015 - 2312. Epub 2015 Aug 26. PMID: 26308290.
- [46] Goldberg K, Bar - Joseph H, Grossman H, Hasky N, Uri - Belapolsky S, Stemmer SM, Chuderland D, Shalgi R, Ben - Aharon I. Pigment Epithelium - Derived Factor Alleviates Tamoxifen - Induced Endometrial Hyperplasia. *Mol Cancer Ther.*2015 Dec; 14 (12): 2840 - 9. doi: 10.1158/1535 - 7163.MCT - 15 - 0523. Epub 2015 Oct 8. PMID: 26450919.
- [47] Smith SK. Regulation of angiogenesis in the endometrium. *Trends Endocrinol Metab.*2001 May - Jun; 12 (4): 147 - 51. doi: 10.1016/s1043 - 2760 (01) 00379 - 4. PMID: 11295569.
- [48] Johns A, Freay AD, Fraser W, Korach KS, Rubanyi GM. Disruption of estrogen receptor gene prevents 17 beta estradiol - induced angiogenesis in transgenic mice. *Endocrinology.*1996 Oct; 137 (10): 4511 - 3. doi: 10.1210/endo.137.10.8828515. PMID: 8828515.
- [49] Heryanto B, Rogers PA. Regulation of endometrial endothelial cell proliferation by oestrogen and progesterone in the ovariectomized mouse. *Reproduction.*2002 Jan; 123 (1): 107 - 13. doi: 10.1530/rep.0.1230107. PMID: 11869192.
- [50] Lash GE, Innes BA, Drury JA, Robson SC, Quenby S, Bulmer JN. Localization of angiogenic growth factors and their receptors in the human endometrium throughout the menstrual cycle and in recurrent miscarriage. *Hum Reprod.*2012 Jan; 27 (1): 183 - 95. doi: 10.1093/humrep/der376. Epub 2011 Nov 10. PMID: 22081249.
- [51] Takenaka K, Yamagishi S, Matsui T, Nakamura K, Jinnouchi Y, Yoshida Y, Ueda S, Katsuki Y, Katsuda Y, Imaizumi T. Pigment epithelium - derived factor (PEDF) administration inhibits occlusive thrombus formation in rats: a possible participation of reduced intraplatelet PEDF in thrombosis of acute coronary syndromes. *Atherosclerosis.*2008 Mar; 197 (1): 25 - 33. doi: 10.1016/j.atherosclerosis.2007.07.041. Epub 2007 Sep 11. PMID: 17850801.
- [52] Nakahara T, Iwase A, Nakamura T, Kondo M, Bayasula, Kobayashi H, Takikawa S, Manabe S, Goto M, Kotani T, Kikkawa F. Sphingosine - 1 - phosphate inhibits H₂O₂ - induced granulosa cell apoptosis via the PI3K/Akt signaling pathway. *Fertil Steril.*2012 Oct; 98 (4): 1001 - 8. e1. doi: 10.1016/j.fertnstert.2012.06.008. Epub 2012 Jul 3. PMID: 22763095.