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Pigment epithelium derived factor (PEDF) expression in the male tract of Wistar rats

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ABSTRACT

Pigment epithelium derived factor (PEDF) expression has been described in many organs as showing neurotrophic, anti-angiogenic, anti-apoptotic, anti-inflammatory, anti-oxidant and pro-cell survival properties. However, references to its activity in the male reproductive system are scarce. We aimed to characterize the expression of PEDF in the male reproductive tract of Wistar rats by using RT-PCR, western blot and immunostaining and also evaluate the effect of flutamide in PEDF expression. We found that PEDF is expressed in the epididymis, prostate and seminal vesicles in Wistar rats, but notably not in the testes. Under the effect of flutamide PEDF expression decreased, recovering by suppressing the antiandrogen. The epididymis is an essential organ in sperm maturation-storages. The role of PEDF in this physiological process has not been fully elucidated yet, but considering that in other systems PEDF has anti-apoptotic, anti-oxidants and pro-cell survival properties, its expression along the epididymis could play a role in the protection of spermatozoa while they are stored.

1. Introduction

Pigment epithelium-derived factor (PEDF) is a secretory glycoprotein member of the serine protease inhibitors family (serpins), but does not exhibit inhibitory activity [1]. This protein was originally isolated from the conditioned medium of human retinal pigment epithelium cells [2]. There many functions attributed to PEDF: neurotrophic actions inducing transformation of Y79 retinoblastoma cells into differentiated neuronal [3]. PEDF also exhibit anti-angiogenic properties antagonizing the effects of vascular endothelial growth factor VEGF [2,4]. Other works reported PEDF anti-inflammatory properties in diabetic nephropathy [5] and anti-oxidant actions [6].

Studies assessing the role of PEDF, specifically in relation to reproductive function, have focused mainly on the female tract. PEDF expression and secretion in human and murine granulosa cells were described as a hormone-dependent process. In these cells, anti-angiogenic properties were observed by antagonizing the VEGF expression in an autocrine and paracrine mode [7,8]. The exposure of granulosa cells to an oxidizing agent such as hydrogen peroxide (H_2O_2) in a dose-dependent manner decreased cell viability and expression levels of PEDF [9]. Apoptosis induced by H_2O_2 was attenuated in these cells by treatment with recombinant PEDF (rPEDF). In the female reproductive tract, PEDF levels were regulated by oestrogens, progesterone and hCG in granulosa cells [8].

PEDF related findings are very scarce in the male reproductive system. Recent studies focused on prostate cancer shown PEDF expression with a negative correlation regarding prostate cancer incidence in human patients [10]. Our group in 2010 described the rat sperm conjugation inside the epididymal lumen [11]. This sperm conjugate, only found in the caudal epididymis, is formed by a dozen sperm bound by their heads and free flagella. On analyzing this phenomenon, we described the presence of PEDF in caudal luminal fluid and their role in mature sperm conjugation [11,12]. So

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far these are the only references we have found regarding the presence of PEDF in the murine epididymis.

Numerous studies have established the strong regulation of male reproductive system by androgens [13,14]. The anti-androgenic agent flutamide has been extensively used in rats to assay the effect of androgens deprivation [15–17]. This background allows us to consider the use of this anti-androgenic agent as a suitable means of study to evaluate the influence of androgens on the expression of PEDF in the male reproductive tract.

The aims of this study were to analyse the expression of PEDF in the male reproductive tract of Wistar rats, both in control animals and in those subjected to androgen deprivation.

2. Materials and methods

Unless otherwise stated, all chemicals and solvents were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Flutamide (alpha-alpha-alpha−trifluro-2-methyl-4′-nitro-*m*-propionatoluidide) was obtained from Gador Laboratory (Argentina). Immunohistochemistry reagents were purchased from Vector Laboratories Oligonucleotides primers were provided by Invitrogen (Waltham, Massachusetts, USA). Molecular biology reagents were purchased from Promega (Madison, WI, USA) and Invitrogen -Thermo Fisher Scientific Inc. (Waltham, Massachusetts, USA).

2.1. Experimental design

Adult male Wistar rats were obtained from the animal facility at the Institute of Histology and Embryology, Mendoza (IHEM). Animals were maintained under controlled conditions and permitted *ad libitum* access to water and standard lab chow. All animals in this study were maintained in accordance with Guiding Principles in the Care and Use of Animals of the US National Institute of Health. The procedures performed were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL http://fcm.uncuyo.edu.ar/cicual). Endorsement N°. 34/2014. Animals were randomly separated into three groups: control (CONT); flutamide group (FLU) and post flutamide (POSTFLU) of 10 animals each. Flutamide was administered to FLU and POSTFLU animals during fifteen days. The FLU group was euthanized using a CO_2 chamber. Immediately the tissues were removed and weighed; samples were fixed and processed for immunohistochemistry, subjected to protein or RNA extraction, according to routine procedures. RNA samples were kept at −80 °C until processing. The remaining groups (POSTFLU and CONT) were maintained for an additional period of 30 days without flutamide administration to assess the recovery of androgenic function. After 30 days, animals were euthanized. Organs were isolated, and immediately processed for immunohistochemistry and extraction of proteins or RNA.

2.2. Flutamide administration

A protocol adapted from Ref. [18] was applied. Briefly, flutamide was administered via intra peritoneal injection. The dose used was 50 mg/kg body weight/day solubilized in 300 μl of the vehicle (1, 2-propanediol) for 15 days uninterrupted. Control animals were injected with 300 μl of the vehicle.

2.3. Body and organ weights

Body weights of both, animals CONT; FLU and POSTFLU were monitored daily. The obtained values were tabulated and averaged. The weight of each of the analysed organs was expressed as a percentage of body weight.

2.4. Serum testosterone levels

Blood was obtained by cardiac puncture from control animals (CONT, N = 4), flutamide treated (FLU, N = 4) and flutamide and recovered (POSTFLU, N = 4). A clot was allowed to form at room temperature and the serum was separated. Samples were analysed by chemiluminescence (one step competition assay, IBL, Hamburg, Germany) according to the manufacturer.

2.5. Immunohistochemistry

Samples were processed by a routine histological technique. Briefly, isolated organs were fixed by immersion in 10% w/v neutral buffered formalin, dehydrated through a series of graded ethanol and paraffin-embedded. Sections were then deparaffinized and heated in sodium citrate 0.001 M for antigen retrieval. Then, sections were stabilized by immersion in glycine 300 mM at room temperature. Endogenous peroxidase was blocked, washed with PBST (Phosphate buffered saline Sigma Cat. P-4417- plus 1% v/v Tween 20) and incubated in blocking solution, 60 min at room temperature. A monoclonal antibody against PEDF (MAB1059 Merck Millipore, Temecula, CA, USA) was used with a polyvalent biotinylated secondary antibody from a Vectastain Kit R.T.U. (Vecta, Burlingame, CA, USA). Later, the extravidin-peroxidase system from the same kit was applied. The reaction was visualized using amino benzidine (peroxidase substrate, Vecta). The preparations were observed and photographed with a Nikon 80i microscope camera and software features Element NIS 3.2 F.

2.6. Western blot analysis

Tissue samples were lysed with 1 ml 1X Sample buffer [19]. Protein concentration was determined by BCA (Bicinchoninic Acid assay, Sigma BCA-1). The samples, 20 μg per lane, were fractionated by SDS-PAGE using 10% acrylamide gels. Proteins were transferred to a nitrocellulose membrane (Amersham protran 0.45 NC) and non-specific binding's sites blocked by incubation with 3% Teleostean fish gelatine. Blots were incubated with Anti-PEDF antibody (MAB1059) 1:1000 or anti-Tubulin B 1:1500 (MP 08A203068) for loading control at 4 °C O.N. Conjugated anti-rabbit IgG (Life technologies 656140) or Biotin conjugated anti-mouse IgG (Sigma B 7404) were used as secondary's antibody (1:10000). Horseradish peroxidase-conjugated extravidine (Sigma) was added (1:750), with a period of incubation of 1 h, at room temperature. After adequate washing detection was accomplished with an enhanced chemiluminescence system (ECL; Amersham Biosciences) and images were obtained using an ImageQuant LAS 4000 series system.

2.7. Relative semi quantitative RT-PCR for PEDF

Total RNA was extracted from individual samples from the three groups of animals under study using TRIzol (Life Technologies) according to the manufacturer's instructions. RNA samples were solubilized in 25 μl UltraPure DNase/RNase-free distilled water (ThermoFisher Scientific) for reverse transcription - polymerase chain reaction (RT-PCR). Concentration and purity of the samples were spectroscopically determined from the absorbance of diluted samples (1:100) at 260/280 nm. Ten micrograms of total RNA were used for retrotranscription with 200 units M-MLV enzyme reverse transcriptase (Invitrogen). Twenty microlitres of the reaction mixture were added following the manufacturer's instructions.

Semi quantitative Multiplex PCR was then performed using the retrotranscription products obtained from the three groups of animals under study. Three pairs of primers designed using Primer3 software (Simgene.com) were used for the amplification by

multiplex PCR. The primers sequences were: Primer 1: Fw: ACATTGATCCCTTCCAGTGC; Rv: CCGGAAACACTTGTGGATCT; product: 242 bp. Primer 2: Fw: TTTGGCTGCCGATCTGTCAA; Rv: CGACCGACCCGTCATTTTTG; product: 490 bp. Primer 3: Fw: GCTCGCAAGCAACTTCACAA; Rv: TCGACCGACCCGTCATTTTT; product: 351 bp. Beta actin (B072-40, Promega) was used as endogenous reference. The PCRs were carried out in a 25 μl final reaction volume containing 2 μl of cDNA, 20 pmoles of each primer, 200 μM dNTPs, 5 mM MgCl2, 1.5 U of Taq DNA polymerase and 1 μl Taq DNA polymerase PCR buffer (Invitrogen). The cycling parameters were as follows: 95 °C, 30 s; 60 °C, 90 s for 28 cycles. Minus RT controls were included in each assay. Amplified products were run on an agarose gel (2% w/v) and dyed with SYBR-safe DNA Gel Stain (Thermo Fisher Scientific). All PCR were performed in triplicate. The images were visualized and photographed on a computer Fuyi LAS4000. The mRNA abundance was determined by measuring the intensity of each band using ImageJ software (National Institute of Health Bethesda, Maryland; https://imagej.nih.gov/ij/).

2.8. Statistical analysis

Data were expressed as the mean ± SEM of at least three independent experiments performed in duplicate. Statistical analysis was performed using the one-way ANOVA followed by Bonferroni post-test. Values of $p < 0.05$ were considered significant. Data were statistically analysed using Prism v 5.0 (http//www.graphpad.com/prism/Prism.htm, San Diego, CA).

3. Results

3.1. PEDF expression in the male reproductive tract of CONT animals

Immunohistochemistry. In epididymal caput, and cauda, the epithelium stained positive for PEDF (Fig. 1B and D Upper panel). The apical border of the principal epithelial cells and the stereocilia are stained (see arrow in Fig. 1B). In cauda sections, the staining is located over the entire cytoplasm of the epithelial cells (Fig. 1D). Non stain was observed in the seminiferous epithelium or peri tubular cells of testis sections (Fig. 1F). Fig. 1H (see arrow) shows a liver section where hepatocyte were stained for PEDF as was previously reported [20], [21]. Fig. 1A, B, 1E, 1G correspond to negative controls where the primary antibody was omitted.

Semi quantitative Multiplex PCR. Fig. 1 (Middle panel) shows PEDF mRNA expressed in all regions along the epididymal duct (caput, corpus and cauda), seminal vesicles, prostate, kidney and liver in control animals. The more intensive bands corresponded to caput and cauda, both for primers 1 and 2 (242 and 490 bp, respectively). Notably, no PEDF mRNA was observed in samples from testis in the control group (CONT) according to the results obtained by immunohistochemistry. In samples from kidney and liver only a weak band of mRNA was observed using primer 1 whose product corresponds to 242 bp.

Western blot: Fig. 1 (Lower panel) shows PEDF in samples obtained from caput and cauda epididymis (50 KDa band). A slight mark is observed in the lane corresponding to testis, but its intensity is remarkably lower than that obtained in both epididymal regions. B Tubulin (47 KDa) was used as loading control.

3.2. Flutamide affects the weight of male reproductive organs

Fig. 2 shows the effect of flutamide administration. The body weight of the controls (CONT) did not show significant differences with respect to the treated animals (FLU). After restoring the androgenic function, body weight increased significantly with respect to controls and FLU treated group (2A). The testicular weight was not significantly modified either during the administration of FLU or after its suspension (2B). Epididymal caput did not vary significantly with the administration of the antiandrogen, but the cauda varied remarkably. In both regions after suspending the administration of flutamide, weight recovery exceeded the levels of the control group (2C and 2D, respectively). The weight of the prostate decreased markedly (2F) and to a lesser extent so did the weight of the seminal vesicles (2E). However, in this latter organ, POSTFLU recovery weight was greater. We assume that it may be due to a possible rebound effect (perhaps because of the increased expression of testosterone receptors due to their blockage by flutamide.). However, this hypothesis was not proven in our work.

3.3. Serum testosterone measurement

As was expected, flutamide caused a marked increase in serum testosterone levels after 15 days of administration compare to the control animals Control Group (CONT): 1.9 ± 0.17 ng/ml. Flutamide (FLU): 9.94 ± 1.01 ng/ml. Thirty days after ceasing the administration, testosterone levels decreased, but did not reach the control values. Post Flutamide (POSTFLU): 5.6 ± 0.53 ng/ml. Reference Values: 1–7. ng/ml. Values are mean ± SEM.

3.4. PEDF expression under flutamide treatment

After the administration of flutamide, the lack of specific staining was observed in all those organs that previously showed PEDF expression using immunohistochemistry (Fig. 3, Left panel). RT-PCR showed no expression of PEDF mRNA in all the organs analysed (a set of 3 primers was used in this multiplex experiment whose products correspond to 490, 351 and 242 bp) and whose sequences are detailed in Materials and Methods. PCR performed using single primers separately also failed to detect PEDF mRNA expression (data not included). These results agree with the lack of staining observed by immunohistochemistry under flutamide treatment, suggesting the regulatory effect of androgens on the expression of this protein (Fig. 3 Right panel: FLU). Thirty days after cessation of flutamide administration (POSTFLU group), the recovery in PEDF mRNA expression was observed by tetraplex PCR at a level similar to that observed in control animals. No expression for PEDF was observed by RT-PCR or by using immunohistochemistry in testis samples (Fig. 3, Right panel: POSTFLU).

4. Discussion

The results obtained in the present work allow us to state for the first time, that PEDF is expressed along the epididymal tract in adult male rats. It was observed by immunohistochemical staining, western blot and RT-PCR. The staining was localized in the apical cytoplasm, and over the stereocilia that lined the epididymal epithelium in principal cells (caput sections) and over the cytoplasm in cauda samples. Surprisingly, there was no marking in the testes, neither in the seminiferous epithelium nor over the spermatozoa near the testicular lumen. This result differs from those obtained previously by Windschüttl et al. [22], who verified by means of immunohistochemistry and proteomics the presence of PEDF in cells and extracellular matrix of the peritubular compartment in human testes. The authors also found PEDF expression in testes of infantile non-human primates (Rhesus monkeys). They propose that PEDF could be involved in the establishment of the avascular nature of seminiferous tubules in human testes. These differences in expression could be attributed to a primate specific expression not observed so far in murine species [23], due to the main characteristics of peritubular cells vary depending on the

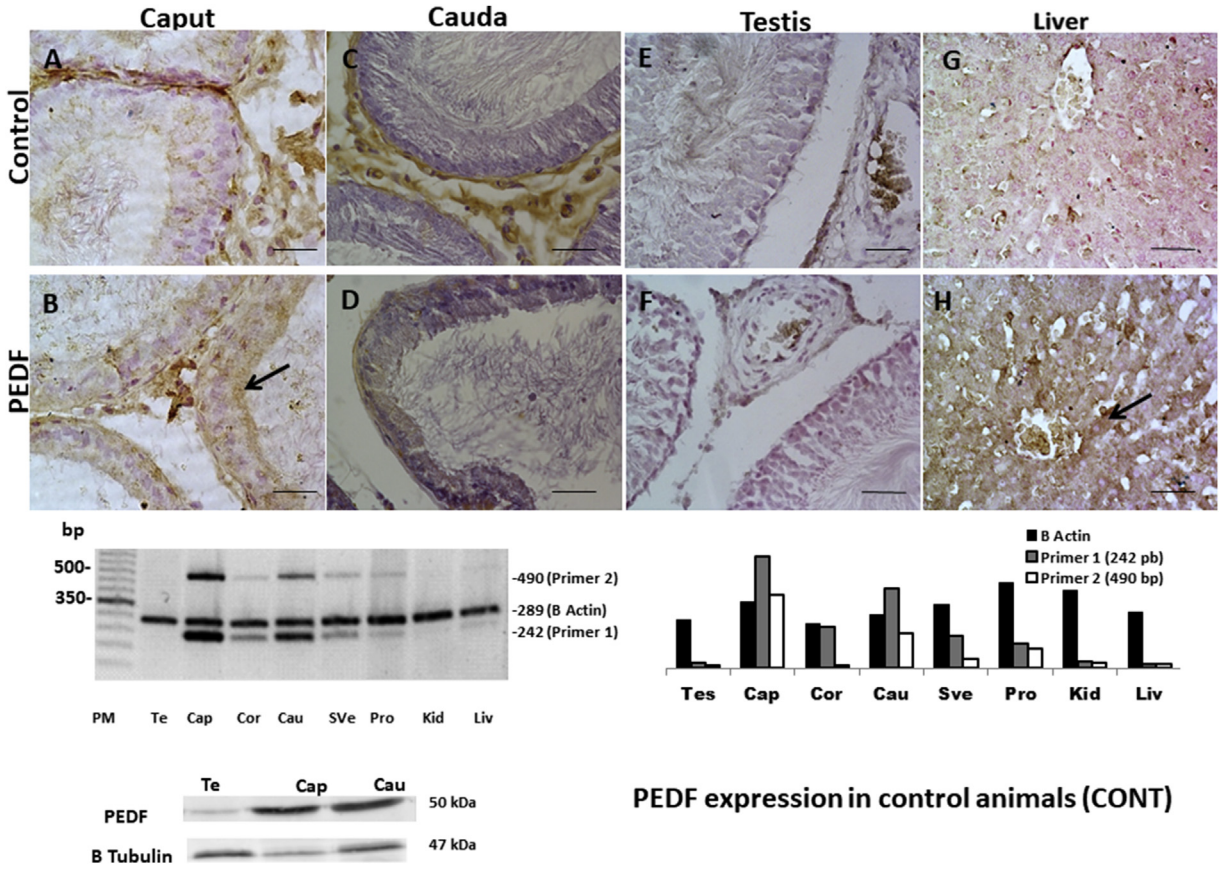

Fig. 1. PEDF expression in the male reproductive tract of control animals. **Upper panel, Immunostaining:** sections of epididymal caput (B) shown stain over the apical border of principal cells (arrow) and over the entire cytoplasm in cauda sections (D). Note that a non-positive reaction was detected in testis samples (F). Figure (H) shows a liver section with positive stain for PEDF over hepatocyte cytoplasm (arrow). Similar sections corresponding negatives controls are shown in Control row (A, C, E and G). Scale bar = 80 μm. **Middle panel: semi quantitative triplex PCR** (representative of three independent experiments) for PEDF and B actin mRNA. Gel is 2% agarose. Two primers 1 (242 bp) and 2 (490 bp) were used. PEDF mRNA was expressed in Caput (Cap); Corpus (Cor); Cauda (Cau); Seminal Vesicles (SVe) and Prostate (Pro). A slight mark was observed on liver and kidney sample (Liv and Kid). There was no expression in Testes (Te). A relative quantification of band intensity was represented for PEDF and B actin mRNA as arbitrary units (right). **Lower panel: western blot.** Testis, Caput and Cauda samples. B tubulin was used as loading control.

species. In laboratory rodents, including rats, hamsters and mice, only one layer of myoid cells is observed in the testis, but in humans there are numerous layers of cells that together with the extracellular matrix proteins forms a compartment that acts in a paracrine way in the regulation of testicular functions [24]. Semi quantitative RT-PCR confirmed the results obtained by immunohistochemistry. PEDF mRNA was detected in samples of epididymis, seminal vesicles, prostate, kidney and liver. Coincidentally, we did not obtain evidence of expression in testicular tissue as we observed by western blot analysis.

Respect to the androgen dependence of PEDF expression, the use of flutamide, a known anti-androgenic agent, allowed us to observe the effect of testosterone deprivation by a decrease in the weight of different organs of the male reproductive system and, as was expected, an increase in serum testosterone levels in treated animals. In the FLU animals group we observed a loss of staining in all the tissues that previously demonstrated a positive reaction for PEDF. Similar findings were observed through the RT-PCR technique. Once the administration of flutamide ceased and after 30 days to recover androgenic activity, we were able to observe restoration in the expression of PEDF mRNA by RT-PCR. With respect to the weight gain shown by the organs analysed after flutamide discontinuation (even greater than those observed in the controls), to our knowledge there are no records in the literature that allow us to explain the data obtained. Recent studies suggest the possible mutation of the androgen receptor as a model to explain the antiandrogen resistance observed in patients with prostate cancer, which restores its affinity for androgens [25]. But this resistance has manifested after 2 or 3 years of treatment, so in our study, this mechanism does not explain the weight gain obtained by suppressing flutamide. So far, to our knowledge, this is the first report about PEDF expression along the epididymis, its androgenic regulation and the lack of expression of this protein in testes.

Regarding the PEDF function, in a number of tissues this protein shows many actions including neurotrophic [3], anti-angiogenic [4], anti-inflammatory [5] and anti-oxidant activity [6]. Concerning the female tract, there are many studies in the bibliography: the expression of PEDF in human and murine granulosa cells and their inverse relationship with oestrogen and progesterone levels that regulate their anti-angiogenic activity [8]; the implication of PEDF in the pathogenesis and treatment of ovarian hyperstimulation syndrome by antagonizing the deleterious effects of VEGF [7]. Other authors analysed the role of PEDF in the generation of ROS in ovarian tissues. Low concentrations of PEDF did not affect the cell viability caused by ROS generation. Finally, an anti-angiogenic and anti-inflammatory effect of PEDF on ovarian hyperstimulation syndrome was proposed [26].

Considering specifically the relationship between PEDF and epididymis, to our knowledge there is only one mention about the

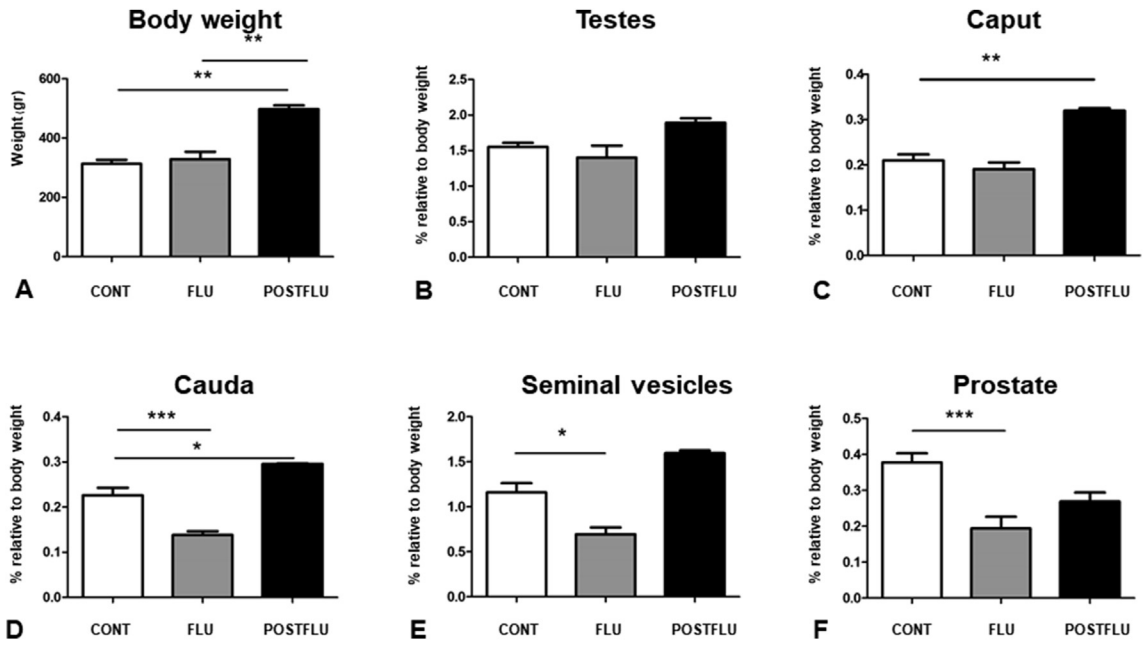

Fig. 2. Effect of flutamide administration on body weight and organs of the male reproductive tract. Body weight was not significantly modified by the administration of flutamide (A). After cessation of treatment, recovery of body weight was notable. Decreased testicular weight (B) and epididymal caput (C) were not significant in response to flutamide. There was a slight increase after restoring the androgenic function in testes but a significant increase in caput. In cauda (D), seminal vesicles (E) and prostate (G), flutamide produced a notable decrease in weight that was recovered to higher levels than the controls in the cauda and seminal vesicles, but not in the prostate.

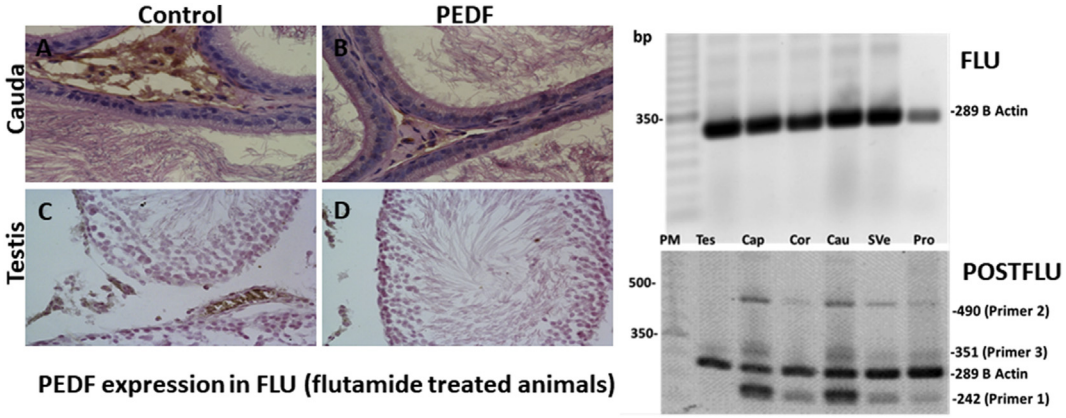

Fig. 3. Flutamide affects the PEDF expression in the male reproductive tract. Left panel, Immunostaining. PEDF immunoreactive stain is lost in sections of epididymis (B). A non positive reaction was detected in testis samples (D). Similar sections to the one depicted in the PEDF column correspond to controls (A and C). Scale bar = 80 μm. **Right panel: Semi quantitative tetraplex PCR** (representative of three independent experiments) for PEDF and B Actin mRNA. Three pairs of primers, 1 (242 bp), 2 (490 bp) and 3 (351 bp), were used. Gel is 2% agarose. (Upper: FLU): the effect of 15 days of flutamide administration. PEDF mRNA was not observed with any of the primers designed to amplify. There is only a band of 289 bp corresponding to B Actin. (Lower: POSTFLU): PEDF mRNA expression after 30-days flutamide suppression. PEDF mRNA was expressed in all tissues analysed at similar levels to those observed in control animals, except in testes, where no expression is observed.

presence of this protein in the epididymal lumen and its relation with sperm conjugation in rats, possibly as a mode of protection of these cells while they are stored [11]; [12]. The present work analysed the expression of PEDF throughout the entire epididymal duct and its dependence on androgenic regulation. At the moment we can conclude that epididymal maturation is a key stage in the spermatozoon life. The role that PEDF would play in epididymal maturation is not yet elucidated. But considering the different functions attributed to this protein in other systems, a wide field of study has opened, with emphasis on the study of PEDF antioxidant and anti-apoptotic properties previously proven in other cellular systems.

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Conflicts of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

[1] S.P. Becerra, A. Sagasti, P. Spinella, V. Notario, Pigment epithelium-derived factor behaves like a noninhibitory serpin. Neurotrophic activity does not require the serpin reactive loop, J. Biol. Chem. 27 270 (1995) 25992–25999. Oct.
[2] J. Tombran-Tink, G.G. Chader, L.V. Johnson, PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity, Eye Res. 53 (1991) 411–414. Sep.
[3] F.R. Steele, G.J. Chader, L.V. Johnson, J. Tombran-Tink, 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. 90 (1993) 1526–1530. Feb 15.
[4] N. Popp, X.K. Chu, D. Shen, J. Tuo, C.C. Chan, Potential therapies in a mouse model of focal retinal degeneration with age-related macular degeneration (AMD)-Like lesions, J. Clin. Exp. Ophthalmol. 4 (2013) 1000296. Oct 1.
[5] J.J. Wang, S.X. Zhang, R. Mott, Y. Chen, R.R. Knapp, W. Cao, J.X. Ma, Anti-inflammatory effects of pigment epithelium-derived factor in diabetic nephropathy, Am. J. Physiol. Ren. Physiol. 294 (2008) F1166–F1173, https://doi.org/10.1152/ajprenal.00375.2007. May.
[6] S. Yamagishi, K. NakamuraK, S. Ueda, S. Kato, T. Imaizumi, 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. 320 (2005) 437–445. Jun.
[7] D. Chuderland, I. Ben-Ami, R. Kaplan-Kraicer, H. Grossman, R. Ron-El, R. Shalgi, The role of pigment epithelium-derived factor in the pathophysiology and treatment of ovarian hyperstimulation syndrome in mice, J. Clin. Endocrinol. Metab. 98 (2013a) E258–E266, https://doi.org/10.1210/jc.2012-3037. Feb.
[8] D. Chuderland, I. Ben-Ami, R. Kaplan-Kraicer, H. Grossman, A. Komsky, R. Satchi-Fainaro, A. Eldar-Boock, R. Ron-El, R. Shalgi, Hormonal regulation of pigment epithelium-derived factor (PEDF) in granulosa cells, Mol. Hum. Reprod. 19 (2013b) 72–81, https://doi.org/10.1093/molehr/gas046. Feb.
[9] H. Bar-Joseph, I. Ben-Ami, R. Ron-El, R. Shalgi, D. Chuderland, Pigment epithelium-derived factor exerts antioxidative effects in granulosa cells, Fertil. Steril. 102 (2014) 891–898, https://doi.org/10.1016/j.fertnstert.2014.06.012. Sep.
[10] Z.H. Guo, H. Huang, T. DU, K.W. Xu, Y. Cao, J.Q. Chen, W. Dong, Y.S. Yao, T.X. Lin, W.L. Xie, C. Jiang, J.L. Han, J. Huang, Inhibition and significance of pigment epithelium-derived factor in the development and metastasis of prostate cancer, Natl. Med. J. China (Peking) 90 (2010) 2980–2983, https://doi.org/10.3760/cma.j.issn.0376-2491.2010.42.009.
[11] M.A. Monclus, A. Cesari, M.E. Cabrillana, T.E. Saez Lancellotti, D.E. Rensetti, M.A. Clementi, P.V. Boarelli, A.E. Vincenti, M.W. Fornés, Protein fraction isolated from epididymal fluid re associates sperm in vitro. Possible role of serpins in rat Rosettes assembling, Mol. Reprod. Dev. 77 (2010) 410–419. May.
[12] M.A. Monclus, M.W. Fornes, Sperm conjugation in mammal reproductive function: different names for the same phenomenon? Mol. Reprod. Dev. 83 (2016) 884–896, https://doi.org/10.1002/mrd.22636. Oct.
[13] S. Breton, Y.C. Ruan, Y.J. Park, B. Kim, Regulation of epithelial function, differentiation, and remodeling in the epididymis, Asian J. Androl. 18 (2016) 3–9, https://doi.org/10.4103/1008-682X.165946. Jan-Feb.
[14] A. Hejmej, B. Bilinska, A role of junction-mediated interactions in cells of the male reproductive tract: impact of prenatal, neonatal, and prepubertal exposure to anti-androgens on adult reproduction, Histol. Histopathol. 29 (2014) 815–830. Jul.
[15] K. Suzuki, K. Ito, K. Kurokawa, T. Suzuki, N. Shimizu, Y. Fukabori, S. Honma, H. Yamanaka, Expression and degradation of rat androgen receptor following castration, testosterone replacement and antiandrogens administration: analysis by Western blot and immunohistochemistry, Tohoku J. Exp. Med. 183 (1997) 159–172. Nov.
[16] J.D. Dhar, S.R. Srivastava, B.S. Setty, Flutamide as an androgen antagonist on epididymal function in the rat, Andrologia 14 (1982) 55–61. Jan-Feb.
[17] J.C. O'Connor, S.R. Frame, G.S. Ladics, Evaluation of a 15 day screening assay using intact male rats for identifying antiandrogens, Toxicol. Sci. 69 (2002) 92–108. Sep.
[18] C.R. Caflisch, Effect of a nonsteroidal antiandrogen, flutamide on intraluminal acidification in rat testis and epididymis, Andrologia 25 (1993) 363–367. Nov-Dec.
[19] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of the bacteriophage T4, Nature 22 (1970) 680–685.
[20] Z. Dai, T. Zhou, C. Li, W. Qi, Y. Mao, J. Lu, Y. Yao, L. Li, T. Zhang, H. Hong, S. Li, W. Cai, Z. Yang, J. Ma, X. Yang, G. Gao, Intracellular pigment epithelium-derived factor contributes to triglyceride degradation, Int. J. Biochem. Cell Biol. 45 (2013) 2076–2086. Sep.
[21] C. Chung, C. Shugrue, A. Nagar, J.A. Doll, M. Cornwell, A. Gattu, T. Kolodecik, S.J. Pandol, F. Gorelick, Ethanol exposure depletes hepatic pigment epithelium-derived factor, a novel lipid regulator, Gastroenterology 136 (2009) 331–340, https://doi.org/10.1053/j.gastro.09.065, e2.
[22] S. Windschüttl, C. Kampfer, C. Mayer, F. Flenkenthaler, T. Fröhlich, J.U. Schwarzer, F.M. Köhn, H. Urbanski, G.J. Arnold, A. Mayerhofer, Human testicular peritubular cells secrete pigment epithelium-derived factor (PEDF), which may be responsible for the avascularity of the seminiferous tubules, Sci. Rep. 5 (2015) 12820, https://doi.org/10.1038/srep12820. Sep. 3.
[23] M. Maekawa, K. Kamimura, T. Nagano, Peritubular myoid cells in the testis: their structure and function, Arch. Histol. Cytol. 59 (1996) 1–13. Mar.
[24] A. Mayerhofer, Human testicular peritubular cells: more than meets the eye, Reproduction 29 (145) (2013) R107–R116, https://doi.org/10.1530/REP-12-0497. Apr.
[25] C. Guo, S. Yeh, Y. Niu, G. Li, J. Zheng, L. Li, C. Chang, Targeting androgen receptor versus targeting androgens to suppress castration resistant prostate cancer, Canc. Lett. (2017), https://doi.org/10.1016/j.canlet.2017.03.022.
[26] I. Miller, D. Chuderland, H. Grossman, R. Ron-El, I. Ben-Ami, R. Shalgi, The dual role of PEDF in the pathogenesis of OHSS: negating both angiogenic and inflammatory pathways, J. Clin. Endocrinol. Metabol. 101 (2014) 4699–4709. Dec.