ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Electron microscopic aspects of the effects of certain prostaglandin analogs on mouse testes

ANCA SAVA[1)], ANDREI GHEORGHE MARIUS MOTOC[2)], CRISTINEL IONEL STAN[1)]

[1)]*Department of Anatomy and Embryology, "Grigore T. Popa" University of Medicine and Pharmacy, Iassy, Romania*
[2)]*Department of Anatomy and Embryology, "Victor Babeş" University of Medicine and Pharmacy, Timisoara, Romania*

Abstract

Prostaglandins were highlighted in the seminal plasma and then in the rest of the male and female genital tract. Prostaglandin analogs, firstly used in obstetrics and gynecology, are now widespread in both sexes, especially in the treatment of gastric and duodenal ulcers, glaucoma, etc. Therefore, we tried to highlight the effects of repeated administration of Cloprostenol and CIPG isopropyl ester (both prostaglandin F2α analogs) for the male gonad. In our experiment, we used Cloprostenol and CIPG isopropyl ester. We used three groups of white, male mice, aged 50–80 days, kept in standard laboratory conditions, which received the same feed. Each group included 12 mice. The first batch was the control group and received no substance at all. The second batch received 25 µg/kg of Cloprostenol dose per body per day, intraperitoneal administration (a single dose per day) on a daily basis for a four weeks period of time. The third batch received a 25 µg/kg CIPG isopropyl ester dose per body/day intraperitoneal administration (a single dose per day) on a daily basis for a four weeks period of time. After 7, 14 and 28 days of treatment, we sacrificed four animals in each of the batches by cutting their carotid arteries. The prostanoid analogs we used, Cloprostenol and CIPG isopropyl ester, have similar actions on male gonad in mice. These analogs induced significant changes in the evolution of the spermatogenesis and spermiogenesis. In relation to the treatment duration there were cellular changes suggesting apoptosis in different stages. With regard to spermiogenesis, the ultrastructural aspects indicate a decrease of the sperm structuring processes, especially in the acrosomal apparatus and chromatin.

Keywords: prostaglandins, testis, spermatogenesis.

Introduction

Initially, prostaglandins were highlighted in the seminal plasma and then in the rest of the male and female genital tract [1, 2]. Numerous actions have been identified, particularly on the female genital tract (luteolysis, myometrial contractions, etc.) and therefore many prostaglandin analogs were synthesized as Meteneprost, Luprostiol, Gemeprost, etc., which are used for their ocitocic action in human therapy [3, 4]. Additionally, many prostaglandin analogs with luteolytic action (Fluprostenol, Prostalene, etc.) are used in veterinary medicine [5, 6]. Prostaglandin analogs, firstly used in obstetrics and gynecology, are now widespread in both sexes, especially in the treatment of gastric and duodenal ulcers, glaucoma, etc. [7–9]. Therefore, we tried to highlight the effects of repeated administration of Cloprostenol and CIPG isopropyl ester (both prostaglandin F2α analogs) for the male gonad.

Materials and Methods

In our experiment, we used Cloprostenol and CIPG isopropyl ester, synthesized in National Institute for Chemical-Pharmaceutical Research and Development (ICCF), Bucharest, Romania. We used three groups of white, male mice, aged 50–80 days, kept in standard laboratory conditions, which received the same feed. Each group included 12 mice.

The first batch was the control group and received no substance at all.

The second batch received 25 µg/kg of Cloprostenol dose per body/day, intraperitoneal administration (a single dose per day) on a daily basis for a four weeks period of time.

The third batch received a 25 µg/kg CIPG isopropyl ester dose per body/day intraperitoneal administration (a single dose per day) on a daily basis for a four weeks period of time.

After 7, 14 and 28 days of treatment, we sacrificed four animals in each of the batches by cutting their carotid arteries. Fragments taken from the testicle were immediately fixed by means of immersion in a 2% glutaraldehyde solution in 0.1 M sodium cacodylate buffer at pH 7.2 for six hours at 4^0C. The fragments were dehydrated in alcohol solutions of a gradual concentration from 50% to absolute alcohol, and afterwards immersed in propylene oxide and then immersed in EPON 812 in gelatin capsules. Polymerization was performed in the thermostat.

Semithin sections were performed with Reichert Om-U2 ultramicrotome and caught on the blade, stained with Toluidine blue, examined and photographed with the Zeiss Axio Scope microscope on Kodak film. After selecting the fields for the electron microscopy examination, thin sections were obtained in the same ultramicrotome, which were caught on Fornvar pre-treated grids. The thin section contrasting was performed by means of uranyl acetate and lead citrate. The examination and the photography were performed with a Philips C100 electron microscope (Philips, Eindhoven, The Netherlands).

Results

With respect to the control group, we observed a fragment of a Leydig cell with smooth endoplasmic reticulum during the ultrastructural examination. We have also noted structures, which were electron-opaque, relatively homogeneous, bound by a membrane unit corresponding to lysosomes (Figure 1). In the depression of Sertoli cells, we observed 1st and 2nd order spermatocytes, spermatides and maturing sperm (Figure 2). At all sacrificed animals did not show any changes of the male gonad.

The batch subjected to our experiment showed the first changes after a week of treatment. The cross section revealed the height increase of endothelial cells. The swollen endothelial cells showed a more abundant cytoplasm with irregularly shaped nuclei, with a shrunken aspect, dense chromatin, demonstrating a toxic effect on the endothelium (Figure 3). In some capillaries, the congestion of erythrocytes that clump together "in rouleau" formation is important, demonstrating a slow flow (Figure 4).

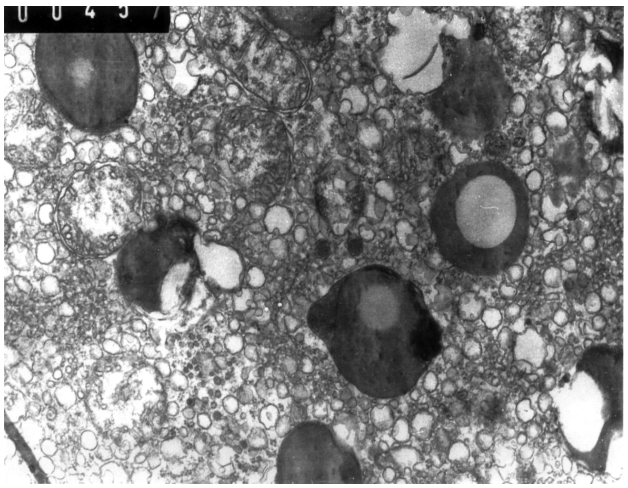

Figure 1 – ***Mouse testicle control group after two weeks of treatment, ×15 000.***

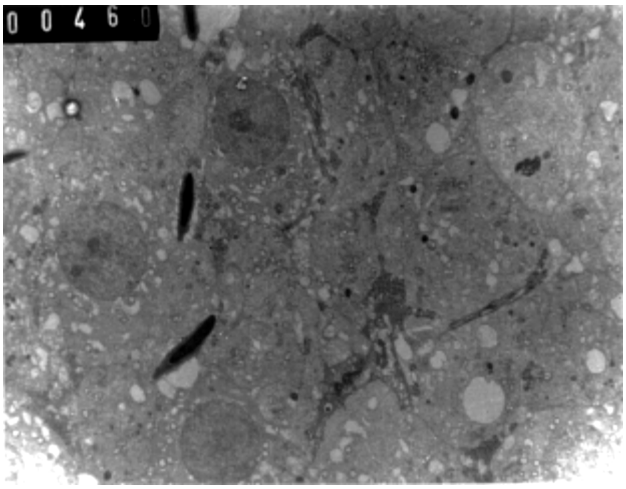

Figure 2 – ***Mouse testicle control group after two weeks of treatment, ×5700.***

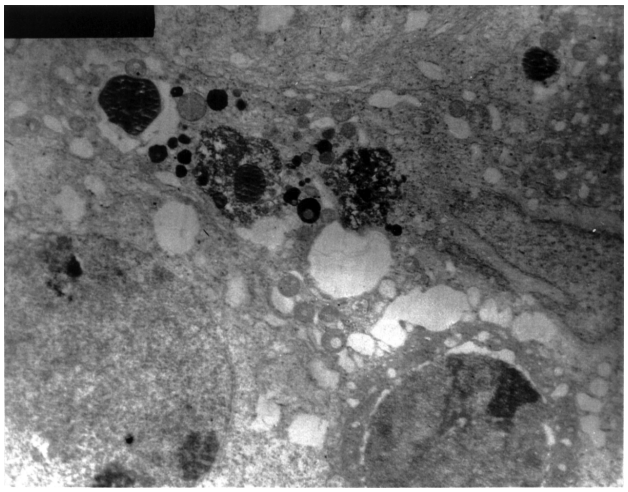

Figure 3 – ***Animal treated with Cloprostenol after one week of treatment, ×5700.***

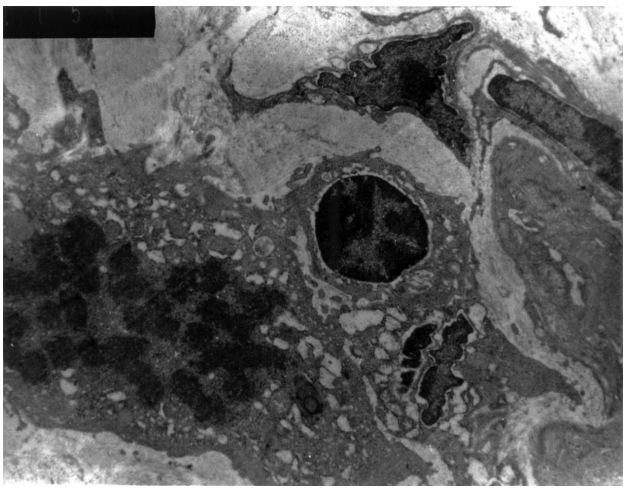

Figure 4 – ***Animal treated with Cloprostenol after one week of treatment, ×5700.***

Two weeks after the treatment, we have mainly selected areas where the interstice was displayed as being enlarged and with a hyaline content. Electron microscopic in these areas, the interstice display cells with ultrastructural features of a macrophage highly loaded with residual bodies, an ultrastructure which characterizes an intense process of phagocytosis (Figure 5). The moderate electronic density of these bodies could advocate in favor of the phagocytosis of apoptotic bodies.

Furthermore, the interstice displayed a large number of fibroblasts. In addition to fibroblasts, fibrocytes occur without obvious changes. Capillaries displayed very thin endothelium areas, sometimes even showing discontinuities (widening of intercellular spaces – Figures 6 and 7).

In the seminiferous tubules, inside the adluminal area, the frequency of structuring sperm is small. Spermatids are rare while in the cytoplasm of Sertoli cells lysosome-like dense bodies frequently occur (Figure 8).

After four weeks of treatment, the changes observed after two weeks are more visible. The lumen of capillaries contains erythrocytes that clump together "in rouleau" formation; the endothelial cells have a well-represented cytoplasm rich in organelles (Figure 9). The Leydig cell is rich in mitochondria and includes a smooth endoplasmic reticulum and rare lipid inclusions (Figure 10).

After four weeks of treatment, there also was evidence

of spermatocytes with condensed cytoplasm and nuclei with a high chromatin condensation, ultrastructural aspects suggesting the early stage of apoptosis (Figure 11). Among spermatides and in relation to extensions of the Sertoli cells, we noticed structuring sperm with obvious acrosomal vacuole. Nevertheless, it was impossible to observe the sperm with acrosome formation and with a chromatin condensation level that is appropriate for this stage of maturation (Figure 12).

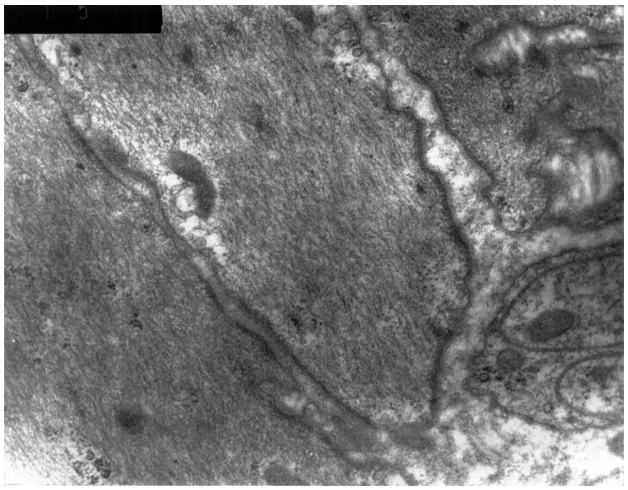

Figure 5 – ***Animal treated with Cloprostenol after two weeks of treatment, ×15 000.***

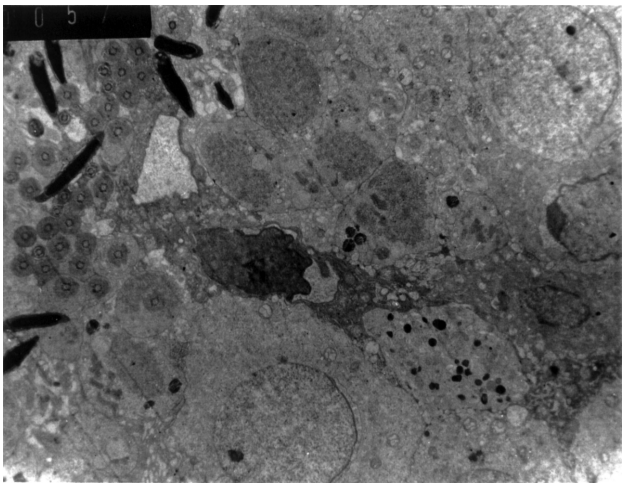

Figure 6 – ***Animals treated with CIPG isopropyl ester, after two weeks of treatment, ×3400.***

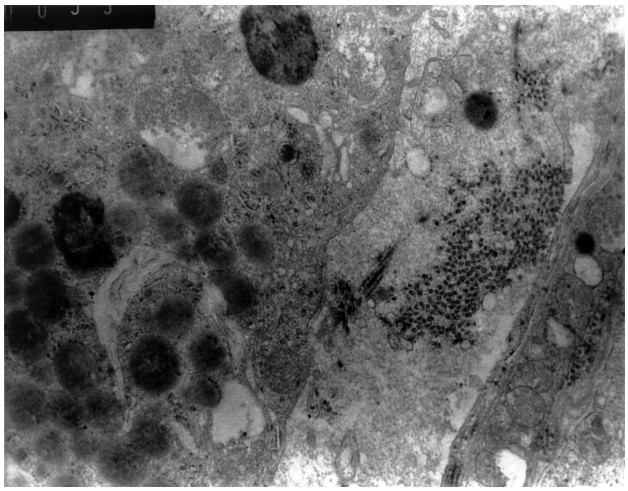

Figure 7 – ***Animals treated with CIPG isopropyl ester, after two weeks of treatment, ×9100.***

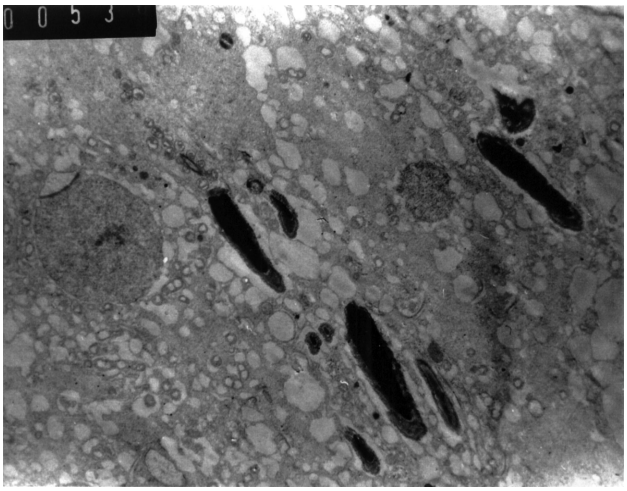

Figure 8 – ***Animal treated with Cloprostenol after two weeks of treatment, ×3400.***

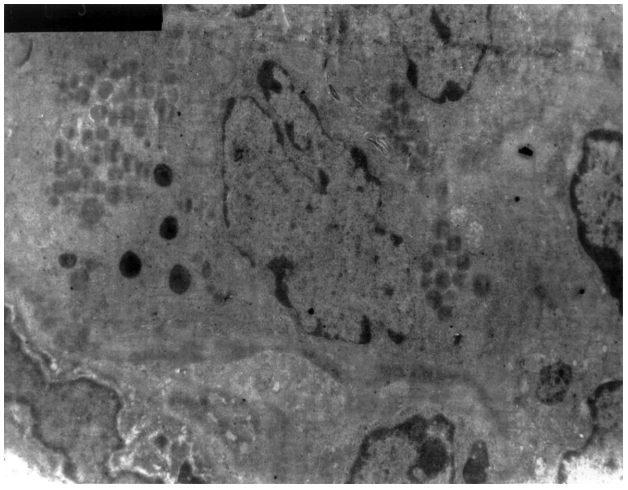

Figure 9 – ***Animal treated with CIPG isopropyl ester after four weeks of treatment, ×5700.***

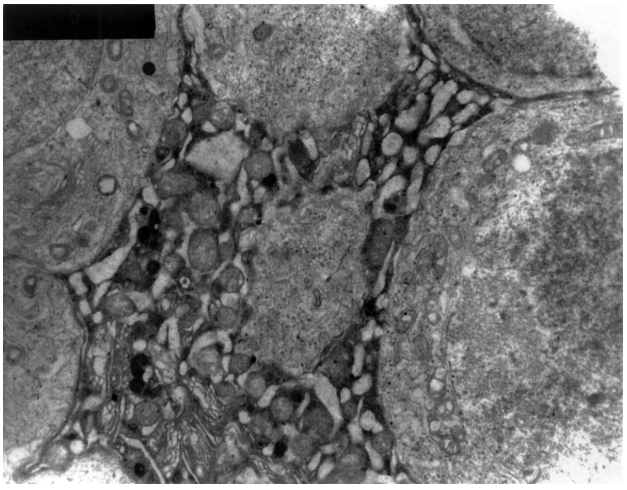

Figure 10 – ***Animal treated with CIPG isopropyl ester after four weeks of treatment, ×7100.***

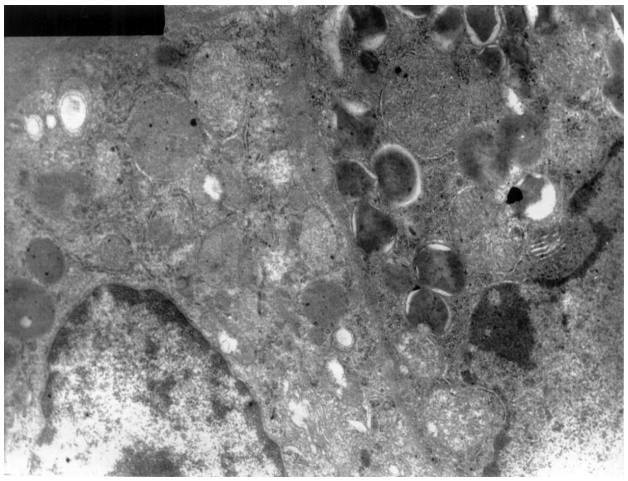

Figure 11 – ***Animal treated with Cloprostenol after four weeks of treatment, ×9100.***

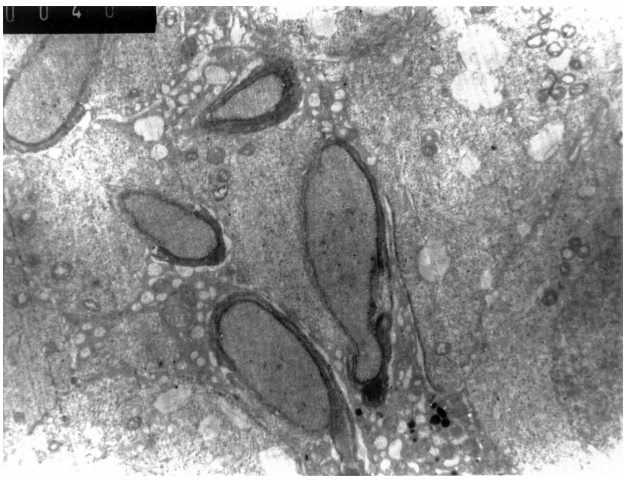

Figure 12 – ***Animal treated with Cloprostenol after four weeks of treatment, ×4500.***

Discussion

Our results are similar to the results of other researchers regarding the inhibitor action of the prostaglandins on the spermatogenesis. In this way, Abatiello *et al.* [1] proved that the prostaglandins F1α and F2α have an important action in decreasing spermatogenesis during the meiotic phase. Histopathological examination revealed increased numbers of exfoliated immature germinal cells in the seminiferous tubules and epididymi of prostaglandin-treated animals. The effects produced by prostaglandin F2α are similar with the effects produced by the anti-spermatogenic factor indenopyridine CDB-4022 [10, 11].

The seminiferous tubules were devoid of spermatozoa and contained only Sertoli cells, spermatogonia, spermatocytes and occasionally spermatids; several multinucleated giant cells were observed in the lumen of the tubules. The Leydig cells were atrophied [12].

Although some researchers suggest the fact that the testicle has reduced effects to induce inflammatory reactions [13–15], our results on the testicle show the capacity of the testicle to sustain an inflammatory action by important influx of macrophages [16, 17].

It is important too, to emphasize the effects of the prostaglandin F2α on the microcirculation on the level of the testicle. Our data suggest an accumulation of fluid in the interstitial space at the same time with the growth of the diameter of the blood capillaries as well as a decrease of the endothelial cells from the capillary wall [18]. After four weeks of treatment, one can notice even the destruction of some capillary vessels.

The effects of the prostaglandin F2α and of their analogous on the microcirculation are controversial. In this way, some authors describe a vasoconstrictor action of some prostaglandin F2α on the kidney microcirculation. The primary sites of action of 8-*epi*-prostaglandin F2α are in preglomerular and possibly mesangial smooth muscle, where it exerts potent constrictor effects. These actions result in reductions in renal perfusion and probably in glomerular capillary surface [19–21]. In rat renal circulation, the constrictor effects of 8-*epi*-PGF2 are caused by its activation of thromboxane receptors [22]. The same vasoconstrictor effect on the cutaneous [23] or pulmonary [24] microcirculation is noticed by some authors.

Other researchers emphasize a vasodilator effect of prostaglandin F2α on the synovial or ophthalmic microcirculation [25–27]. Singh & Dominic [28] demonstrated that the administration of prostaglandin F2α caused marked suppression of spermatogenesis and significant reduction in the weights of the testis, epididymis and accessory sex glands, like Winnall *et al.*, too [29]. Our results suggest a slight vasodilator effect of prostaglandin F2α on the testicular microcirculation accompanied with a retraction of the endothelial cells from the capillary wall. Similar results were obtained by Takahashi *et al.* [30] and Sada *et al.* [31].

Another interesting aspect of our study is related to a possible action inducting the apoptosis exerted by the prostaglandin F2α on the mouse testicle, just like other results from the medical literature. Thus, Zannoni *et al.* [32] report the apoptotic action of the prostaglandin F2α on the porcine corpus luteum while Wang *et al.* ascertain the same action on the rodent corpus luteum [33]. The mechanisms through which the prostaglandin F2α stimulates the apoptosis are not yet elucidated, even some theories are proposed [34].

Conclusions

The prostanoid analogs we used, Cloprostenol and CIPG isopropyl ester, have similar actions on male gonad in mice. These analogs induced significant changes in the evolution of the spermatogenesis and spermiogenesis. In relation to the treatment duration there were cellular changes suggesting apoptosis in different stages. With regard to spermiogenesis, the ultrastructural aspects indicate a decrease of the sperm structuring processes, especially in the acrosomal apparatus and chromatin. Referring to aspects related to the interstice, capillary endothelium had a surprising ultrastructure suggesting an active transcytoplasmic transport under the action of particular present stimuli. The accumulation of interstitial fluid as an important influx of macrophages is obvious. The Leydig cells suggest a stimulation of the androgen synthesis according to the increasing duration of treatment and of the administered dose.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Abatiello ER, Kaminsky M, Weisbroth S. The effect of prostaglandins and prostaglandin inhibitors on spermatogenesis. Int J Fertil, 1975, 20(3):177–182.

[2] Frungieri MB, Gonzalez-Calvar SI, Matzkin ME, Mayerhofer A, Calandra RS. Sources and functions of prostaglandins in the testis: evidence for their relevance in male (in)fertility. Anim Reprod, 2007, 4(3/4):63–69.

[3] Ruan YC, Zhou W, Chan HC. Regulation of smooth muscle contraction by the epithelium: role of prostaglandins. Physiology (Bethesda), 2011, 26(3):156–170.

[4] Purohit GN, Shekher C, Kumar P, Solanki K. Induced termination of pregnancy in domestic farm animals. Iranian J Appl Anim Sci, 2012, 2(1):1–12.

[5] Murta D, Batista M, Trindade A, Silva E, Henrique D, Duarte A, Lopes-da-Costa L. *In vivo* notch signaling blockade induces abnormal spermatogenesis in the mouse. PLoS One, 2014, 9(11):e113365.

[6] Volkmann J, Müller D, Feuerstacke C, Kliesch S, Bergmann M, Mühlfeld C, Middendorff R. Disturbed spermatogenesis associated with thickened lamina propria of seminiferous tubules is not caused by dedifferentiation of myofibroblasts. Hum Reprod, 2011, 26(6):1450–1461.

[7] Tanaka K, Tamura H, Tanaka H, Katoh M, Futamata Y, Seki N, Nishimune Y, Hara T. Spermatogonia-dependent expression of testicular genes in mice. Dev Biol, 2002, 246(2):466–479.

[8] Alam MJ, Khatun M, Begum M, Hossain M, Akhter J, Paul UK, Begum T. Role of vitamin E on antispermatogenic effects of indomethacin on number of sperm containing seminiferous tubules of testes in Long Evans rats. Banglad J Anat, 2009, 7(1):5–9.

[9] Freitas MJ, Fardilha M. Intraflagellar protein 88 interactome analysis: a bioinformatics approach highlights its role in testis and sperm function. J Biophys Chem, 2014, 5(3):99–106.

[10] Selvaraju S, Nandi S, Subramani TS, Raghavendra BS, Rao SBN, Ravindra JP. Improvement in buffalo (*Bubalus bubalis*) spermatozoa functional parameters and fertility *in vitro*: effect of insulin-like growth factor-I. Theriogenology, 2010, 73(1):1–10.

[11] Kumar A, Shekhar S, Dhole B. Thyroid and male reproduction. Indian J Endocrinol Metab, 2014, 18(1):23–31.

[12] Jarad SA, Al-Samawy ERM, Al-Badran ASH. Effects of L-arginine on spermatogenesis of the diabetic rat. Bas J Vet Res, 2011, 10(2):19–32.

[13] Korfanty J, Stokowy T, Widlak P, Gogler-Piglowska A, Handschuh L, Podkowiński J, Vydra N, Naumowicz A, Toma-Jonik A, Widlak W. Crosstalk between HSF1 and HSF2 during the heat shock response in mouse testes. Int J Biochem Cell Biol, 2014, 57:76–83.

[14] Saeed SA, Anwar N, Khan KM, Sarfraz N. Effect of chronic treatment with a cyclooxygenase inhibitor on reproductive parameters in male rat. J Ayub Med Coll Abbottabad, 2009, 21(3):66–71.

[15] Venza I, Giordano L, Piraino G, Medici N, Ceci G, Teti D. Prostaglandin E2 signalling pathway in human T lymphocytes from healthy and conjunctiva basal cell carcinoma-bearing subjects. Immunol Cell Biol, 2001, 79(5):482–489.

[16] Zhang J, Fujii S, Wu Z, Hashioka S, Tanaka Y, Shiratsuchi A, Nakanishi Y, Nakanishi H. Involvement of COX-1 and up-regulated prostaglandin E synthases in phosphatidylserine liposome-induced prostaglandin E2 production by microglia. J Neuroimmunol, 2006, 172(1–2):112–120.

[17] Farhat A, Philibert P, Sultan C, Poulat F, Boizet-Bonhoure B. Hematopoietic-prostaglandin D2 synthase through PGD2 production is involved in the adult ovarian physiology. J Ovarian Res, 2011, 4:3.

[18] Parent J, Fortier MA. Expression and contribution of three different isoforms of prostaglandin E synthase in the bovine endometrium. Biol Reprod, 2005, 73(1):36–44.

[19] Lazarus M, Eguchi N, Matsumoto S, Nagata N, Yano T, Killian GJ, Urade Y. Species-specific expression of microsomal prostaglandin E synthase-1 and cyclooxygenases in male monkey reproductive organs. Prostaglandins Leukot Essent Fatty Acids, 2004, 71(4):233–240.

[20] Kotarska K, Lenartowicz M, Przybyło M, Gołas A, Styrna J. Increased prostaglandin E_2-EP2 signalling in cumulus cells of female mice sired by males with the Y-chromosome long-arm deletion. Reprod Fertil Dev, 2013, 25(6):900–906.

[21] Zhang GL, Zhang XF, Feng YM, Li L, Huynh E, Sun XF, Sun ZY, Shen W. Exposure to bisphenol A results in a decline in mouse spermatogenesis. Reprod Fertil Dev, 2013, 25(6):847–859.

[22] Schell C, Albrecht M, Spillner S, Mayer C, Kunz L, Köhn FM, Schwarzer U, Mayerhofer A. 15-Deoxy-delta 12-14-prostaglandin-J2 induces hypertrophy and loss of contractility in human testicular peritubular cells: implications for human male fertility. Endocrinology, 2010, 151(3):1257–1268.

[23] Zerani M, Catone G, Quassinti L, Maccari E, Bramucci M, Gobbetti A, Maranesi M, Boiti C, Parillo F *In vitro* effects of gonadotropin-releasing hormone (GnRH) on Leydig cells of adult alpaca (*Lama pacos*) testis: GnRH receptor immunolocalization, testosterone and prostaglandin synthesis, and cyclooxygenase activities. Domest Anim Endocrinol, 2011, 40(1):51–59.

[24] Rowley AF, Vogan CL, Taylor GW, Clare AS. Prostaglandins in non-insectan invertebrates: recent insights and unsolved problems. J Exp Biol, 2005, 208(Pt 1):3–14.

[25] Caton MPL, Crowshaw K. Pharmaceutical exploitation. In Curtis-Prior PB (ed). Prostaglandins – biology and chemistry of prostaglandins and related eicosanoids. Churchill Livingstone, Edinburgh–London–Melbourne–New York, 1988, 671–683.

[26] Einarsson SG. Comparative trial with natural prostaglandin and an analogue (Cloprostenol) in induction parturition in sows. Acta Vet Scand Suppl, 1981, 77:321–326.

[27] Hild SA, Reel JR, Larner JM, Blye RP. Disruption of spermatogenesis and Sertoli cell structure and function by the indenopyridine CDB-4022 in rats. Biol Reprod, 2001, 65(6): 1771–1779.

[28] Singh SK, Dominic CJ. Prostaglandin F2 alpha-induced changes in the sex organs of the male laboratory mouse. Exp Clin Endocrinol, 1986, 88(3):309–315.

[29] Winnall WR, Ali U, O'Bryan MK, Hirst JJ, Whiley PA, Muir JA, Hedger MP. Constitutive expression of prostaglandin-endoperoxide synthase 2 by somatic and spermatogenic cells is responsible for prostaglandin E2 production in the adult rat testis. Biol Reprod, 2007, 76(5):759–768.

[30] Takahashi K, Nammour TM, Fukunaga M, Ebert J, Morrow JD, Roberts LJ 2nd, Hoover RL, Badr KF. Glomerular actions of a free radical-generated novel prostaglandin, 8-*epi*-prostaglandin F2 alpha, in the rat. Evidence for interaction with thromboxane A2 receptors. J Clin Invest, 1992, 90(1):136–141.

[31] Sada K, Shirai M, Ninomiya I. Effects of prostaglandin F2 alpha and prostacyclin on pulmonary microcirculation in the cat. J Appl Physiol (1985), 1987, 62(3):1124–1132.

[32] Zannoni A, Bernardini C, Rada T, Ribeiro LA, Forni M, Bacci ML. Prostaglandin F2-alpha receptor (FPr) expression on porcine corpus luteum microvascular endothelial cells (pCL-MVECs). Reprod Biol Endocrinol, 2007, 5:31.

[33] Wang Z, Tamura K, Yoshie M, Tamura H, Imakawa K, Kogo H. Prostaglandin F2alpha-induced functional regression of the corpus luteum and apoptosis in rodents. J Pharmacol Sci, 2003, 92(1):19–27.

[34] Pointis G, Gilleron J, Carette D, Segretain D. Physiological and physiopathological aspects of connexins and communicating gap junctions in spermatogenesis. Philos Trans R Soc Lond B Biol Sci, 2010, 365(1546):1607–1620.

Corresponding author

Anca Sava, Associate Professor, MD, PhD, Department of Anatomy and Embryology, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universităţii Street, 700115 Iassy, Romania; Phone +40744–303 678, Fax +40232–301 633, e-mail: dr_anca_sava@yahoo.com

Received: February 15, 2014 *Accepted: September 4, 2015*