Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 12
Sofia • 2007

Effects of Fibroblast Growth Factors (FGF's) 1, 2 and 7 on the First Wave of Mouse Prospermatogonial Proliferation

Y. Martinova

Institute of Experimental Morphology and Anthropology with Museum Bulgarian Academy of Sciences, Sofia

The onset of spermatogenesis in prepubertal males is marked by the first wave of mitosis in quiescent prospermatogonia. Since Sertoli cells at that time are nonmature and do not mediate hormonal regulation signals, it is hypothesized that this event is under the direct control of paracrine growth factors FGF 1 or FGF 2. We have used a Day 2 mouse testis organ culture assay to identify factors that may control this process. Recombinant FGF 1, FGF 2 and FGF 7 were tested. The DNA synthesis in quiescent prospermatogonia was identified immunocytochemically by using Cell Proliferation kit. In control testes the percentage of labelled germ cells was 10, while after 24 h cultivation in presence of 100 pg/ml FGF 2 the mitotic activity of prospermatogonia increased up to 57%. FGF 1 and FGF 7 stimulated mitotic activity to a lesser extend compared with FGF 2. It is concluded that growth factors from FGF's family play a key role in paracrine regulation of the first wave of prepubertal spermatogonial proliferation in mammals.

Key words: Fibroblast Growth Factors, prespermatogenesis.

Introduction

Different factors are known to stimulate DNA synthesis, proliferation and/or differentiation of germ cells in the adult testes. In the rat testis activin A increases ^{3}H-thymidine incorporation by differentiating spermatogonia [7], whereas inhibin decreases their number [8]. IL-1α, IGF – I and IGF – II stimulate DNA synthesis of differentiating spermatogonia in adult seminiferous tubules in vitro [9]. Thus several growth factors appear to regulate the proliferation of spermatogonia in adult testes but none of these data demonstrate the stimulation of DNA synthesis by the "totipotent" prospermatogonial cells on neonatal animals [5]. Using Sertoli cell secreted media isolated from rat prepubertal Sertoli cells we stimulated prospermatogonial proliferation up to 5-10 fold over the controls in organ culture [4]. We hypothesized that Sertoli cells secrete growth factors, probably FGF's family, acting as a local mitogenic factors on male germ cells.

Fibroblast growth factors make up a large family of polypeptide growth factors, found out from nematodes to humans. In vertebrates 22 members of FGF's family range in molecular mass from 17 to 34 kDa and share 13 — 71% amino acid identity. FGF's have a high affinity for heparin sulfate proteoglicans and require heparin sulfate to activate one

of four cell-surface FGF receptors [11]. FGFs are involved in many biological processes acting through kinase receptors. To date, based on recent evidences, FGF anf TGFβ family members are the only growth factors implicated in regulation of DNA synthesis in prospermatononial stem cells during the first mitotic wave in postnatal testicular development.

The aim of the present work was to study the effect of some members of FGF's family, namely recombinant FGF 1, FGF 2 and FGF 7 on mouse prospermatogonial proliferation.

Materials and Methods

Materials

Male conventional 2 days old mice were supplied by animal breeding farm of the Bulgarian Academy of Sciences (Sofia). Dulbecco's modified Eagle's medium (DMEM) and bovine serum albumin (BSA) (Sigma), OCT compound (Miles, Scientific), Procelloidin (Fluka Chemica-Biochemica), Organ culture dishes (Falkon, Becton-Dickinson), Cell proliferation kit (Amersham) were used. Recombinant Fibroblast growth factors 1, 2 and 7 were kindly provided by Prof. Anthony R. Bellve (Columbia University, New York, USA).

Tissue culture

Tissue culture were prepared as described previously [6]. Briefly, 2-day-old mouse testes were cut into 2 segments, placed on permeable celloidine membrane and placed in an organ culture dish containing DMEM supplemented with 2% BSA and 5-bromo-2-deoxyuridine (BrdU) (control). In the experimental groups the medium was additionally supplemented with FGF 1, 2 or 7 in doses of 1, 3, 10, 30, 100 and 300 pg/ml. The explants were cultured at 37°C with 5% CO_2. At 24th h the explants were immersed in OCT compound, snap-frozen in liquid nitrogen and cryosectioned at 5 μm.

Immunocytochemistry

Sections mounted on slides were fixed in Carnoy's fixative. The incorporated BrdU was detected by using Cell proliferation kit. The sections were countersatined with Harris's haematoxylin and mounted in Canada balsam.

Statistic analysis

At least 100 prospermatogonia were counted in three different testicular segments, each done in triplicate. Statistical analysis was performed by Student's t-test.

Results

On day 2 post partum (p.p.) mouse testis is composed of seminiferous cords and intersitial tissue. In the cords two cell types are well distinguished: Sertoli cells and prospermatogonia. Nonmature Sertoli cells are situated on the basal membrane. They are cylindric in shape, small in size (5-7 μm in diameter) with dark nuclei. In 2-day-old mouse testis Sertoli cells actively proliferate and intensively incorporate BrdU (Fig. 1 and Fig. 2). Prospermatogonia are located in the center of the seminiferous cords. They are big,

round cells (about 20 μm in diameter) with pale nucleus and 2-3 nucleoli (Fig. 1, Fig. 2). In control testes of all groups the percentage of labelled prospermatogonia was about 10 (Fig. 3, 4, 5). Dose-response curve of FGF1 application showed stimulation of prospermatogonial germ cell proliferation up to 27% at a dose of 100 pg/ml (Fig. 3). FGF 2 stimulated BrdU incorporation up to 57% at a dose of 100 pg/ml (Fig. 4). The effect of FGF 7 was similar to FGF 1 with maximum stimulation up to 31% at a dose of 30 pg/ml (Fig. 5). After incubation with maximally effective doses of FGF 2 many prospermatogonial cells were localized between Sertoli cells on the basal membrane of the testicular cords (Fig. 2).

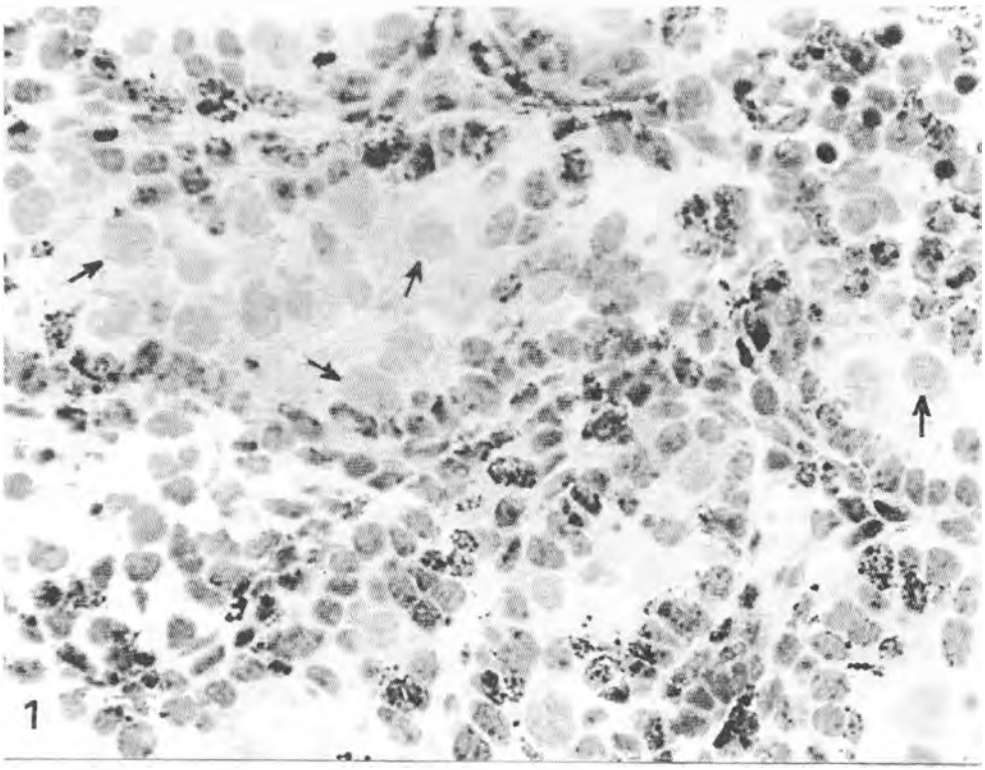

Fig. 1. Section from 2-day-old mouse testis, incubated 24 h in DMEM and BrdU only (control). Prospermatogonia are not labeled (arrows) (× 100)

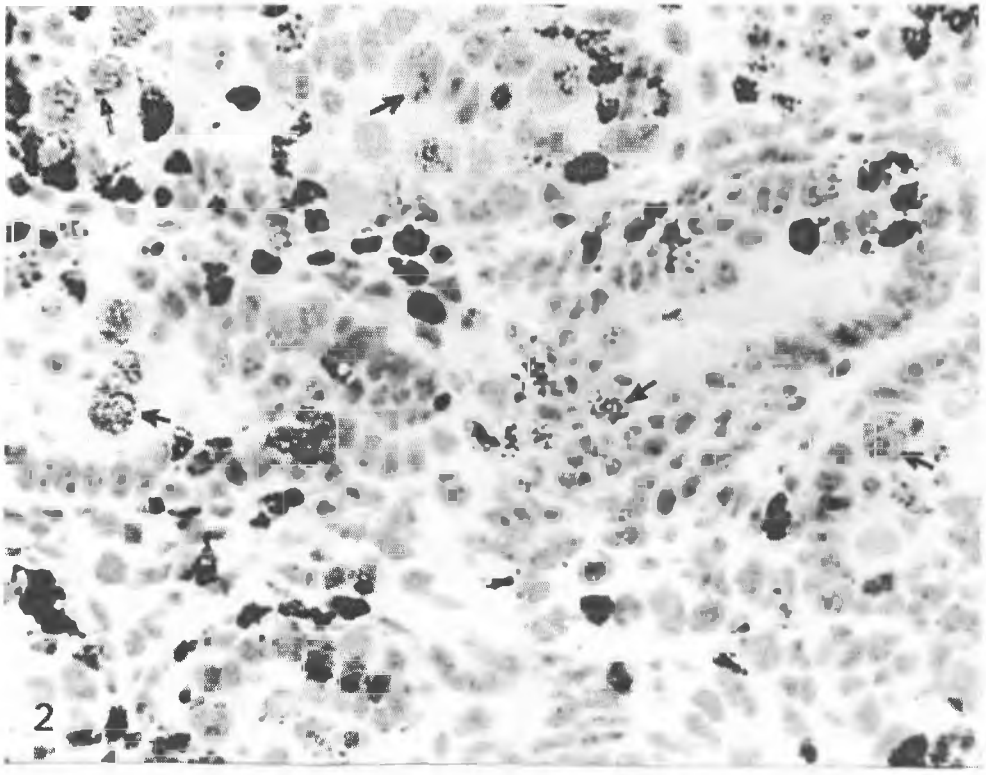

Fig. 2. Section from 2-day-old mouse testis, incubated 24 h in presence of DMEM, BrdU and 100pg/ml FGF 2. Labelled prospermatogonia in the center of the seminiferous cords and between Sertoli cells on the basal membrane (arrows) (× 100)

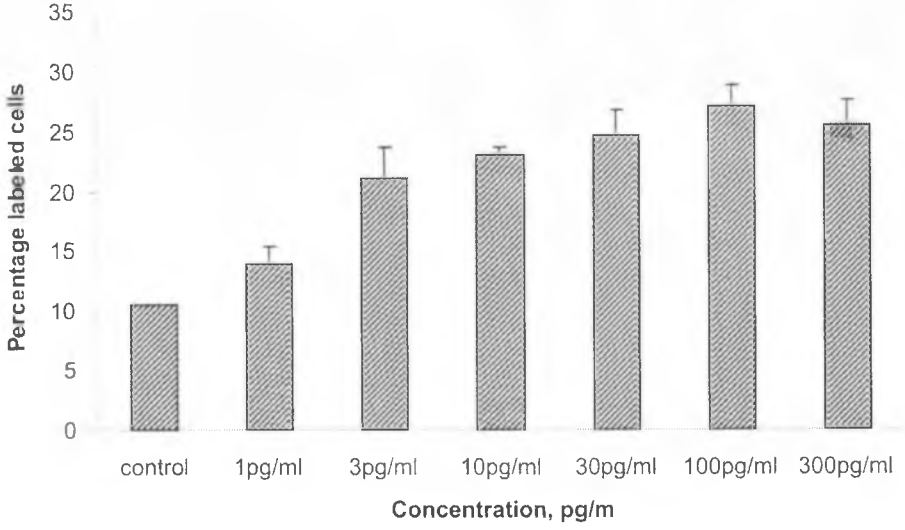

Fig. 3. Percentage (mean ± SD) of labelled prospermatogonia of 2-day-old mouse testis, 24 h after culture in presence of different doses FGF 1

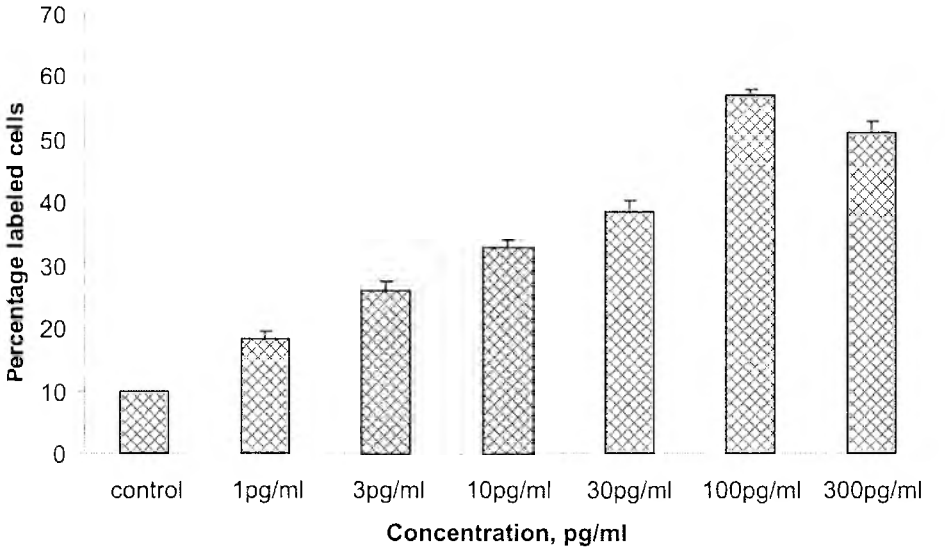

Fig. 4. Percentage (mean ± SD) of labelled prospermatogonia of 2-day-old mouse testis, incubated 24 h in presence of different doses FGF 2

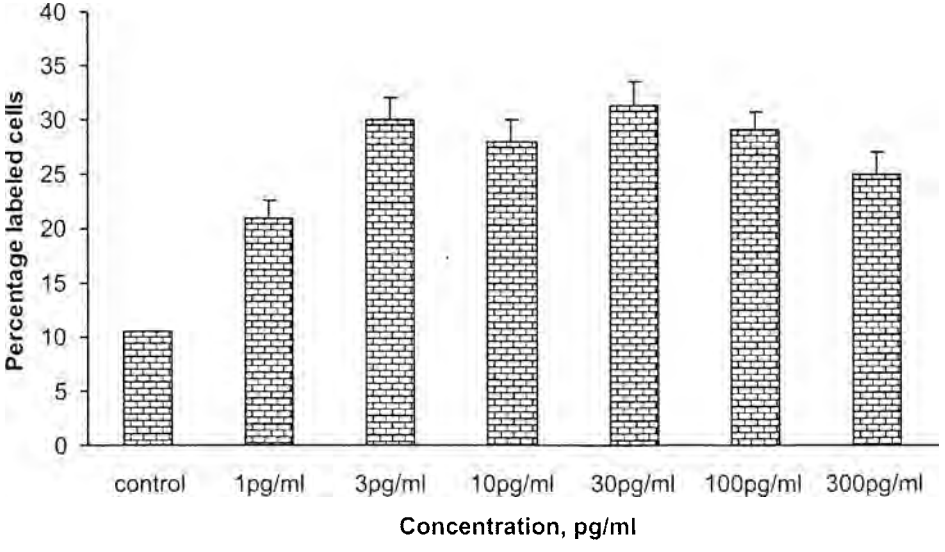

Fig. 5. Percentage (mean ± SD) of labelled prospermatogonia of 2-day-old mouse testis, 24 h after culture in presence of different doses FGF 7

Discussion

It is known that growth factors in general stimulate cell proliferation and/or differentiation by interacting with cell-surface receptors. Using RT-PCR we showed previously that FGF 1 and FGF 2 mRNA is detected in day-4 mouse testis. We suggested that at lest two FGFs are present in the mouse testis at the onset of spermatogenesis (FGF 1 and FGF 2) and prospermatogonia have at least one FGF receptor prior to the onset of spermatogenesis — FGFR — 2 [12]. Lately it was found an expression of mRNA for FGFR — 1, 2, 3 and 4 in fetal, immature and adult testis [2]. It is suggested that ligands FGFs 1 — 5 and 8 can signal through these receptors [3]. Our results indicate that not only FGF — 1 and FGF- 2 but FGF — 7 as well is able to stimulate prospermatogonial DNA synthesis and probably acts through the above mentioned receptors. In addition FGF-7 stimulates DNA synthesis in exocrine pancreatic cells in diabetic and control rats *in vitro* [10], thus being a universal mitogen for a wide variety of cell types.

The application of FGF 2 resulted in changed localization of many prospermatogonia from central location to periphery in the seminiferous cords. It is known that after increasing in gonial cell number, the ratio between germ and Sertoli cell is changed and the process of differentiation starts [1]. The first sign of germ cell diferentiation is change in their localization from the center to the periphery of the cords between Sertoli cells.

In conclusion FGF 1, FGF 2 and FGF 7 stimulate the first wave of mouse prospermatogonial proliferation. FGF 2 is the most efective growth factor acting not only on germ cell proliferation but on male germ cell differentiation as well.

References

1. Bellve, A. R. The molecular biology of mammalian spermatogenesis. – In: Oxford Reviews of Reproductive Biology (Ed. C. A. Finn). Oxford, Oxford University Press, 1979, 159-261.
2. Cancilla, B., G. P. Risbridger. Differential localization of fibroblast growth factor receptor - 1, -2, -3 and - 4 in fetal, immature and adult rat testes. – Biol. Reprod., **58**, 1998, 1138-1145.
3. Cancilla, B., A. Davies, M. Ford – Perriss, G. P. Risbridger. Discrete cell- and stage-specific localization of fibroblast growth factors and receptor expression during testis development. – J. Endocrinol., **164**, 2000, 149-159.
4. Kancheva, L., Y. Martinova, V. Georgiev. Prepubertal Sertoli cells secrete a mitogenic factor(s) that stimulates germ and somatic cell proliferation. – Mol. Cell. Endocrinol., **69**, 1990, 121-127.
5. Kubota, H., M. R. Avabrock, R. L. Brinster. Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells. – Proc. Natl. Acad. Sci. USA, **101**, 2004, 16395-16396.
6. Martinova, Y., D. Nikolova, A. Bellve. Mullerian inhibitory substance down-regulates seminiferous growth factor-stimulated rat prespermatogonial cell proliferation in vitro. – Acta Morphol. Anthropol., **6**, 2001, 45-50.
7. Mather, J. P., K. Attie, T. Woodruff, G. Rice, D. Phillips. Activin stimulates spermatogonial proliferation in germ-Sertoli cell cocultures from immature rat testis. – Endocrinology, **127**, 1990, 3206-3214.
8. Moore, A., L. Krummen, J. Mather. Inhibins, activins, their binding proteins and receptors — interactions underlying paracrine activity in the testis. – Mol. Cell. Endocrinol., **100**, 1994, 81-86.
9. Nakayama, Y., T. Yamamoto, S. Abe. IGF-I, IGF-II and insulin promote differentiation of spermatogonia to primary spermatocytes in organ culture of newt testes. – Int. J. Dev. Biol., **43**, 1999, 343-347.
10. Ogneva, V., Y. Martinova. The effect of fibroblast growth factors on cell proliferation in pancreas from normal and streptozotocin-treated rats in vitro. – Diabetes Research and Clinical Practice, **57**, 2002, 11-16.
11. Ornitz, D., N. Itoh. Fibroblast growth factors. – Genome Biol., **2**, 2001, Reviews 3005.
12. Seidensticker. M., Y. Martinova, A. Bellve. Fibroblast growth factor and the onset of spermatogenesis. – In: Proc. Northeast Reg. Confer. on Developmental Biology, 1996, Marine, USA.