osmium tetroxide in the same buffer, and embedded in epoxy resin according to standard procedures. Sections (1 μm in thickness) were stained with toluidine blue for light microscopic examination.

The following criterion was used to evaluate the effect of various conditions on testicular fragments. Five random toluidine blue-stained 1-μm-thick plastic sections from each of five cultured testis fragments originating from 2 eels were examined, and the number of cysts containing pre-proliferated spermatogonia (type A and early-type B spermatogonia) or proliferating spermatogonia (late-type B spermatogonia) was counted. The results were expressed as a percentage of cysts of late type B spermatogonia per total cysts observed. Isolated type A spermatogonia, or groups of two germ cells surrounded by Sertoli cells, were counted as cysts.

Statistics

The results were expressed as means and SEMs. Differences in means within each group were measured by Duncan's multiple range test.

Results

Before cultivation, most of the germ-cells in the eel testis were type A and early type B spermatogonia (99.2%) (Fig. 1A). These testes were cultured for 15 days in medium with or without human recombinant activin A (10 ng/m*l* and 100 ng/m*l*), activin B (10 ng/m*l* and 100 ng/m*l*) or 11-ketotestosterone (10 ng/m*l*). After cultivation, late type B spermatogonia appeared in cultured testis with 11-ketotestosterone, activin A and activin B (Fig. 1C and Fig. 2A, B) in both fish. Late type B spermatogonia had dense and heterogeneous nuclei. The appearance of late-type B spermatogonia indicates that spermatogonia are proliferating, so the appearance of late-type B spermatogonia in cysts was used as the criterion of proliferation of spermatogonia. The activin B recombinants (10 ng/m*l* and 100 ng/m*l*) and 10 ng/m*l* of 11-ketotestosterone (for positive control) were almost equally effective; mitosis occurred in 30 to 50% of cysts (Fig. 3A, B). Furthermore, mitotic figures were observed frequently in the medium with activin B (Fig. 2B). There was no significant difference between the effect of activin A and activin B in the appearance of cysts containing late-type B spermatogonia (Fig. 3A, B), but treatment with activin B was more effective than that with activin A in increasing the number of late-type B spermatogonia in one cyst (Fig. 2A, B). On the other hand, testicular cultures without activin recombinants or 11-ketotestosterone still had only type A and early-type B spermatogonia which were premitotic (Fig. 1B and Fig. 3A, B).

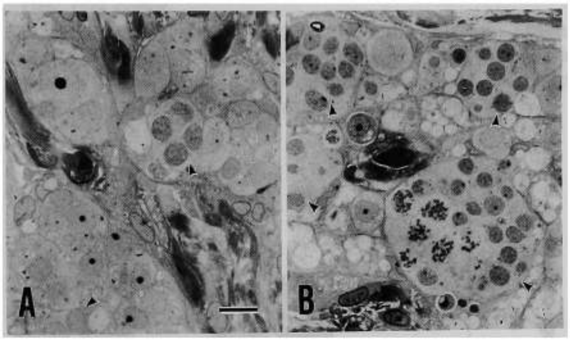

Fig. 2. Light micrographs of toluidine blue-stained 1-μm sections of testes cultured with human recombinant activin A (A) and human recombinant activin B (B) at 10 ng/m*l* for 15 days.
Arrowheads indicate late type B spermatogonia. (Bar = 10 μm)

Discussion

Activin β_B subunit is one of the components of dimeric growth factors which belong to the transforming growth factor-βs (TGF-βs) family.[11] In teleost, activin β_A and β_B had been sequenced from the goldfish genomic DNA partially.[12] It is possible that the activin β_B subunit from eel testis was estimated to be a component of activin B, activin AB and/or inhibin B protein. Activin (especially activin A) and inhibin are developmentally and physiologically important factors.[13] In spermatogenesis, activin A also induce DNA synthesis of rat spermatogonia and preleptotene spermatocyte,[14,15] whereas the detailed action of activin B and AB have not been clarified yet. In this study,

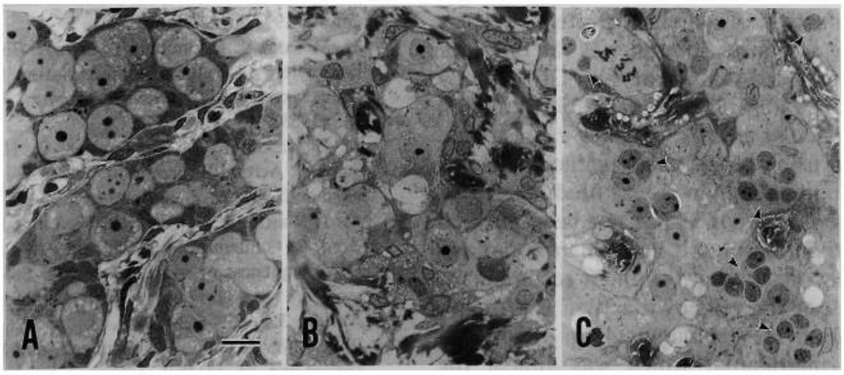

Fig. 1. Light micrographs of toluidine blue-stained 1 μm sections of testes before culture (A), cultured in medium without hormones (B) and cultured in medium with 11-ketotestosterone (C) at 10 ng/m*l* for 15 days.
Arrowheads indicate late type B spermatogonia. (Bar = 10 μm)