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Review

Initiation and stimulation of spermatogenesis *in vitro* by mammalian follicle-stimulating hormone in the Japanese newt, *Cynops pyrrhogaster*

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ABSTRACT In order to elucidate the molecular mechanisms by which spermatogenesis is regulated, especially the roles of hormones and somatic cells in the initiation and promotion of spermatogenesis, we developed an organ culture system with a chemically defined medium. When newt testes fragments rich in secondary spermatogonia were cultured in control medium for three weeks, most of the testicular cysts still remained as secondary spermatogonia. On the other hand, in the medium supplemented with follicle-stimulating hormone (FSH) alone, DNA syntheses in secondary spermatogonia and Sertoli cells were stimulated and secondary spermatogonia differentiated into primary spermatocytes (zygotene-pachytene) in more than half of the cysts by the second week. When newt testes fragments rich in primary spermatocytes were cultured in a control medium for three weeks only round spermatids were observed at the most advanced stage. On the other hand, in the medium supplemented with FSH alone, elongated spermatids appeared by the second week. Neither the addition of luteinizing hormone (LH) nor androgens (testosterone and 5α-dihydrotestosterone) to the control medium stimulated differentiation for either step. Consistent with these findings was the fact that radioreceptor assays revealed high affinity specific binding sites for FSH but none for LH for either stage of the testes (secondary spermatogonia and primary spermatocytes). Preliminary results indicate that FSH does not bind to germ cells but to somatic cells (most probably Sertoli cells). These and our unpublished data suggest that FSH triggers proliferation and differentiation of spermatogonia into elongated spermatids by acting on Sertoli cells which in turn act on germ cells.

KEY WORDS: *initiation of meiosis, nuclear elongation, FSH, organ culture, newt*

Introduction

Spermatogenesis is one of the most attractive systems of differentiation and morphogenesis. Spermatogenesis is initiated by the proliferation of spermatogonia, followed by the differentiation into primary spermatocytes which proceed through meiotic divisions and give rise to spermatids which undergo complex morphogenetic changes, culminating in mature sperm. Generally, several hormones and somatic cells are involved in these steps: it is well established in mammals that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are secreted from the pituitary and work on Sertoli cells and Leydig cells, respectively (Steinberger, 1971; Means *et al.*, 1980; Ritzen *et al.*, 1981; Hodgson *et al.*, 1983; Griswold *et al.*, 1988). Leydig cells secrete androgens which work on Sertoli cells and myoid cells (Fritz, 1978; Sanborn *et al.*, 1983; Skinner, 1991). It is believed that activated Sertoli cells interact with germ cells to stimulate spermatogenesis (Parvinen *et al.*, 1986; Griswold *et al.*, 1988; Skinner, 1991). However, it is poorly understood when FSH and androgens are required or how Sertoli cells interact with germ cells.

In order to elucidate the molecular mechanisms by which spermatogenesis is regulated, it would be ideal to establish a system in which spermatogenesis proceeds *in vitro*. In mammals, organ culture systems have permitted the differentiation of rat gonocytes (Steinberger and Steinberger, 1970) and mouse type A spermatogonia (Aizawa and Nishimune, 1979) into the pachytene stage of primary spermatocytes. However, the essential factors which might be produced by Sertoli cells and stimulate spermatogenesis have not been determined.

Abbreviations used in this paper: FSH, follicle-stimulating hormone; LH, luteinizing hormone; BrdU, 5-bromo-2'-deoxyuridine; HCG, human chorionic gonadotropin; 3ß-HSD, 3ß-hydroxy-Δ^5-steroid dehydrogenase; cAMP, cyclic AMP.

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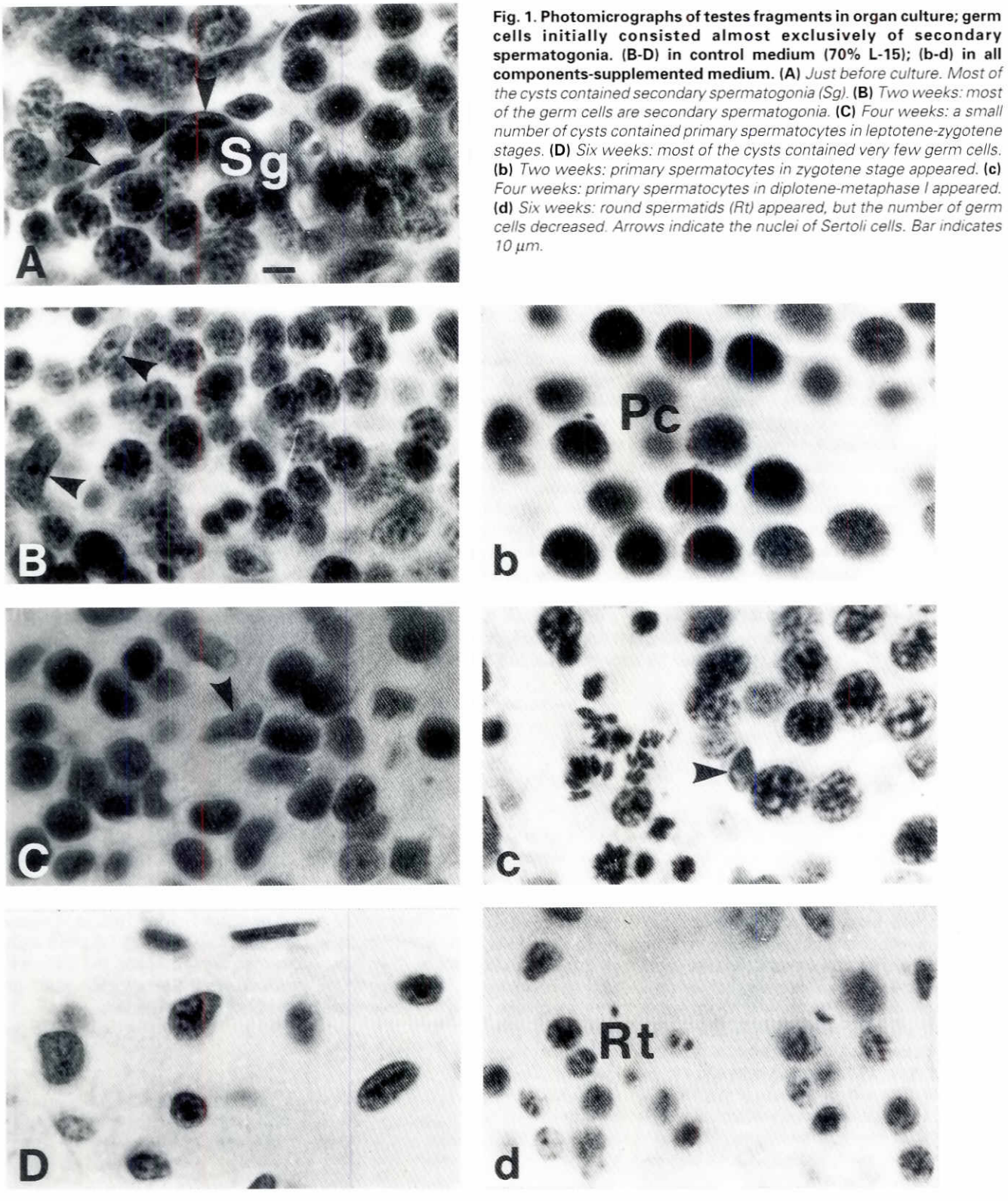

Fig. 1. Photomicrographs of testes fragments in organ culture; germ cells initially consisted almost exclusively of secondary spermatogonia. (B-D) in control medium (70% L-15); (b-d) in all components-supplemented medium. (A) *Just before culture. Most of the cysts contained secondary spermatogonia (Sg).* **(B)** *Two weeks: most of the germ cells are secondary spermatogonia.* **(C)** *Four weeks: a small number of cysts contained primary spermatocytes in leptotene-zygotene stages.* **(D)** *Six weeks: most of the cysts contained very few germ cells.* **(b)** *Two weeks: primary spermatocytes in zygotene stage appeared.* **(c)** *Four weeks: primary spermatocytes in diplotene-metaphase I appeared.* **(d)** *Six weeks: round spermatids (Rt) appeared, but the number of germ cells decreased. Arrows indicate the nuclei of Sertoli cells. Bar indicates 10 μm.*

Amphibians have several advantages for the *in vitro* analysis of the mechanism of spermatogenesis. First, mammalian gonadotropins are effective *in vivo* (Callard *et al.*, 1978; Lance and Callard, 1980; Lofts, 1987); injections of FSH stimulated Sertoli cells and spermatogenesis, while addition of LH caused activation of Leydig cells and spermiation both in anurans (Burgos and

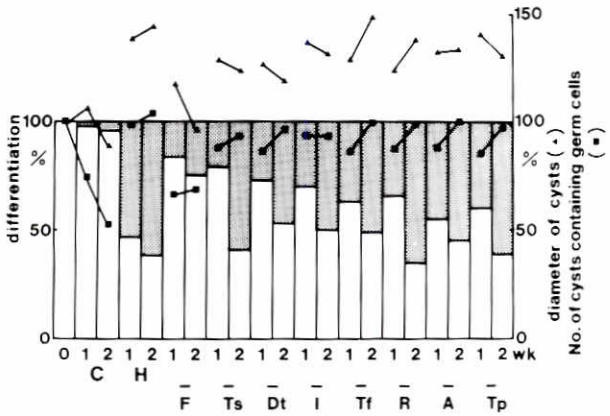

Fig. 2. Percentage of differentiated cells *(white column, secondary spermatogonia; dotted column, primary spermatocytes), diameter of cysts (triangles) and number of cysts containing germ cells (squares) after culture for 1 and 2 weeks in the control medium (C), all components-supplemented medium (H) and all components-supplemented medium depleted whether of FSH (-F), testosterone (-Ts), 5α-dihydrotestosterone (-Dt), insulin (-I), transferrin (-Tf), retinol acetate (-R), L-ascorbic acid (-A) or DL-α-tocopherol (-Tp).*

Ladman, 1957; Lofts, 1961) and urodeles (Andrieux *et al.*, 1973; Moore, 1975; Tanaka and Takikawa, 1984). In fact, Kubokawa and Ishii (1980) demonstrated the presence of specific and saturable binding sites for radioiodinated rat FSH in the testes of the Japanese newt, *Cynops pyrrhogaster*. Second, differentiation of primary spermatocytes to early, mid-spermatid stages in cell suspension cultures with chemically defined media has been established for the Japanese newt, *Cynops pyrrhogaster* (Abé, 1981, 1987, 1988; Nishikawa and Abé, 1983) and *Xenopus laevis* (Risley and Eckhardt, 1979; Risley, 1983; Abé and Asakura, 1987). Third, urodelan testes consist of cysts in which spermatogenesis proceeds synchronously and germ cells at a certain stage (spermatogonia, primary spermatocytes and spermatids) are located in a restricted region (Lance and Callard, 1980). Therefore it is possible to isolate a region where germ cells at a certain stage are localized, depending on the season. Hence, we examine the effect of several hormones and vitamins on spermatogenesis in organ culture of testicular fragments rich in secondary spermatogonia and those rich in primary spermatocytes. We show that secondary spermatogonia differentiate into primary spermatocytes and that primary spermatocytes differentiate to elongated spermatids with high viability in the presence of mammalian FSH alone.

Differentiation of secondary spermatogonia into primary spermatocytes by FSH (Ji *et al.*, 1992)

Testes fragments rich in secondary spermatogonia (Fig. 1A) were cultured at 22°C on a Nuclepore filter which was floated on culture medium in a 60 mm plastic dish. In control medium (70% Leibovitz-15) most of the germ cells remained as secondary spermatogonia for two weeks (Figs. 1B and 2). The diameter of the cysts in control medium decreased a little by the second week, presumably due to the decrease in the number of germ cells. The number of cysts containing germ cells (viability of germ cells) decreased linearly to about a half in the control medium during two weeks of culture. By the fourth week, some primary spermatocytes in leptotene-zygotene stages appeared in a few cysts, but a large number of germ cells were lost (Fig. 1C). After six weeks, germ cells were barely observable (Fig. 1D). On the other hand, when testes fragments were cultured in an "all components-supplemented" medium — hormones: (10 μg/ml bovine insulin, 10 μg/ml human transferrin, 5 μg/ml porcine FSH, 30 ng/ml testosterone and 30 ng/ml 5α-dihydrotestosterone); vitamins: (30 ng/ml retinol acetate, 50 μg/ml L-ascorbate and 200 ng/ml DL-α-tocopherol) —, secondary spermatogonia differentiated into zygotene-pachytene stages in more than half of the cysts within two weeks (Figs. 1b and 2). Moreover, the cyst size increased to about 1.5 times as the original size and the viability of germ cells remained almost constant (Fig. 2). They then differentiated into diplotene-metaphase I stages by the 4th week (Fig. 1c). By the 6th week round spermatids were observed but the number of germ cells decreased remarkably (Fig. 1d). These results show that over a two week culture period germ cells survived and differentiated in the all components-supplemented medium, whereas in control medium, very few germ cells differentiated and, in fact, many died.

To determine which components in the all components-supplemented medium were effective in promoting the differentiation of spermatogonia to primary spermatocytes, specific components were removed from the all components-supplemented medium, and the percentage of differentiated cells, the diameter of cysts and the viability of germ cells were determined (Fig. 2). Removal of each of the 8 components except FSH from the all components-supplemented medium did not result in significant reduction of the three parameters. But only when FSH was depleted, did the three

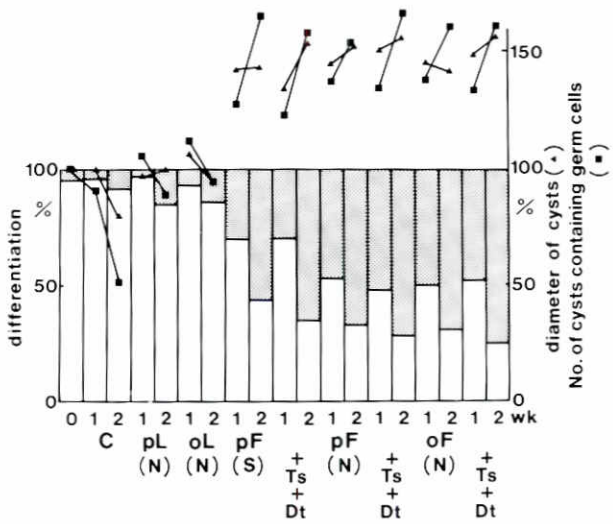

Fig. 3. Percentage of differentiated cells, *diameter of cysts and number of cysts containing germ cells after culture for 1 and 2 weeks in various media (control medium supplemented with components indicated). pL(N), porcine LH from NIH; oL(N), ovine LH from NIH; pF(S), porcine FSH from Sigma; pF(N), porcine FSH from NIH; oF(N), ovine FSH from NIH. The concentrations of FSH and LH were 5 μg/ml, respectively. The concentrations of testosterone and 5α-dihydrotestosterone were 30 ng/ml, respectively.*

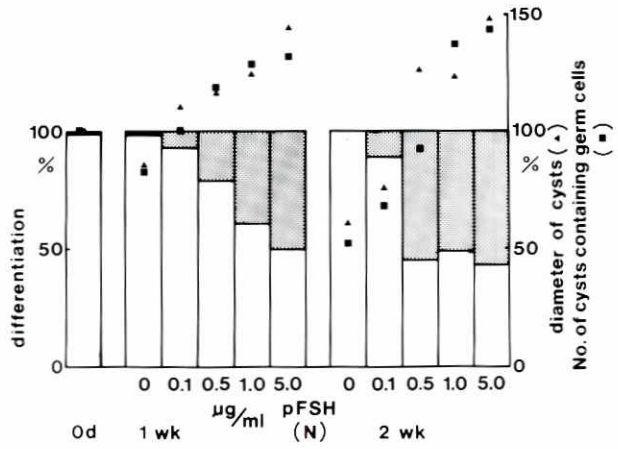

Fig. 4. Dose-dependent effect of porcine FSH (NIH) on the percentage of differentiated cells, diameter of cysts and number of cysts containing germ cells after culture for 1 and 2 weeks.

parameters show significant decrease. These results indicated that FSH is indispensable for the differentiation of spermatogonia to primary spermatocytes.

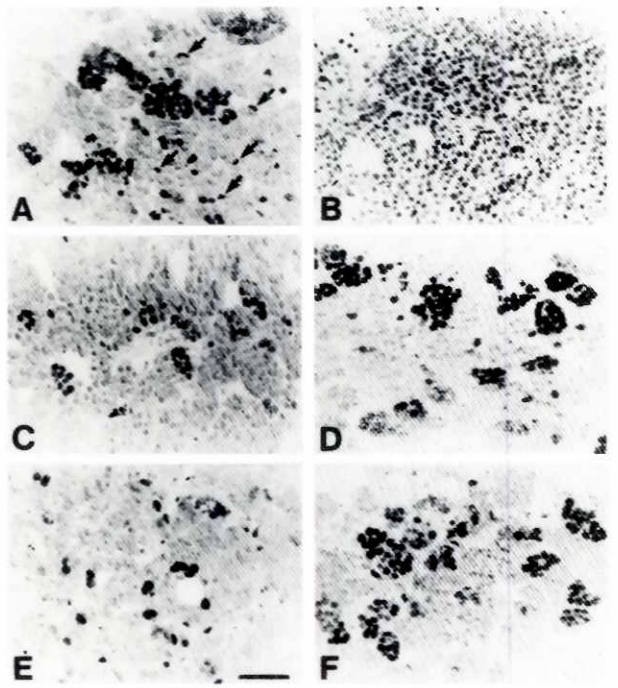

Fig. 5. Detection of BrdU-incorporating cells by immunohistochemistry. **(A)** *BrdU was incorporated into testes fragments in organ culture for 3 h on day 0* **(A)**, *7* **(C and D)** *and 8* **(E and F)**, *washed off, fixed in Bouin solution, embedded together in paraffin and processed through histological procedures. Sections (5 μm) were stained with anti-BrdU monoclonal antibody, followed by peroxidase-conjugated anti-mouse IgG. Arrows show some labeled Sertoli cells.* **(B)** *The section just adjacent to (A) was stained by hematoxylin and eosin. All of the germ cells were secondary spermatogonia.* **(C)** *After culture for 7 days in the absence of FSH.* **(D)** *After culture for 7 days in the presence of FSH.* **(E)** *After culture for 8 days in the absence of FSH. Bar indicates 100 μm.* **(F)** *After 1 day following addition of FSH to the fragments which had been cultured in the absence of FSH for 7 days. Magnification is the same for all photographs.*

To determine whether FSH alone is sufficient or both FSH and androgens are required for the differentiation of spermatogonia to primary spermatocytes, the effect of adding FSH, LH (several sources) and androgens, separately or in combination was examined. Addition of porcine FSH alone (5 μg/ml, Sigma) to the control medium promoted differentiation of spermatogonia to primary spermatocytes to the same extent as the all components-supplemented medium (Fig. 3). Also, porcine or ovine FSH from NIH strongly promoted differentiation. However, porcine or ovine LH (5 μg/ml) from NIH had very little effect. Neither testosterone alone nor 5α-dihydrotestosterone alone promoted differentiation at all concentrations examined. Moreover, synergistic effect of testosterone or 5α-dihydrotestosterone with FSH (5 μg/ml) was not found at the concentration range examined (3-300 ng/ml). The extent of differentiation was dependent on the concentration of porcine FSH (NIH) (Fig. 4). After a week of culture the percentage of primary spermatocytes, diameter of cysts and viability of germ

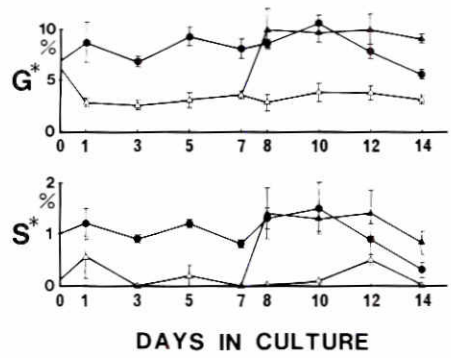

Fig. 6. Percentage of labeled spermatogonia (G*) and Sertoli cells (S*) during culture. *Symbols: (●) in the continuous presence of FSH; (Δ) in the continuous absence of FSH; (▲) FSH was added on day 7 following continuous absence of FSH. Standard errors among 18 fragments in triplicate experiments were shown.*

cells increased in proportion to the increase in FSH concentration. At 2 weeks, concentrations of 0.5 μg/ml and greater promoted differentiation in more than 50% of the cysts. Thus, FSH alone is sufficient for promoting the differentiation of spermatogonia to primary spermatocytes.

To examine the possibility that steroids other than testosterone or 5α-dihydrotestosterone were produced by the fragments exposed to FSH and promoted differentiation, cyanoketone, a specific inhibitor of 3β-hydroxy-Δ^5-steroid dehydrogenase (3β-HSD) (Young *et al.*, 1982), was added to the culture medium containing FSH. Cyanoketone at concentrations of 0.1-10.0 μg/ml did not inhibit the differentiation of spermatogonia to primary spermatocytes that was promoted by FSH. These results excluded the possibility that FSH promoted the differentiation of spermatogonia to primary spermatocytes via stimulating the production of sex steroids and their metabolites in the Δ^4 pathway.

Miura *et al.* (1991a,b) have shown that human chorionic gonadotropin (HCG) induced all stages of spermatogenesis in organ culture of testes fragments from the Japanese eel *(Anguilla japonica)* and that 11-ketotestosterone, a major androgen in teleost

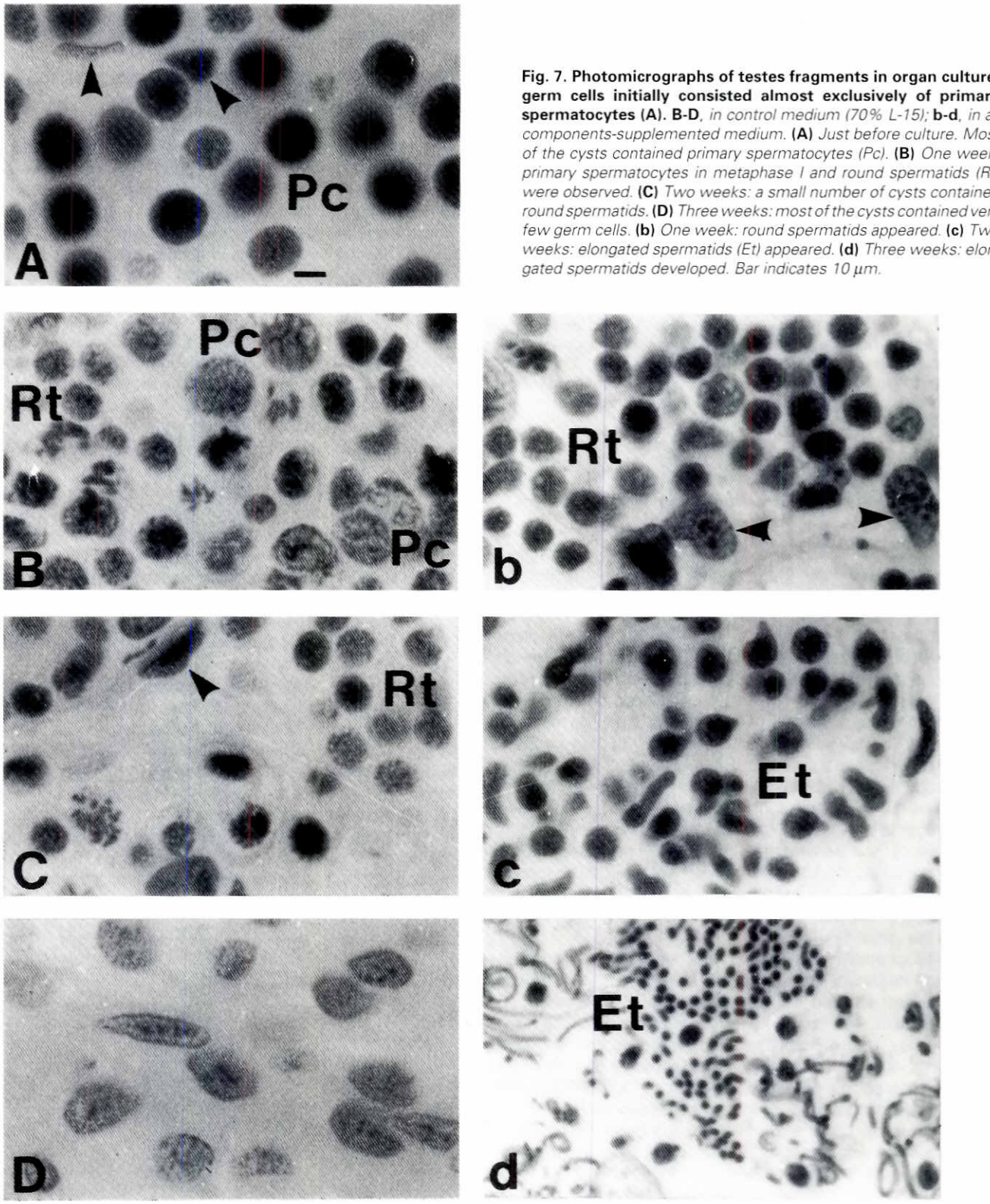

Fig. 7. Photomicrographs of testes fragments in organ culture; germ cells initially consisted almost exclusively of primary spermatocytes (A). B-D, *in control medium (70% L-15);* **b-d**, *in all components-supplemented medium.* **(A)** *Just before culture. Most of the cysts contained primary spermatocytes (Pc).* **(B)** *One week: primary spermatocytes in metaphase I and round spermatids (Rt) were observed.* **(C)** *Two weeks: a small number of cysts contained round spermatids.* **(D)** *Three weeks: most of the cysts contained very few germ cells.* **(b)** *One week: round spermatids appeared.* **(c)** *Two weeks: elongated spermatids (Et) appeared.* **(d)** *Three weeks: elongated spermatids developed. Bar indicates 10 μm.*

fish, could substitute for HCG, with a marked activation of Sertoli cells. From these results, Miura *et al.* (1991b) suggested the following sequence of events for the hormonal induction of spermatogenesis in eel testes: gonadotropin stimulates the Leydig cells to produce 11-ketotestosterone, which in turn activates the Sertoli cells to stimulate premitotic spermatogonia. Thus, the mechanism by which spermatogonia are stimulated to proliferate may be different in amphibians and teleost fishes.

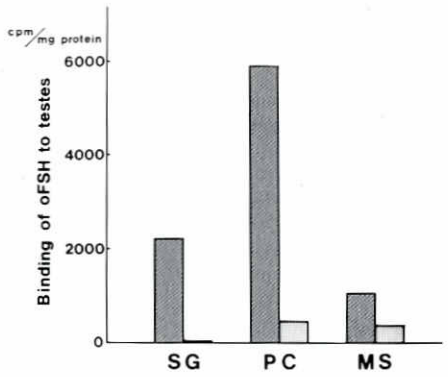

Fig. 8. Binding of ovine FSH to newt testes in three different stages: spermatogonia-rich stage (SG), primary spermatocyte-rich stage (PC) and mature sperm-rich stage (MS). *Left column in each stage shows specific binding and right column shows nonspecific binding. Standard deviations were smaller than 70 cpm per mg protein in a triplicate experiment.*

Stimulation and proliferation of secondary spermatogonia and Sertoli cells by FSH (Ji and Abé, 1994)

In order to examine if FSH stimulates DNA synthesis in spermatogonia and Sertoli cells, testes fragments in culture were incubated in 5-bromo-2'-deoxyuridine (BrdU) for 3 h on each of the following days: 0,1,3,5,7,8,10,12 and 14. Sections were stained with anti-BrdU monoclonal antibody, followed by peroxidase-conjugated anti-mouse IgG. Testes fragments, incubated with BrdU during the initial hours of culture, showed some labeled spermatogonia and Sertoli cells (Fig. 5A). Most of the spermatogonia were labeled as a cluster which consisted of 10-30 cells per section and corresponded to a cyst (Fig. 5A). On day 0, 7-8% of the total spermatogonia were labeled and during a culture period of 2 weeks in the continuous presence of FSH, the percentage of labeled spermatogonia was almost constant (Fig. 6). In the absence of FSH, however, the percentage of the labeled cells decreased by half by the first day, and thereafter was maintained at this low level until day 14. These results indicate that about three times more spermatogonia continue proliferation in the presence of FSH than those in the absence of FSH.

We next examined if FSH could stimulate proliferation of quiescent spermatogonia (Figs. 5C-F and 6). After a week in culture, few spermatogonia showed DNA synthesis in the absence of FSH (Figs. 5C and 6). Addition of FSH to the testes fragments induced, after one day, a remarkable increase in the number of labeled spermatogonia (Fig. 5F) to the same extent as that attained when FSH was present continuously from day 0 (Fig. 6). Thus, FSH induced the proliferation of quiescent secondary spermatogonia.

In contrast to the case of spermatogonia, most of the Sertoli cells were labeled as single cells and asynchronously in a cyst (Fig. 5). The percentage of the labeled Sertoli cells in the presence of FSH was about 1% and significantly higher than that in the absence of FSH throughout the culture period (Fig. 6). Addition of FSH to the fragments which had been cultured without FSH for 7 days remarkably increased the number of labeled Sertoli cells after one day to the same level as that in the continuous presence of FSH from day 0 (Fig. 6). The combined results indicate that FSH stimulates DNA synthesis in both spermatogonia and Sertoli cells and also induces quiescent spermatogonia and Sertoli cells to proliferate.

Differentiation of primary spermatocytes to elongated spermatids by FSH

When testes fragments rich in primary spermatocytes (Fig. 7A) were cultured in control medium for one week, primary spermatocytes in metaphase I and round spermatids were observed (Fig. 7B). By the second week, a small number of cysts contained round spermatids, though the germ cells began to be lost (Fig. 7C). After three weeks of culture, it was very difficult to find germ cells in cysts but the most advanced stage was round spermatids (Fig. 7D). The diameter of the cysts in control medium decreased remarkably by the third week, presumably due to the decrease in the number of germ cells. The viability of the germ cells also decreased drastically after 3 weeks in the control medium. On the other hand, when testes fragments were cultured in the "all components-supplemented medium", round spermatids appeared after one week (Fig. 7b). By the end of two weeks, some cysts contained elongated spermatids (Fig. 7c). By the third week elongated spermatids continued to develop, but the number of germ cells decreased a little and the arrangement of the elongated spermatids became somewhat irregular (Fig. 7d). The cyst size remained at the original level and the viability increased to a level higher than the original level after 3 weeks. These results show that during three weeks the spermatocytes survived and differentiated into elongated spermatids in the all components-supplemented medium, whereas, in a control medium, only some spermatocytes differentiated into round spermatids but most of the germ cells died during the culture.

In order to ascertain which components in the all components-supplemented medium were effective in promoting the differentiation of primary spermatocytes to elongated spermatids, specific components were added to the control medium. In the media to which was added one of the vitamins — A (30 ng/ml), C (50 μg/ml) and E (200 ng/ml) — or androgens — testosterone (30 ng/ml) and 5a-dihydrotestosterone (30 ng/ml) —, no differentiation into the elongated spermatid-stage was observed after three weeks, and the diameter of the cysts and viability of germ cells decreased drastically as in the control medium. Addition of only transferrin (10 and 100 μg/ml) had no stimulatory effect on the three parameters, while insulin (10 and 100 μg/ml) slightly improved the parameters.

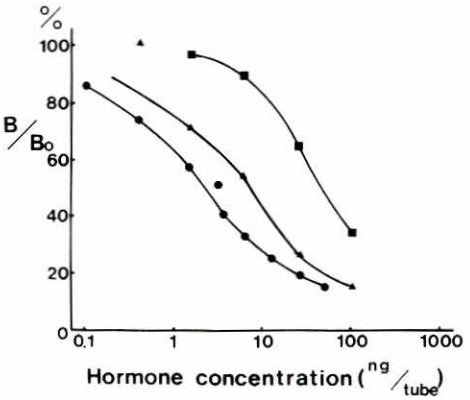

Fig. 9. Competitions by various hormones to specific binding of [^{125}I]oFSH to testicular receptors. *Symbols: ●: ovine FSH; ▲: rat FSH; ■, human FSH; ◊, ovine LH.*

On the other hand, in the medium added with FSH (5 μg/ml) alone, elongated spermatids appeared after 2 weeks of culture in almost the same number of cysts as in all components-supplemented medium. Also the diameter of cysts and viability of germ cells were as significantly high as those in all components-supplemented medium. All three parameters, namely, the percentage of elongated spermatids, diameter of cysts and viability of germ cells increased in proportion to the increase of FSH concentration. On the other hand, neither luteinizing hormone (LH, 5 μg/ml) nor androgens had significant effect of promoting differentiation. The combination of FSH (5 μg/ml), testosterone and 5α-dihydrotestosterone (3-300 ng/ml) had no more of a stimulatory effect than did FSH alone. This result showed that FSH did not act to promote differentiation synergistically with testosterone and 5α-dihydrotestosterone. These results indicated that the vitamins, transferrin and androgens were dispensable, insulin had some effect on improving the viability of germ cells but FSH alone was indispensable and sufficient for the differentiation of primary spermatocytes to elongated spermatids.

The effect of cyanoketone, a specific inhibitor of 3ß-hydroxy-Δ^5-steroid dehydrogenase (3ß-HSD), on the differentiation in the culture medium containing FSH was found to be negative. This result excluded the possibility that FSH promoted the differentiation of spermatocytes to elongated spermatids via stimulating the production of sex steroids and their metabolites in the Δ^4 pathway.

The effect of mammalian gonadotropins on spermatogenesis was studied *in vivo* and *in vitro* in various species of urodelan amphibians, and the results are consistent with our findings. Injections of mammalian LH into hypophysectomized *Pleurodeles* stimulated 3ß-HSD reactions in the glandular tissue but caused no change in germ cells, whereas addition of FSH stimulated spermatogenesis but caused no effect on glandular tissue (Andrieux *et al.*, 1973). Injections of mammalian FSH stimulated proliferation of secondary spermatogonia and their differentiation into primary and secondary spermatocytes in hypophysectomized *Ambystoma tigrinum* larvae, whereas the secondary spermatogonial cysts degenerated unless FSH-treatment was continued (Moore, 1975). Also, in *Triturus cristatus carnifex*, treatment of the hypophysectomized adults with FSH and LH promoted maturation of spermatogonia to the pachytene stage (Vellano *et al.*, 1974). In *Cynops pyrrhogaster*, injection of ovine FSH stimulated spermatogenesis (Tanaka and Takikawa, 1984).

In the absence of FSH, primary spermatocytes differentiated to round spermatids. This is consistent with our previous results that in cell suspension culture without any hormones or serum, purified primary spermatocytes undergo two meiotic divisions to give rise to round spermatids which form flagella and acrosomes but never initiate nuclear elongation even after a month's culture (Abé, 1981, 1987, 1988; Nishikawa and Abé, 1983). There seem to be two possible reasons why primary spermatocytes do not differentiate to elongated spermatids in organ culture without FSH. One is that the population of germ cells which could reach elongated spermatids-stage was very low due to the significant decrease in the viability of germ cells with time. Another one is that there is a barrier between round spermatid-stage and elongated spermatid-stage which could be overcome only by the addition of FSH.

Binding of FSH to newt testicular receptors

Binding of radioiodinated ovine FSH to testicular receptor preparations (crude plasma membrane preparation) was measured for samples in three different stages (secondary spermatogonia, primary spermatocytes and mature sperm) (Ji *et al.*, 1992). Specific binding of [^{125}I]oFSH was highest in primary spermatocyte-rich and lowest in mature sperm-rich samples (Fig. 8). Specific binding of radioiodinated ovine LH to testicular receptor preparations was not detectable.

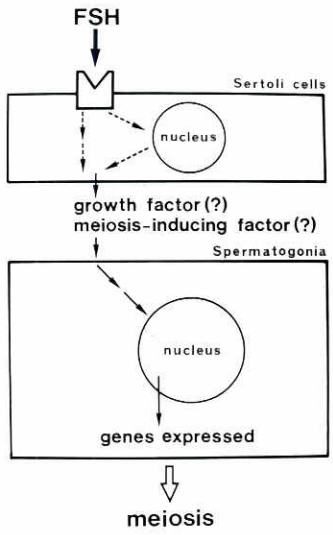

Fig. 10. A scheme depicting the action of FSH on Sertoli cells which then activate spermatogonia to stimulate their proliferation and entering into meiosis.

The abilities of various gonadotropins to inhibit specific binding of [^{125}I]oFSH were determined in receptor preparations from testes in the primary spermatocyte-stage (Fig. 9) (Ji *et al.*, 1992). Rat and human FSH inhibited the binding of oFSH to the testicular receptors and their competition curves were parallel with that of oFSH. Ovine LH showed little inhibition even at an extremely high concentration (1 μg/tube). These results indicate that the newt testes contain specific binding sites of oFSH which do not bind oLH.

In order to examine which cell types FSH binds to, we separated germ cells from somatic cells by centrifugation of dissociated cells in Metrizamide solution. Preliminary results showed that [^{125}I]oFSH barely bound to germ cells but did bind to somatic cells (most probably Sertoli cells).

How does FSH work on newt spermatogenesis?

The *in vitro* studies mentioned above revealed three main findings: (1) mammalian FSH stimulates DNA synthesis in both secondary spermatogonia and Sertoli cells; (2) FSH alone is required and sufficient for the differentiation of secondary spermatogonia into elongated spermatids; and (3) newt testes contain receptors specific to FSH. How does FSH work to stimulate proliferation of secondary spermatogonia and their differentiation into primary spermatocytes and elongated spermatids? First of all, our preliminary results showed, as mentioned above, that FSH bound to somatic cells (most probably Sertoli cells). So, it seems quite probable that FSH activates Sertoli cells. This is consistent with our recent finding that cAMP content in somatic cells which had been incubated in FSH was 2-3 times as high as that not incubated in FSH, whereas cAMP content in germ cells was not affected by incubation in FSH (Ji *et al.*, in preparation). Hence, it seems reasonable to assume that FSH works on the membrane receptors on Sertoli cells within which the cAMP level is then elevated, followed by activation of signal transduction cascade,

finally some genes are turned on and the protein products are synthesized and transported to the plasma membrane where they are anchored or secreted (Fig. 10).

To further analyze the interaction of germ cells with somatic cells, we measured [^{3}H]thymidine incorporation into a reaggregation of a pure population of secondary spermatogonia and a mixed population of secondary spermatogonia and somatic cells in the presence and absence of FSH. Preliminary results indicate that, in the presence of FSH, somatic cells stimulate DNA synthesis of secondary spermatogonia, while, in the absence of FSH, somatic cells did not. In addition, reaggregation of primary spermatocytes and somatic cells showed differentiation of more number of round spermatids in the presence of FSH than in the absence, while reaggregation of germ cells alone resulted in similar extent of differentiation in the presence and absence of FSH (Ji *et al.*, in preparation). Hence, it is reasonable to postulate that Sertoli cells, after exposure to FSH, could stimulate secondary spermatogonia and primary spermatocytes by secreting molecules or by membrane-bound molecules which act on germ cells. Some genes in the spermatogonia are then expressed for the cells to enter into growth and meiosis (Fig. 10).

Further analysis should provide insight into the molecular mechanisms by which spermatogonia are stimulated to proliferate and differentiate into mature sperm in amphibians.

Acknowledgments

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