IN VITRO STEROID SECRETION BY SERTOLI/GERM CELL UNITS (SPERMATOCYSTS) DERIVED FROM DOGFISH (SQUALUS ACANTHIAS) TESTIS

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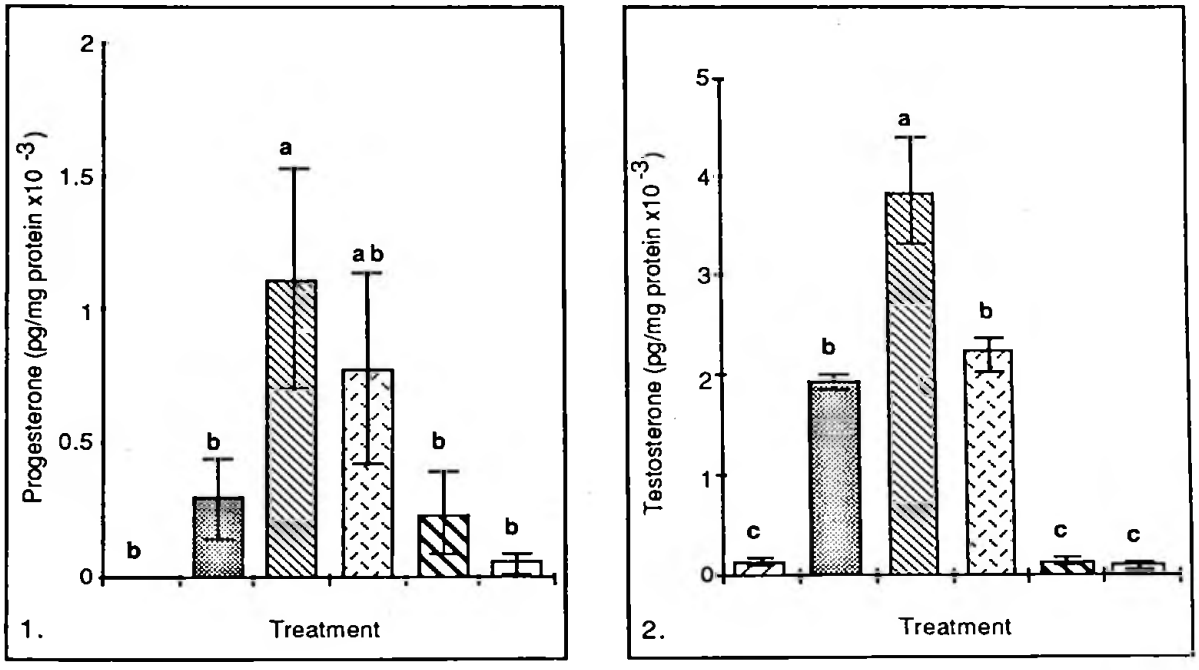
A key to understanding the relationship between steroidogenesis and spermatogenesis is to define qualitatively and quantitatively the steroid microenvironment of each germ cell stage. Due to the complex organization of the mammalian testis, germ cells cannot be segregated by stage without disrupting their relationship to endocrine (Sertoli and Leydig) cells. By contrast, the spiny dogfish is an ideal model for investigating stage-dependent biochemical changes. Although all important features of spermatogenesis and steroidogenesis are identical to those in mammals, the organization of the testis differs: (a) Sertoli cells and germ cells, all synchronized in the same stage, form discrete anatomic units (spermatocysts); (b) as spermatocysts in successive developmental stages progress through the testis, a readily visible zonation is discerned under the dissecting microscope; (c) electron microscopic observations ascribe primary steroidogenic functions to a single cell type, the Sertoli cell, whereas Leydig cells are sparse and undeveloped at this phyletic level. Previous studies in this laboratory have used Squalus testis to demonstrate stage-dependent variations in key enzymes of androgen and estrogen biosynthesis (Callard et al., Endocrinology 177:1328, 1985); however, it remains to be determined whether enzymatic activities, as measured in cell subfractions by tracer methodology, accurately reflect steroid production by intact cells from endogenous precursors and how these changes are regulated. We recently reported techniques for isolating, staging and culturing spermatocysts from Squalus testis (DuBois and Callard, The Bulletin 27:41, 1987) and here we present evidence of their steroid secretory activity.

For each experiment, spermatocysts were isolated from testes of 1-3 animals as described previously (DuBois and Callard, op cit). Only cysts from testicular Zone III (postmeiotic stages) were collected for these preliminary experiments, because steroidogenic activity is known to be maximal at this developmental stage. Cysts were suspended in culture medium composed of Dulbecco's Modified Eagle's Medium/HAM Nutrient Mixture F-12 (DME/F-12, 1:1), 20 mM Hepes, 2% fetal bovine serum, penicillin (1 u/ml), streptomycin (1 µg/ml), insulin/transferrin/ selenite (25 ng/ml each), linoleic acid (100 µg/ml) and adjusted to 280 mM NaCl and 350 mM urea, pH 7.4, and incubated in microwell plates at 17°C in a 5% CO_2 atmosphere for 24 hr. Cysts and medium were then separated and the latter used for determination of steroids by specific radioimmunoassay according to procedures in routine use in this laboratory (Pasmanik et al., Endocrinology 122(4):1349, 1988). Cysts were used for determination of protein by the method of Lowry.

Results shown in Figures 1 and 2 are representative of 3 separate culture experiments. Although progesterone (P_4) secretion was undetectable when cysts were cultured in basic medium, addition of 25-hydroxycholesterol (25-OH, 60 µM) markedly increased output. By contrast, testosterone (T) secretion was detectable even in the absence of added 25-OH, although substrate addition further increased levels >10 fold. Estradiol was at the limits of detection under these conditions (not shown). Addition of dibutyryl cyclic AMP (1 mM) together with the phosphodiesterase inhibitor 3-isobutyl-1-methyl xanthine (MIX 1 mM) markedly stimulated secretion of P_4 and T in the presence, but not the absence, of 25-OH, (Fig 1 and 2). Variable effects on P_4 and T were obtained with forskolin, a known activator of adenyl cyclase. As shown here increases in P_4 were marginal and T was not responsive but in other experiments effects with forskolin were observed. In a separate experiment (not shown), spermatocysts incubated with 25-OH for 6 days progressively increased P_4 secretion (516 $\pm$95 vs 2393 $\pm$1089 pg/mg protein/ 24 hr on d 1 and 6, respectively) and remained responsive to cAMP throughout this period. A different effect of time in culture was observed with T, levels declining progressively to 33% of the initial 24-hr secretory rate by day 6 .

These data support the view that Squalus Sertoli cells actively convert cholesterol to P_4 and T and, in this respect, differ from mammalian Sertoli cells which derive T mainly from adjacent Leydig cells. Although steroid production in vitro is minimal in the absence of 25-OH, a soluble form of cholesterol, lipoprotein-mediated uptake of circulating cholesterol presumably is the source of substrate in vivo. Mammalian studies have shown that gonadotropins and other circulating messengers

act via cAMP to activate steroidogenesis at several points in the pathway. Thus, increased P_4 secretion by spermatocysts in response to cAMP suggests that $P450_{scc}$ (cholesterol--->pregnenolone) has been stimulated. Whether there is an additional effect on $P450_{17a}$ (P_4--->T) cannot be determined from these studies since T output would be expected to increase as a consequence of enhance P_4 synthesis. On the other hand, differential effects of forskolin and time in culture on P_4 versus T suggests differential regulatory mechanisms. Data presented here are evidence that isolated spermatocysts remain functional under defined conditions in vitro and open new possibilities for studying stage-related changes in steroid secretion and the consequences of steroid action on spermatogenesis. (Supported by a grant from NIH HD16715).



(Figure 1) Progesterone and (Figure 2) testosterone production by isolated spermatocysts. Values are means + SEM of triplicate cultures. Means with different letters are significantly different ($p \leq 0.05$) No treatment; 25 OH - Cholesterol; 25 OH - Cholesterol + cAMP/MIX; 25 OH - Cholesterol + Forskolin; cAMP/MIX; Forskolin