The appearance of a vitellogenin band was monitored by sodium-dodecyl-sulfate gel electrophorens (7-15%). Vitellogenin was not detectable in 25-50 ul samples of male and early pregnancy female plasma but low levels were detectable in late pregnancy female plasma. Treatment with estradiol-17β alone or with estradiol 17β + progesterone failed to induce detectable vitellogenin synthesis in males and early pregnancy females. However, injection of estradiol-17β readily induced vitellogenin production in late pregnancy females (Fig. 1) and plasma levels continued

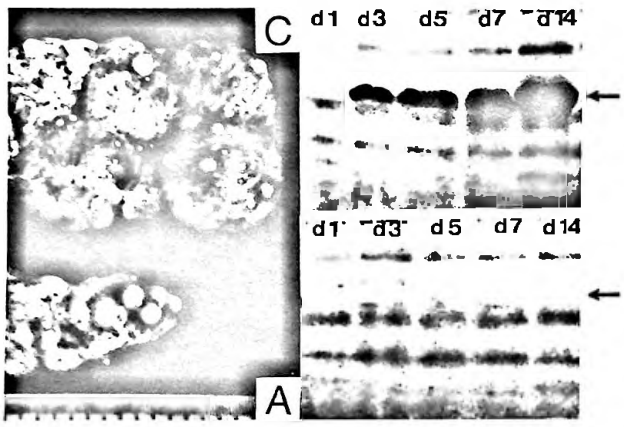

Figure 1. Portion of the ovary and gel electrophoretic pattern of plasma from different reproductive stages of the spiny dogfish Squalus acanthias. The left upper panel shows a portion of the ovary from a Stage C pregnancy fish with growing follicles and left lower from a Stage A pregnancy fish with small follicles. The right panel shows the electrophoretic pattern of the plasma from animals run on 0.1% sodium dodecyl sulphate, 7-15% polyacrylamide gels. Fish were injected with 3 x 1 mg of estradiol-17β in sesame oil and estrogen-induced vitellogenesis was observed only in Stage C animal (above) and not in Stage A animal (below).

to increase reaching maximum 15 days after the first injection. Concomittant injection of progesterone with estradiol-17β, appeared to reduce the estrogen-induced response. Since early pregnant S. acanthias appear to possess active corpora lutea (Lance and Callard, Gen. Comp. Endocrinol. 13, 255-267, 1979) and progesterone has been demonstrated in elasmobranch plasma, the insensitivity of early pregnancy females to estradiol may be due to the presence of this steroid in the circulation. Further investigations of peripheral plasma progesterone concentration at various stages of pregnancy are required to support this suggestion. Supported by NSF grant #PCM 78-08201. *Recipient of an NSF Undergraduate Research Participation Award.

ESTROGEN SYNTHESIS IN DOGFISH TESTIS

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Although the ovary and placenta are generally considered to be the major sources of circulating estrogen, the testis can also synthesize large amounts of estrogen (Engel, Handbook of Physiology, Section 7, Vol. 2, Pt. 1, p. 467, 1974). Androgens, testosterone (T) and androstenedione (A), are the immediate precursors of estradiol-17β (E_2) and estrone (E_1), respectively, and, in all estrogen-synthesizing tissues, the reaction is governed by a P_{450} enzyme complex termed aromatase that is found predominantly in microsomal subfractions. Both Leydig (interstitial) and Sertoli (tubular) cells have been implicated in testicular estrogen synthesis (Canick et al, Endocrinology 104, 285, 1979). The functional significance of aromatization in the testis has yet to be demonstrated, but it has been suggested that estrogen formed in situ governs testicular androgen production by a local feedback mechanism or regulates germ cell proliferation and maturation.

In mammals, all spermatogenetic stages are intermixed in every tubular cross section. Therefore, it is not possible to relate steroid biochemistry to specific developmental stages, although cycles of apparent secretory activity have been observed in Leydig and Sertoli cells in morphological and histochemical studies (Lofts and Bern, Steroids in Non-Mammalian Vertebrates, (ed. D. Idler, p. 37, 1972). In certain non-mammalian vertebrates, namely urodeles and elasmobranchs, germ cells in successive stages of maturation are topographically segregated (Lofts and Bern, op. cit., 1972), and zonation is clearly visible under a dissecting microscope. It should be possible to use these species as models for correlating morphology and function of the endocrine cells with spermatogenetic progression.

A preliminary experiment was carried out using dogfish testis. The semen of Squalus acanthias has high concentrations of estrogen (Simpson et al, J. Endocrinol. 26, 489, 1963), and testicular homogenates synthesize substantial amounts of E_1 and E_2 from ^{3}H-A (Callard et al., Endocrinology 103, 2283, 1978). We report here that microsomal aromatase activity was significantly greater in regions having spermatogonia and spermatocytes than in regions with mature spermatozoa. Effects of mammalian gonadrotropins were also observed.

Male sharks captured in July were untreated or injected intravenously 1 and 3 days prior to sacrifice with ovine FSH (500 μg); ovine LH (1 mg); or saline. Under a dissecting microscope, testes were sectioned transversely and separated into 3 regions (mesoventral → dorsolateral) according to Holstein (Z. Zellforsch. 93, 265, 1969) as follows: (I) zones 1-5, stem cells and spermatogonia; (II) zones 6-7, spermatocytes and spermatids; (III) zone 8, mature spermatozoa. Procedures for incubation and analysis have been described in detail (Callard et al, Endocrinology 100, 1214, 1977). Tissues were homogenized in phosphate/sucrose buffer containing dithiothreitol (10 mM) and microsomal subfractions (100,000 x g x 60 min) prepared by differential centrifugation. Aromatase activity was assayed by incubating 3-5 mg of microsomal protein for 30 and 60 min at 22°C in buffer (500 μl) containing (^{3}H-7) -androstenedione (266 nM) and an NADH/NADPH generating system. Controls were boiled microsomes or substrate-free incubates to which (^{3}H) -E_1 (100,000 cpm) was added for determination of recovery. To compare substrate preferences, microsomes prepared from whole cross-sections were incubated with ^{3}H-A, -T, or -19-hydroxy-androstenedione (19-OH-A) (all 266 nM). Formed ^{3}H-E_1 and E_2 were measured after extraction, chromatography on thin layer silica gel plates, and double phenolic partition. Radioactivity corresponding to E_1 and E_2 after this procedure was verified in selected samples after dilution with authentic unlabeled steroid and recrystallization to constant specific activity. After correction for procedural losses, results were expressed as fmol of steroid produced/mg microsomal protein/hr.

Estrogen production was linear during 60 min of incubation, and Table 1 shows that A and 19-OH-A were more efficiently aromatized than T. When microsomal aromatase activity was measured in dogfish testis by region, the following distribution was observed: II > I > III (Table 2). Comparison of regions I and II in single control or

Table 1. Comparison of substrate aromatization by dogfish testicular microsomes

	fmol/mg/hr[a]		
	E_1	E_2	$E_1 + E_2$
Androstenedione	19.0	27.1	46.1
Testosterone	3.0	17.9	20.9
19-hydroxy-androstenedione	15.7	34.6	50.3

[a]Values based on 2 determinations for a single microsomal preparation.

Table 2. Regional distribution of microsomal aromatase activity in dogfish testis

	fmol/mg/hr[c]		
	E_1	E_2	$E_1 + E_2$
I.	37.9	51.1[b]	89.8[b]
	± 4.5	± 2.6	± 6.4
II.	51.2	71.7[a]	122.9[a]
	± 2.6	± 5.7	± 6.7
III.	15.5[a,b]	37.5[b]	52.9[a,b]
	± 2.9	± 7.5	±10.5

[a] $P =< .05$ compared to zone I

[b] $P =< .05$ compared to zone II

[c] ± Standard error of mean based on 3 microsomal preparations and 6 determinations.

gonadotropin-injected animals, showed variations in E_1, E_2 and total estrogen production were less than 25%; however, in region III, aromatase activity increased with FSH or LH pre-treatment from 29.6 to 55.4 and 73.8 fmol E_1 and E_2/mg protein/hr, respectively.

Results of this preliminary study show that estrogen production is greatest in the region in which germ cells are undergoing meiotic division and lowest in the region having mature spermatozoa. Regions with proliferating spermatogonia have intermediate enzyme activity. Prismatic epithelial cells with characteristics of endocrine cells have been described in Squalus and during spermatogenesis undergo morphological changes that are consistent with cycles of secretion (Holstein, op. cit., 1969). These are considered to be analogous to mammalian Sertoli cells and, since conventional Leydig cells are not found in sharks, are a likely source of aromatase. Steroid metabolism in germ cells cannot be ruled out, however, because steroidogenic enzymes have been demonstrated histochemically in shark spermatozoa (Ozon and Collenot, C.R. Soc. Biol. 261, 3204, 1955). The changing ratio of Sertoli cells to germ cells in the progression from spermatogonia (1:1) to spermatids (1:64) must also be taken into account in interpreting these data since enzyme activity is based on total cell protein.

Whether mammalian gonadotropins are recognized by elasmobranch gonadotropin receptors is still open to question. In this experiment, ovine FSH or LH pretreatment appeared to double aromatase activity of testicular m microsomes in region III, and this observation is consistent with the stimulatory effects of gonadotropins on estrogen production in rat testis (Canick et al, Endocrinology 104, 285, 1979).

As in mammals, T is somewhat less efficiently aromatized than A; however, 19-OH-A, an intermediate in estrogen synthesis, is not a better precursor than A in this shark system as it is in mammals. This difference may be due to incubation temperature or to inherent characteristics of the aromatase complex from phylogenetically diverse species.

Results reported here are part of an investigation surveying the testes of non-mammalian species for the regional distribution of steroidogenic activity. Supported by NSF-PCM 78-23214.