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CHANGES IN SERUM LEVELS OF GONADOTROPIN, OESTRADIOL 17β AND VITELLOGENIN DURING THE FIRST AND SUBSEQUENT REPRODUCTIVE CYCLES OF FEMALE RAINBOW TROUT

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ABSTRACT

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The present study investigates the relationship between changes in serum levels of gonadotropin, oestradiol 17β and vitellogenin (by measurement of phosphoprotein phosphorus and calcium) during the annual reproductive cycle of the female rainbow trout. Two series of experiments were conducted: the first using 2-year-old previously immature fish, and the second using 3-year-old trout which had spawned during the previous reproductive cycle. The fish were maintained throughout the experiments in light-proof aquaria under simulated normal seasonal light cycles at a constant temperature of 9°C and feeding rate of 0.5% body weight/per day.

The fish in both series of experiments spawned by hand-stripping in mid-January at exactly the same time as other fish maintained in outside tanks under ambient conditions. Thus, photoperiod is confirmed as the major environmental determinant of reproduction in rainbow trout. A similar sequence of neuroendocrine changes was observed in both the first- and second-time spawning fish, thus indicating that similar pituitary—ovarian control mechanisms are in operation in all maturing fish irrespective of age.

The initial endocrine event was an increase in the serum level of gonadotropin during the first stages of oocyte development. In the second series of experiments, which were begun in February, it appeared that minor alterations in both gonadotropin and oestradiol 17β levels were occurring as early as March in the annual cycle. Following these changes in gonadotropin, oestradiol 17β levels were increased to reach a maximum in the summer during the period of active exogenous vitellogenesis. After the subsequent induction of vitellogenin secretion, as evidenced by raised calcium and phosphoprotein phosphorus levels, oestradiol 17β values fell and this decrease appeared to elicit a much greater increase in gonadotropin levels at the time of final oocyte maturation, ovulation and spawning. Vitellogenin levels did not fall until spawning had occurred. The relationship of these changes to the overall control of trout reproduction is discussed.

INTRODUCTION

Although there is considerable histological evidence for a relationship between pituitary gonadotropic cell activity and ovarian cycles in fish

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(Robertson and Wexler, 1962a, b; Sage and Bromage, 1970; Cook and Van Overbeeke, 1972), it is only with the advent of more specific and sensitive assays for gonadotropins and oestrogens that studies of the dynamic changes in blood levels of these hormones and hence their relative roles in reproduction have become possible.

Using such techniques, Breton et al. (1972) demonstrated significantly higher levels of plasma gonadotropin in goldfish on the day of ovulation, and in a later study produced similar findings for the rainbow trout and tench (Breton et al., 1975). In seasonal studies of both Atlantic and Pacific salmon and brook and brown trout, Crim et al., (1973, 1975) showed that plasma levels of gonadotropin were raised during maturation. Billard et al. (1978) have also demonstrated plasma and pituitary gonadotropin changes in the brown trout during ovarian development. In the same way, authors have reported seasonal changes in the levels of ovarian steroids (Schreck and Hopwood, 1974; Wingfield and Grimm, 1977; Yaron et al., 1977; Billard et al., 1978; Whitehead et al., 1978a), and higher levels of oestradiol 17β have been correlated with ovarian development (Lambert et al., 1978).

Although these data clearly establish the separate involvement of both gonadotropin and oestradiol 17β in teleost reproduction, only integrated studies of the interrelationships of these hormones during gonadal development enable a more complete understanding of the complex mechanisms of control. Both Fostier et al. (1978) and Whitehead et al. (1978a, b) in studies of the interplay of gonadotropin and oestradiol 17β have shown that there is a marked increase in circulating gonadotropin levels during the period of final oocyte maturation and ovulation which is always preceded by a fall in the levels of oestradiol 17β; similar data are available for the brown trout (Billard et al., 1978; Crim and Idler, 1978). The reciprocal changes in oestradiol 17β gonadotropin as ovulation approaches suggest a similar negative feedback relationship to that found in higher vertebrates.

These studies have in the main been concerned with the latter stages of ovarian development and consequently in the present work an investigation is made of the serial changes in serum levels of gonadotropin, oestradiol 17β and phosphoprotein phosphorus and total calcium (as measures of vitellogenin) during the complete reproductive cycle. Both first- and second-spawning fish were studied, as Henderson (1963) has suggested that the hypothalmic—hypophyseal—gonadal mechanisms may respond differently in fish of different ages.

MATERIALS AND METHODS

Female rainbow trout, *Salmo gairdneri*, of Danish origin with a natural spawning time of January—February were used in this study. Two series of experiments were conducted using different groups of fish drawn from the same original stock. In the first, previously immature 2-year-old fish weigh-

ing approximately 1.3 kg were used, but in the second, 3-year-old fish which weighed approximately 2.5 kg and had spawned once before during the preceding season were utilized. The two series of experiments were commenced in April and February, respectively, and both were completed in February of the following year. Both groups of fish were maintained in 800-l light-proof aquaria under simulated normal seasonal light cycles provided by 40-W fluorescent tubes (200 lux at the water surface) controlled by time clocks adjusted once per week.

The water temperature was constant at 9°C with a dissolved oxygen of 100% saturation in the effluent and pH of 6.6. The fish were fed at a level of 0.5% body weight/day with BP Nutrition Mainstream diet. All fish were blood sampled at the start of the experiment in February and each month thereafter. Methods for sampling and subsequent assay of gonadotropin, oestradiol and calcium are described by Bromage et al. (1982a). Fish were also examined each month for signs of sexual maturation. Spawning was said to have occurred when ripe eggs were expelled after gentle hand pressure on the abdomen, i.e., stripping. Differences between means were tested statistically by either a Student's t-test or an F-test if the variances were dissimiliar.

RESULTS

The fish in both series of experiments spawned by hand-stripping in mid-January, at exactly the same time as broodstock maintained outside in open ponds under ambient conditions. During their reproductive cycles, changes were observed in the serum levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium.

The changes in first-spawning fish are shown in Table I and graphically in Fig. 1. In April, at the beginning of the experiment, low levels of gonadotropin (9.9 ± 1.8 ng/ml), oestradiol 17β (122 ± 11 pg/ml), phosphoprotein phosphorus (15.3 ± 5.5 μg/ml), and total calcium (10.5 ± 0.8 mg%) were observed.

Serum levels of gonadotropin rose to 13.2 ± 0.8 ng/ml in July and gradually fell to 5.5 ± 0.6 ng/ml by November. Levels in July were significantly different from those in November ($P < 0.01$). Prior to spawning in mid-January, levels rose rapidly to a peak of 37.1 ± 14.0 ng/ml. Levels in February were also significantly different from those in November ($P < 0.01$).

During June and July, serum oestradiol levels increased from basal to 4808 ± 1105 pg/ml in October, and then returned to resting levels before the onset of spawning in mid-January. Levels in October were significantly different from those in April ($P < 0.001$).

To enable examination of the interrelationships of these parameters the data are represented schematically in Fig. 1. As ovarian development progressed during the months of July to February, there was a significant correlation between the serum levels of gonadotropin and oestradiol 17β

TABLE I

Series 1 experiments: levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus, and total calcium in serum of female rainbow trout under a normal 12-month seasonal photoperiod regime (n = 7)

	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.
Gonadotropin (ng/ml)	9.9	8.6	11.2	13.2	11.4	10.9	8.3	5.5	6.1	16.5	37.1
± S.E.	1.8	1.4	1.3	0.8	1.8	1.4	1.8	0.6	1.4	2.8	14.0
Oestradiol 17β (pg/ml)	122	128	234	371	886	3471	4808	4107	2729	310	130
± S.E.	11	11	29	50	126	549	1105	666	667	109	12
Phosphoprotein phosphorus (μg/ml)	15.3	20.0	39.3	34.7	54.3	93.3	176.3	206.6	255.9	400.9	132.9
± S.E.	5.4	2.0	8.0	6.6	7.5	14.9	25.7	38.8	55.3	66.9	23.1
Total calcium (mg%)	10.5	11.9	14.2	14.3	15.6	21.9	33.0	36.1	43.6	57.5	30.3
± S.E.	0.8	0.2	0.2	0.5	0.5	1.0	3.5	5.0	7.2	5.4	2.8

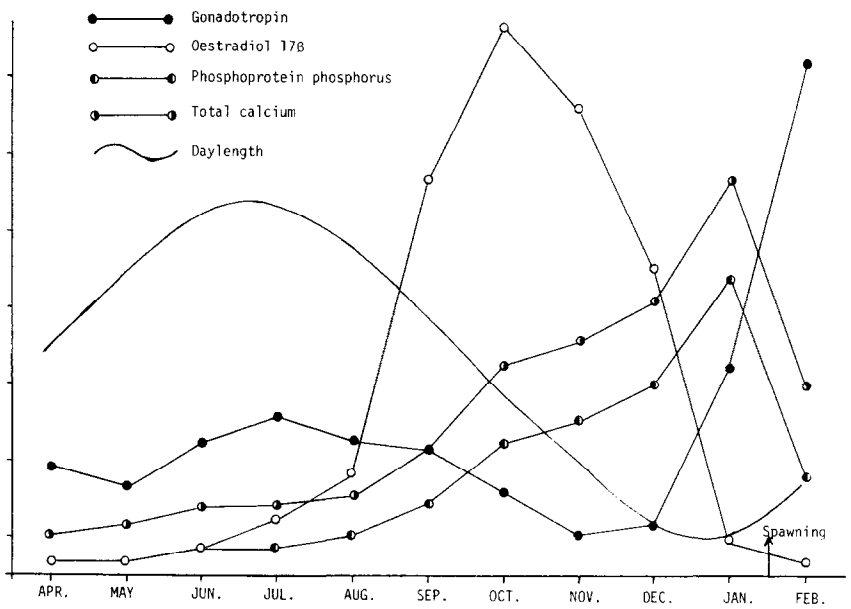

Fig. 1. Series 1 Experiments. Schematic representation of changes in levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium in serum of female rainbow trout under a normal 17-month seasonal photoperiod regime. The fish in this series were immature at the start of the experiment.

($P < 0.001$, $r = 0.3569$, $n = 103$). No correlation was found between these two hormones during the earlier stages of development (April—July) although both showed parallel increases during this period.

By September, the serum levels of phosphoprotein phosphorus and total calcium had begun to increase, reaching peaks of 400.9 ± 66.9 μg/ml and 57.5 ± 5.4 mg%, respectively, in January. Both serum levels of phosphoprotein phosphorus and total calcium in January were significantly different from their respective levels in April ($P < 0.001$).

During the months of July to October, there was a significant correlation between the serum levels of oestradiol and total calcium/phosphoprotein phosphorus ($P < 0.001$, $r = 0.6448$, $n = 26$). No such correlation was found earlier (April—July) or later (November—February) in the year. Throughout the reproductive cycle a significant correlation was also observed between the levels of total calcium and phosphoprotein phosphorus ($P < 0.001$, $r = 0.8411$, $n = 75$).

A similar interrelationship of changes in the levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium was observed in fish maturing for the second time although the absolute levels reached were somewhat different from those of first-spawning fish (Table II). In addition there appeared to be a slight elevation of both oestradiol and gonadotropin levels in March which was not observed in the immature fish because this experiment was not begun until April.

TABLE II

Series 2 experiments: levels of gonadotropin, oestradiol 17β, phosphoprotein phosphorus and total calcium in serum of female rainbow trout under a normal 12-month seasonal photoperiod regime (n = 8)

	18 Feb.	17 Mar.	19 Apr.	18 May	14 June	20 July	25 Aug.	27 Sep.	4 Nov.	6 Dec.	10 Jan.	1 Feb.
Gonadotropin (ng/ml)	10.3	15.4	17.1	20.1	22.2	17.0	11.4	9.3	9.8	8.0	25.9	44.8
± S.E.	1.4	4.3	3.1	3.6	1.9	2.7	2.7	2.1	1.7	2.1	15.5	16.3
Oestradiol 17β (pg/ml)	73	364	158	241	244	222	976	2453	2556	1366	552	97
± S.E.	11	93	63	60	109	37	296	531	647	353	103	17
Phosphoprotein phosphorus (μg/ml)	178.5	68.0	67.0	79.3	83.0	134.6	107.6	168.3	151.6	186.0	108.0	190.0
± S.E.	41.1	9.7	11.4	21.7	44.4	29.2	15.2	25.2	18.9	34.8	46.7	49.8
Total calcium (mg%)	29.8	15.0	12.9	14.7	17.2	15.6	16.9	22.0	22.4	26.4	29.1	26.1
± S.E.	5.0	1.3	1.7	1.7	4.2	3.1	2.1	2.1	2.0	4.6	6.1	5.3

DISCUSSION

The present study clearly demonstrates similar sequences of endocrine events in female rainbow trout spawning for the first and subsequent occasion. Similar profiles have also been recorded in previously immature and also second-spawning fish exposed to modified seasonal and constant light cycles (Whitehead et al., 1978a, b; Bromage et al., 1982a, b).

It should be emphasized that under all the experimental regimes cited above and the seasonal cycles reported here that the initial event was an increase in the serum levels of gonadotropin early in the year during the period of early oocyte development, after which they returned to low levels. Prior to spawning, levels rose rapidly to a much higher value presumably during the period of final oocyte maturation and ovulation.

Although there is a wide agreement relating to the presence of high gonadotropin values in association with the later stages of ovarian development in salmonids (Crim et al., 1973, 1975; Billard et al., 1978; Bromage et al., 1982a, b; Scott and Sumpter, 1983) there is less agreement regarding levels during the remainder of the cycle. Thus, undetectable or low levels were found during gonadal recrudescence and vitellogenesis but far higher levels at ovulation in the brown and brook trout and two species of salmon (Crim et al., 1973, 1975). In a similar study Billard et al. (1978), working on the brown trout, have reported raised gonadotropin levels during the early and later portions of the spawning cycle with lower levels in the middle of the year during the first part of vitellogenesis. Fostier et al. (1978), Whitehead et al. (1978b) and Bromage et al. (1982a) have also observed higher levels of gonadotropin in rainbow trout during early ovarian development, final oocyte maturation and ovulation, but with reductions in mid-cycle. In contrast, Scott and Sumpter (1983), also working with rainbow trout, showed that high levels of gonadotropin were only present at the time of ovulation.

Although this controversy may be partly explained by differences in assay procedure, sensitivity or specificity or possibly in sampling frequency of the different studies, such disagreements will not be fully resolved until it is clear how many gonadotropins are present in fish. Several workers propose the existence of only one gonadotropin (e.g., Burzawa-Gerard et al., 1975; Breton et al., 1976; Sumpter et al., 1978) whereas others suggest that there are two (Idler et al., 1975; Ng and Idler, 1979). The present data provided by the results of a homologous radioimmunoassay offers some support to the presence of a single gonadotropin with different roles at different stages of ovarian development.

In the present work, after the initial increases in gonadotropin in both groups, levels fell, during which time serum oestradiol values increased. Levels of oestradiol continued to rise until October, then returned to basal values just prior to spawning in January, at which time a second and much higher peak in gonadotropin was observed. The highly significant negative

correlations between the levels of these two hormones from July to February indicate that during the latter half of the cycle oestradiol exerts a similar negative feedback on gonadotropin secretion to that seen in higher vertebrates. This followed a period during the first part of vitellogenesis when the gonadotropin increase was followed by an increase in oestradiol. A similar cycle in levels of oestradiol 17β has also been demonstrated in the rainbow trout by Lambert et al. (1978), and Fostier et al. (1978) have reported that oestradiol 17β fell to a low level before oocyte maturation at a time when plasma gonadotropin was high. A similar pattern of changes in plasma oestradiol 17β and gonadotropin has also been observed in the brown trout by Billard et al. (1978) and Crim and Idler (1978) although the latter study was not continued to the completion of the spawning cycle. Thus, the rainbow trout, like other teleost fish, has raised oestradiol 17β levels, probably as a result of an earlier increase in gonadotropin, at a time when the developing oocytes are actively incorporating yolk.

Following the observed increase in serum oestradiol, serum levels of phosphoprotein phosphorus and total calcium were raised from an initial basal level to a peak just prior to spawning. Throughout the period of investigation there was a highly significant correlation between the serum levels of phosphoprotein phosphorus and total calcium, confirming their co-operative importance in the vitellogenin complex. During the period from July to October, a highly significant correlation was also observed between the serum levels of oestradiol 17β and total calcium/phosphoprotein phosphorus, thus indicating that oestradiol 17β is involved in the induction of vitellogenesis. After October, however, as oestradiol levels fell to basal, levels of serum phosphoprotein phosphorus and total calcium continued to increase, reaching a maximum just prior to spawning. Whether this reflects a continuation of vitellogenin production after return of oestradiol to basal levels, as has been reported elsewhere (Elliott et al., 1979), or whether it indicates that circulating vitellogenin is not fully taken up during this period, is not clear.

The results from both series of experiments were essentially the same, indicating that similar hypothalmic—hypophyseal—ovarian mechanisms operate in maturing fish, irrespective of whether they are spawning for the first or second occasion. However, in addition in the previously spawned fish there were modest increases in both gonadotropin and oestradiol levels in March well before the appearance of vitellogenin in the serum, and this, together with unpublished data of Breton, suggest that these hormones may already be exerting a significant influence at this early stage of ovarian development.

In summary, therefore, the results demonstrate that the changing levels of gonadotropin and oestradiol during maturation are functionally interrelated, and suggest that both positive and negative feedback mechanisms of control are involved. An increase in serum levels of oestradiol occurs early in oocyte development, probably as a result of an early increase in

gonadotropin; as oestradiol levels increase, values of gonadotropin fall from a previously elevated level. The resulting increase in oestradiol stimulates vitellogenesis as evidenced by increases in serum phosphoprotein phosphorus and total calcium. Towards the final stages of maturation, falling oestradiol levels could provide the stimulus for the rapid increase in serum levels of gonadotropin and the consequent oocyte maturation and ovulation, this final interplay between these two hormones marking the end of the complex sequence of events required to produce a fertilizable oocyte.

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