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NEW DATA ON THE POSSIBILITIES OF CONTROLLING REPRODUCTION IN TELEOST FISH BY HORMONAL TREATMENT

by

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R E S U M E

—Ce document comporte une revue de quelques résultats récents concernant les traitements hormonaux destinés à induire ou à accélérer les stades de fin de gamétogenèse chez quelques poissons téléostéens.

Chez la Truite arc-en-ciel et le Brochet des recherches détaillées mettant en oeuvre des techniques in vivo et in vitro ont conduit à une meilleure connaissance du contrôle endocrinien de la maturation ovocytaire. En conséquence, des traitements hormonaux exogènes comprenant l'administration d'une dose de GTH, seule ou suivie d'une injection de 17α-hydroxy-20β-dihydroprogesterone induisent la maturation ovocytaire et l'ovulation chez la Carpe, la Truite et le Brochet avec des taux de fécondation supérieurs à 75 %. Des traitements avec des stéroïdes sexuels ou GTH stimulent la spermiation chez les mâles (Truite, Brochet et poisson rouge). —

Chez la Dorade, l'administration de HCG dosée selon le stade de vitellogenèse, induit l'achèvement de l'ovogenèse et la maturation ovocytaire suivie d'ovulation selon une séquence quotidienne qui se prolonge pendant 2 mois. Les ovules ainsi obtenus sont fécondés à 80-100 % et le taux d'éclosion est de 80-90 %.

A B S T R A C T

— Some recent findings concerning hormonal treatments inducing or accelerating final stages of gametogenesis in some teleost fish are reviewed.

In the rainbow trout and northern pike detailed research using in vivo and in vitro techniques led to a better understanding of the endocrine control of final stages of oogenesis. As a result, exogenous hormonal treatments, composed of a piscine gonadotropin administered alone or followed by 17α-hydroxy-20β-dihydroprogesterone injection were found to be effective inducers of precocious oocyte maturation and ovulation in the carp, trout and pike. In all species fertilization rates of ovulated oocytes were high (more than 75 %). In the males of the trout, the pike and the goldfish, treatment with fish gonadotropin or with steroid hormones stimulated spermiation. —

In the gilthead seabream, a very weak treatment with HCG, dosed according to the oocyte vitellogenetic stage in the treated female, induced the completion of oogenesis ; a daily rhythm of maturation, ovulation and spawning spontaneously continued up to two months after the appropriate hormonal treatment. Spawned eggs were highly fertilizable (80-100 %) and hatching rates were high (80-90 %).

KEY WORDS : Induced spawning - Ovulation, Spermiation - Teleosts

MOTS CLES : Ponte induite - Ovulation, Spermiation - Téléostéen

INTRODUCTION

In fish culture programs,one of the basic problems to be taken into consideration is the exogenous control of reproduction in the cultured species. Many of the fish serving in such programs do not breed spontaneously in captivity. In most of the cases this results from failure of the females to complete the ovarian cycle under captivity conditions. Various hormonal treatments can serve in inducing the completion of oogenesis, ovulation and spawning of viable eggs in these fish. In addition, it is of a great advantage to artificially advance or extend the natural breeding season of a given fish ; this may enable the supply of fry over a longer period of the year. Appropriate hormonal treatment may also induce spermiation in males and increase the gametes viability (in both sexes) and quantity (in males), thus enhance fertilization and hatching rates.

In the present paper, some recent developments concerning the exogenous hormonal control of the reproductive cycle in the fresh water species Salmo gairdneri (rainbow trout), Esox lucius (northern pike) and Cyprinus carpio (common carp) and in the marine species Sparus aurata (gilthead seabream) are reviewed. Problems are raised in vue of utilisation of the described findings for artificial propagation in Tuna.

I. GENERALITY ON THE ENDOCRINE CONTROL OF FINAL STAGES OF THE SEXUAL CYCLE

In the rainbow trout, the northern pike and the goldfish Carassius auratus (a cyprinid fish closely related to the carp), intensive in vitro research (summarized in JALABERT, 1976) supplied many details as to the nature of the hormonal control of the last stages of oogenesis, i.e. maturation and ovulation of oocytes. It was demonstrated that the process of oocyte maturation is mainly under the control of pituitary gonadotropin. The action of the gonadotropin on oocyte maturation is mediated through steroid hormones, and most probably through the 17α-hydroxy-20β-dihydroprogesterone (17α-20 β P). A different factor, triggering the initiation of follicule contractions, is required for the ovulation of oocytes. Other factors, mainly neurohypophyseal hormones, were shown to be the mediators of the spawning process in some teleost species (reviewed by HELLER, 1972).

These findings led to the introduction of some exogenous hormonal treatments aiming in inducing or accelerating final stages of oogenesis in the studied fish.

2. INDUCTION OF REPRODUCTION BY HORMONAL TREATMENTS IN SOME TELEOST REPRESENTATIVES

2.1. Cyprinids

In the common carp, oocyte maturation and ovulation do not occur spontaneously under captivity conditions. Hypophysation (injection of pituitary extracts) is routinely used to spawn common carp females. Nevertheless this technique is associated with some difficulties. The quality of the injected pituitaries is variable, resulting from differences in the age and gonadal stage of the donor fish and in the methods of collection and storage of the pituitaries. Therefore the appropriate dose for an effective treatment is not constant.

In addition, hypophysation is effective only if high doses of pituitary extracts are used (about 3-6 mg pituitary extract per 1 kg of body wt.) and only if the fish are preacclimated to water temperatures exceeding 20°C. These two last facts make the hypophysation technique relatively expensive. Therefore, in the common carp, a new maturation and ovulation inducement treatment was introduced (JALABERT et al., 1977). This treatment is composed of a low "priming" dose of carp pituitary extract (0.6 mg/kg body wt.) followed by an injection of a cheap crude preparation of 17α-20 β P (2 mg/kg body wt.)(Table 1). This technique was found to be very effective in inducing maturation and ovulation of highly fertilizable oocytes, in fish acclimated to cold water (13-15°C) ; therefore its application enables the replacement of the classical hypophysation technique by a much more economical treatment.

In this group (cyprinids), stimulation of spermiation by hormonal treatment was carried out mainly in the goldfish (YAMAZAKI and DONALDSON, 1968 ; BILLARD, 1976, 1977). Among various hormones tested, carp gonadotropin (c-GTH) is more potent than trout gonadotropin (t-GTH)(Fig. 1) suggesting a species specificity in the action of GTH on spermiation. However human chorionic gonadotropin (HCG) is also efficient (table 2), but it should be pointed out that HCG has no effect on spermatogenesis strictly speaking (BILLARD and ESCAFFRE, 1973). Progesterone is more potent than androgens (Table 2, BILLARD, 1976) in increasing both volume of sperm and number of spermatozoa yielded. In common carp, sperm release is stimulated by injection of carp pituitary extract (CLEMENS and GRANT, 1965).

2.2. Northern pike

The northern pike is an important species in fresh water fish culture programs. Under confinement, oocyte maturation and ovulation do not occur in this species. The males of the same species normally produce a very low volume of sperm, mainly towards the end of the breeding season. Hypophysation with dried carp pituitaries (4-8 mg/kg body wt.) was reported to induce ovulation in females (SORENSON et al., 1966) and sperm release in males (ANWAND, 1963). Both studies lack details concerning the use of the technique. Recently, following the above described *in vitro* findings, it was found that a dose of 0.1 mg/kg body wt. of partially purified salmon gonadotropin administered to females having oocytes with the germinal vesicle in a subperipheral position, induced oocyte maturation and ovulation (Table 1, de MONTALEMBERT et al., 1978a, b). Ovulation and fertilization rates were high (89 % and 83 % respectively). The gonadotropin dose was reduced when it was followed by an injection of 17α-20β P, in which case fertilization rates of ovulated oocytes were found to be lower. The same study (de MONTALEMBERT et al., 1978 b) demonstrated that progesterone treatment of male fish caused stimulation of sperm release ; an increase of 2-3 times was observed in the sperm volume delivered from hormonal treated males (Fig. 2). These procedures brought to the simplification and standardization of the artificial control of reproduction in the northern pike. However a large scale and economical production of partially purified fish gonadotropins is not yet available.

2.3. Rainbow trout

In the rainbow trout ovarian and testicular cycles are completed spontaneously in captivity. Therefore most efforts are directed towards extending the natural breeding season, either by environmental (BRETON and BILLARD, 1977 ; WHITEHEAD et al., 1978) or by hormonal (JALABERT et al., 1978) manipulations. In the later study it was demonstrated that a treatment with 17α-20β P could produce fertilizable mature oocytes 4-6 weeks before the natural spawning time, when administered to females in which oocytes were before the peripheral germinal vesicle (GV) migration stage. In most of the treated females ovulation did not follow maturation. On the other hand ovulation did follow maturation if a priming dose of trout pituitary extract (0.5 mg/kg body wt.) was administered

to the fish two days before the steroid injection (Table 1). In this case ovulated eggs were highly fertilizable (87 %). Injection of 17α-20β P alone was able to induce oocyte maturation followed by ovulation when administered to fish at later stages of oocyte development (subperipheral GV). These in vivo findings correlate with the above mentioned in vitro ones (JALABERT, 1976) and show that a gonadotropin dependent specific factor is required for triggering ovulation ; this factor should be synthetized before 17α-20β P action, either spontaneously (if the steroid is administered after the endogenous increase of plasma gonadotropin level) or as a result of an exogenous priming gonadotropin treatment (if the steroid is administered before the endogenous increase of plasma gonadotropin level). In males, stimulation of spermiation is obtained after an intraperitoneal injection of various hormones mainly t-GTH and progesterone.

2.4. Seabream

The gilthead seabream is a promising fish for mariculture purposes. This species does not spawn spontaneously in captivity. Detailed histological study (ZOHAR, 1977) showed that in these conditions ovarian development is not completed. Oocyte develop to the last stages of vitellogenesis and then undergo rapid atresia. On the other hand testicular development is completed. It was already demonstrated that in the gilthead seabream HCG is a very effective oocyte maturation and ovulation inducer (BARNABE and RENE, 1973 ; ALESSIO et al., 1975, 1976 ; ARIAS, 1976 ; BARNABE, 1976 ; SAN FELIU, 1976 ; VILLANI, 1976). In some of the studies (BARNABE and RENE, 1973 ; BARNABE, 1976) effective HCG doses were found to be 800 IU/kg body wt. where as in the others they ranged from 3500 to 15000 IU/kg body wt. In most of the cases, treated females were stripped manually 48-96 hr. after the last hormonal injection and eggs were artificially inseminated. The stripping manipulation frequently resulted in the emission of either immature or aged eggs.

It was already mentioned (BARNABE, 1976) that a hormonal treatment leading to a natural spawning would be of a great advantage in the gilthead seabream, since it avoids any stressful handling of treated females and usually results in much higher fertilization and hatching rates of spawned eggs. In order to approach the physiological course of the reproductive process in the gilthead seabream a research was carried out, aiming in calibrating HCG doses used to spawn females and correlating them to the ovarian developmental stage of the treated female (GORDIN and ZOHAR, 1978). Using a biopsy technique for sampling oocytes from treated females, it was demonstrated that the quantity of HCG needed to induce oocyte maturation, ovulation and natural spawning was in inverse relation to the vitellogenetic stage of the oocytes at the beginning of the treatment (Fig. 3).Optimal HCG doses for induction of ovulation and natural spawning in females with oocytes at the last stages of vitellogenesis ranged from 100-300 IU/kg body wt. ; in females with oocytes at earlier stages of vitellogenesis (up to two months before the completion of vitellogenesis) successful spawning occured after a treatment with HCG doses reaching 1200 IU/kg body wt. A high percentage of the females treated with low HCG doses (100-300 IU/kg body wt.) exhibited a diurnal cycle of spawning ; each 24 hours few ten thousands eggs were laid during a period of up to two months following the treatment (ZOHAR and GORDIN, in preparation). Total number of viable eggs spawned by individual females reached 0.7-1.5 millions. Fertilization and hatching rates were high (80-100 % and 80-90 % respectively). Using the appropriate hormonal treatments,the spawning season of the gilthead seabream was extended to five and a half months (in comparison to the approximated six weeks natural one in the eastern mediterranean). These results demonstrate the availability of a simple hormonal treatment for controlling reproduction in the captivity reared gilthead seabream which enables the constant supply of larvae over a long period of the year.

3. CONCLUSION

Few general lines may be drawn concerning future research. The present review describes some aspecific biological phenomena such as the induction of last stages of oogenesis in the pike by salmon gonadotropin and in the gilthead seabream by human gonadotropin. Nevertheless it should be emphasized that the existence of differences in the mechanisms of gonadotropic hormones action between teleostean and mammalian species as well as between different teleostean families is well established (reviewed by BRETON et al., 1973). Therefore, the endocrinological processes controlling reproduction should be thoroughly investigated in as many fish as possible , especially in those species having potential for aquaculture, including different tuna species. Information gained in this way may help us, as it was demonstrated in the present review, to build up an appropriate hormonal treatment for controlling reproduction in the investigated species. In a future mariculture program dealing with any of the tuna species (as well as with any other fish species) one should be able to complete the entire life cycle of the captivity reared fish, or to alter it according to its convenience. Hence it is very probable that one would need to utilise hormonal treatments in order to overcome the "reproduction barrier" and thus to improve the possibilities of a successful fish culture.

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Table 1 : The effects of various hormonal treatments on maturation and ovulation in the carp, pike and trout (summarized from JALABERT et al., 1977,1978, de MONTALEMBERT et al., 1978 a, b).

Treated species	Treatments			% fish undergoing maturation	Day	% fish undergoing ovulation	Day
	Day 0	Day 1	Day 2				
Cyprinus carpio	Priming C.P.E.(1) 0.6 mg/kg	Hypophysation 5.4 mg/kg	——	70 (part) (6)	2	70 (part)	3-4
	17α-20β P(2) 2 mg/kg	——	——	0		0	
	Priming C.P.E. 0.6 mg/kg	17α-20β P 2 mg/kg	——	50-70 (norm) (5) 30-50 (part)	1-2	50-70 (norm)	2
	Physiological saline	Physiological saline	——	0		0	
Esox lucius	P.P.S.G.(3) 0.1mg/kg	——	——	43 (norm), 67(part)	1-3	43 (norm) 67 (part)	4
	P.P.S.G. 0.03 mg/kg	——	——	100 (part)	4	0	
	17α-20β P 3 mg/kg	——	——	100 (part)	2-4	33 (part)	4
	P.P.S.G. 0.03 mg/kg	17α-20β P 3 mg/kg	——	100 (part)	1-3	100 (part)	4
	Physiological saline	Physiological saline	——	0		0	
Salmo gairdneri	T.P.E.(4) 0.5 mg/kg	——	Physiological saline	25	6	25	8-15
	Physiological saline	——	Physiological saline	27	11-15	27	15-18
	17α-20β P 3 mg/kg	——	17α-20β P 3 mg/kg	100	6-11	25	8-15
	T.P.E. 0.5 mg/kg	——	17α-20β P 3 mg/kg	100	6-11	59	8-15

(1) : C.P.E. Carp pituitary extract
(2) : 17α-20 β P 17α-hydroxy-20 β-dihydroprogesterone
(3) : P.P.S.G. Partially purified salmon gonadotropin
(4) T.P.E. trout pituitary extract
(5) Norm. normal maturation, ovulation
(6) part. partial maturation, ovulation

Table 2 : Effects of various hormones on spermiation in goldfish (after BILLARD, 1976)

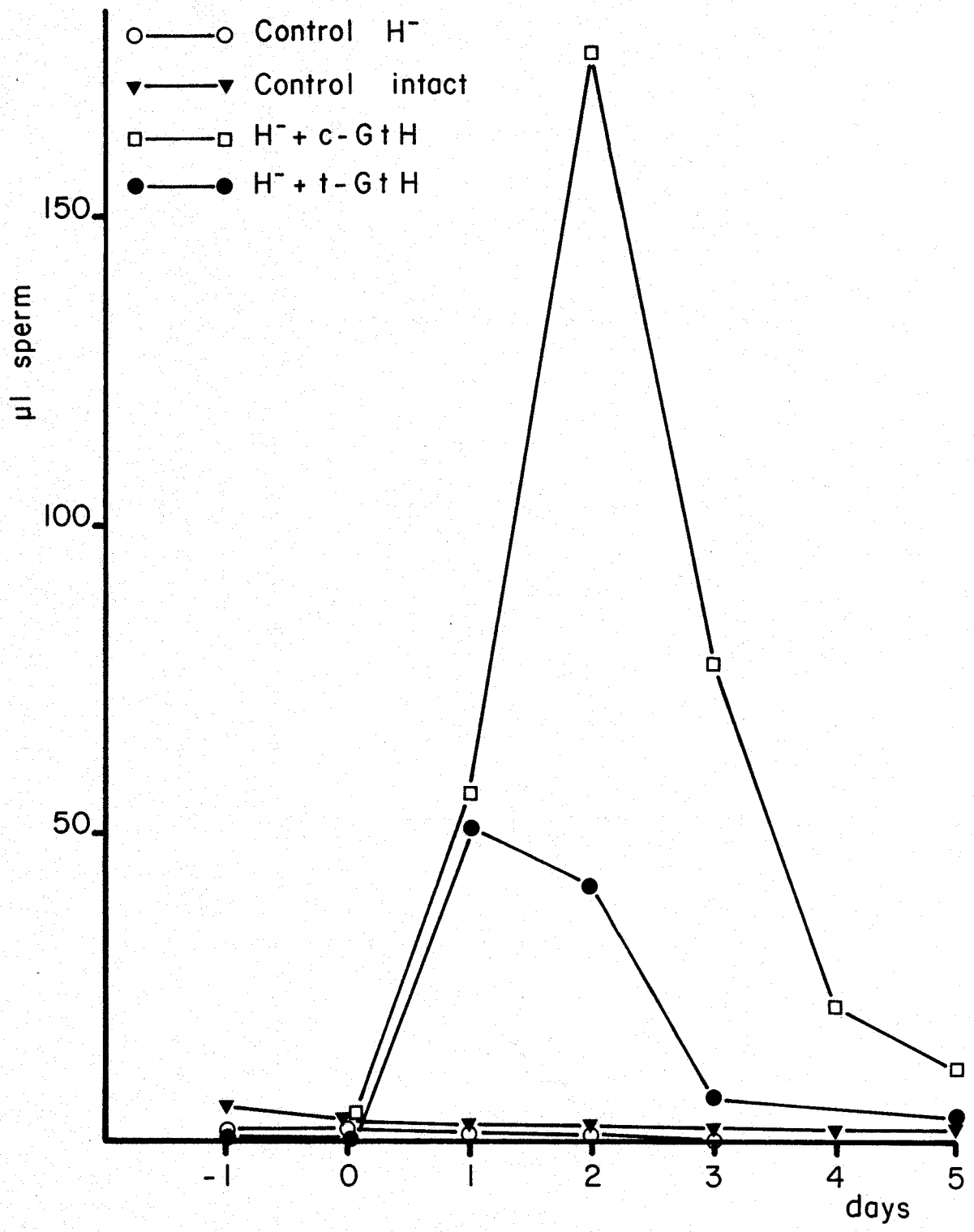
OLH : Ovine Luteinizing Hormone
E_2 17β : Estradiol 17β

Treatment	Dose µg/g b.w.	Nb of fish response/total	Mean individual sperm production µl±SEM	Duration of response (days)	Volume of sperm at day of maxi. response
Intact control	0	5/5	11.8±I.56	-	3.2±0.6
Hypophysectomized (H^-)	0	0/5	0	-	-
H^- + carp pituitary extract	50	10/10	72.5±8.30	3.2	42.6±6.7
H^- + OLH	200	3/5	0.8±0.37	< 1	-
H^- + HCG	10	5/5	61.8±5.72	6.2	27.6±2.6
H^- + Methyl-testosterone	10	5/5	33.0±1.58	5.0	15.0±1.2
H^- + E_2 17 β	10	5/5	8.6±2.25	3	4.4±1.2
H^- + Progesterone	10	5/5	41.8±9.5	5	15.8±6.8

Figure 1 : Stimulation of spermiation in the hypophysectomized (H^-) goldfish (Carassius auratus) following intraperitoneal injections of carp gonadotropin (c-GTH) and trout gonadotropin (t-GTH). The H^- control fish were injected with saline solution. Fish were treated on day 0. n = 10 in each group - Dose of GTH : 1mg/kg body weight.

Figure 2 : Stimulation of spermiation (measured by the delivered sperm volume) in the intact northern pike (Esox lucius) following intraperitoneal injections of progesterone and testosterone. Control fish were injected with the hormone carrier. Arrows indicate time of treatment. Lighter curves represent individual fish whereas darker ones represent mean values. (de MONTALEMBERT et al, 1978b). Dose of stenoids : 100 mg/kg body weight.

Figure 3 : HCG doses effective in inducing ovulation and natural spawning in the gilthead seabream (Sparus aurata), in relation to the oocyte diameter of the treated females, throughout the course of the extended breeding season (from ZOHAR and GORDIN, in preparation).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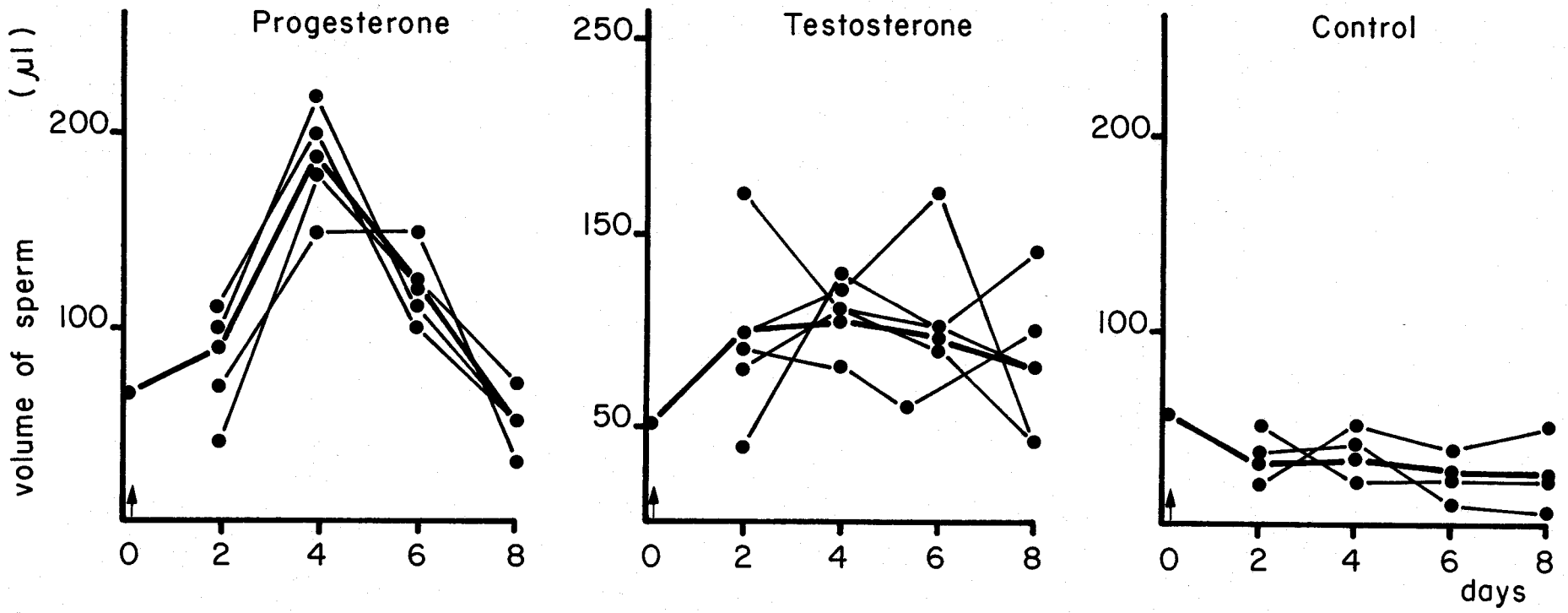
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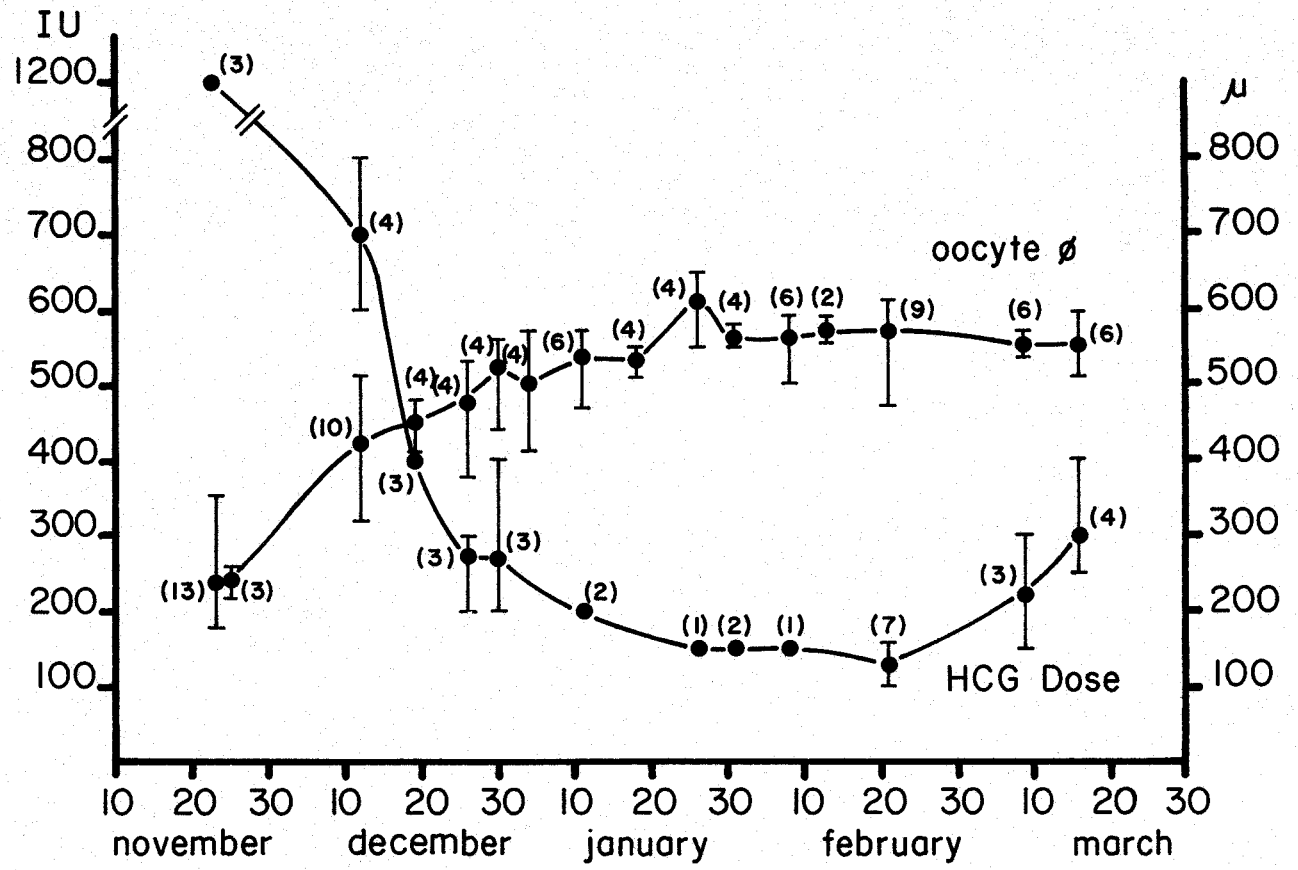
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