

## ARTIFICIAL INDUCTION OF GONADAL MATURATION AND OVULATION IN THE JAPANESE EEL (*ANGUILLA JAPONICA* T. ETS.).

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### ABSTRACT

8 injections of carp pituitary extract (CPE, 5 pit. / fish / 10 days) induced ovarian maturation in female eel, gonadosomatic index (GSI) increasing significantly to 43-55 %. 4 injections of CPE (1-2 pit. / fish / 10 days) induced spermatogenesis and spermiation in male eel, GSI increasing to 2.1-2.8 %. After serial injections of CPE, serum gonadotropin (GtH) levels increased significantly, indicating that the exogeneous GtH stimulated gonadal development and maturation in the Japanese eel. 8 implantations of 17  $\alpha$ -methyltestosterone (MT) or androstenedione (ADSD) (50  $\mu$ g / g body weight / 15 days) stimulated ovarian maturation in female eel, GSI increasing significantly to 38-49 %. 4 implantations of MT or ADSD (50  $\mu$ g / g body weight / 15 days) induced spermatogenesis and spermiation in the male eel, GSI increasing to 2.1-3.5 %. MT or ADSD implantations significantly elevated pituitary and serum GtH contents. ADSD or MT implantations resulted in the significant elevation of serum estradiol levels in female eel and serum testosterone levels in male eels. In female eel, ADSD implantations significantly increased mammalian-like-gonadotropin-releasing hormone (mGnRH) content in brain and pituitary. These results demonstrated that the positive feedback effects of sex steroids on the brain (hypothalamus)-pituitary-gonad axis play an important role on the induction of gonadal development and maturation in the Japanese eel, and the endogeneous GtH is the key hormone during these physiological processes. In the matured female eel, ovulation and spawning can be induced 18-21 hours following an injection of CPE alone or in combination with [D-Ala<sup>6</sup>, Pro<sup>9</sup>-NET]-luteinizing hormone-releasing hormone (LHRH-A) and dopamine antagonist domperidone (DOM).

**Key-words** : hormonal induction, gonadal maturation, ovulation, *Anguilla japonica*.

### INDUCTION ARTIFICIELLE DE LA MATURATION DES GONADES ET DE L'OVULATION CHEZ L'ANGUILLE JAPONAISE (*ANGUILLA JAPONICA* T. ETS.).

### RÉSUMÉ

8 injections d'extrait hypophysaire de carpe (CPE, 5 hypophyses / poisson / 10 jours) induisent la maturation ovarienne chez l'anguille femelle, le rapport gonadosomatique (RGS = GSI) augmentant significativement jusqu'à 43-55 %. 4 injections de CPE

(1-2 hypophysés / poisson / 10 jours) induisent la spermatogenèse et la spermiation chez l'anguille mâle, le RGS augmentant jusqu'à 2,1-2,8 %. Après des injections répétées de CPE, les taux de gonadotrophine (GtH) sérique augmentent significativement, indiquant que la GtH exogène stimule le développement gonadique et la maturation de l'Anguille japonaise. 8 implants de 17  $\alpha$ -méthyltestostérone (MT) ou d'androsténédione (ADSD) (50  $\mu$ g / g poids du corps / 15 jours) stimulent la maturation ovarienne chez l'anguille femelle, le RGS augmentant significativement jusqu'à 38-49 %. 4 implants de MT ou ADSD (50  $\mu$ g / g poids du corps / 15 jours) induisent la spermatogenèse et la spermiation chez l'anguille mâle, le RGS augmentant jusqu'à 2,1-3,5 %. Les implants de MT ou ADSD élèvent significativement les taux hypophysaires et sériques de GtH. Les implants de MT ou ADSD résultent en une élévation significative des taux d'estradiol sérique chez la femelle et de testostérone sérique chez le mâle. Chez l'anguille femelle, les implants d'ADSD augmentent significativement le contenu cérébral et hypophysaire en gonadolibérine de type mammalienne (mGnRH). Ces résultats démontrent que le rétrocontrôle positif des stéroïdes sexuels sur l'axe cerveau (hypothalamus)-hypophyse-gonades joue un rôle important dans l'induction du développement gonadique et de la maturation chez l'Anguille japonaise et que la GtH endogène est l'hormone clé durant ces processus physiologiques. Chez l'anguille femelle mûre, l'ovulation et la ponte peuvent être induites 18-21 heures après une injection de CPE seul ou en combinaison avec un agoniste de la gonadolibérine [D-Ala<sup>6</sup>, Pro<sup>9</sup>-NET]-LHRH (LHRH-A) et un antagoniste de la dopamine, le domperidone (DOM).

**Mots-clés** : induction hormonale, maturation gonadique, ovulation, *Anguilla japonica*.

## INTRODUCTION

The Japanese eel, *Anguilla japonica*, is a highly valued species in the aquaculture of China. The eel culture in China developed rapidly in recent years, the annual production of cultured-eel amounted to 70-90 thousand tons ; especially in southern China, in the Pearl-river delta and Han-river delta area of Guangdong Province, several large eel-culture farms were established, the annual yield of cultured-eel exceeded 50 thousand tons, account for 60 % of total production of China. Due to the development of large scale of eel farming, more and more glass eels are needed. Currently, about 60-80 tons of glass eels (equals 400-500 million glass eels) are demanded for culture in China. However, eel larva is entirely dependent on the capture of wild. The reproductive cycle of the Japanese eel, like that of the European eel (*A. anguilla*), is characterized by a long delay before sexual maturation, and, even at the beginning of the reproductive migration to the sea, the gonads remain immature ; furthermore, if the reproductive migration is blocked, such as by captivity, sexual maturation will never occur. Thus, the discovery of means of inducing ovarian development to the prespawning stage, to enable production of fertile eggs and viable larvae, would be a major achievement. Treatment of Japanese eels with exogenous gonadotropin (GtH), such as carp pituitary extract (CPE) or human chorionic gonadotropin (HCG) has been demonstrated to induce gonadal development as well as ovulation and spermiation, but viable larvae have not been produced so far (OHTA *et al.*, 1996). Chronic treatment with testosterone (T) can stimulate ovarian development of Japanese eel to the prespawning stage, and provide a new lead on the difficult problem of induction of gonadal maturation of the Japanese eel (LIN *et al.*, 1991).

This paper introduces our studies on the exogeneous and endogeneous gonadotropin (GtH) stimulated gonadal development and maturation, and the hormonal treatment inducing ovulation in the Japanese eel.

## MATERIAL AND METHODS

Freshwater stage Japanese silver eels (female body weight 380–560 g, body length 55–67 cm, male body weight 120–240 g, body length 42–53 cm) were captured from October to December near the estuary of the Pearl River, Guangdong Province, China, and transported to the laboratory. In the laboratory, the eels were maintained indoor in tanks at room temperature (range 18–26 °C) under a natural photoperiod regime with recycling artificial seawater (salinity 30–35 ppt). Eels were acclimated to laboratory conditions more than one month. All experiments were conducted on late-January till May.

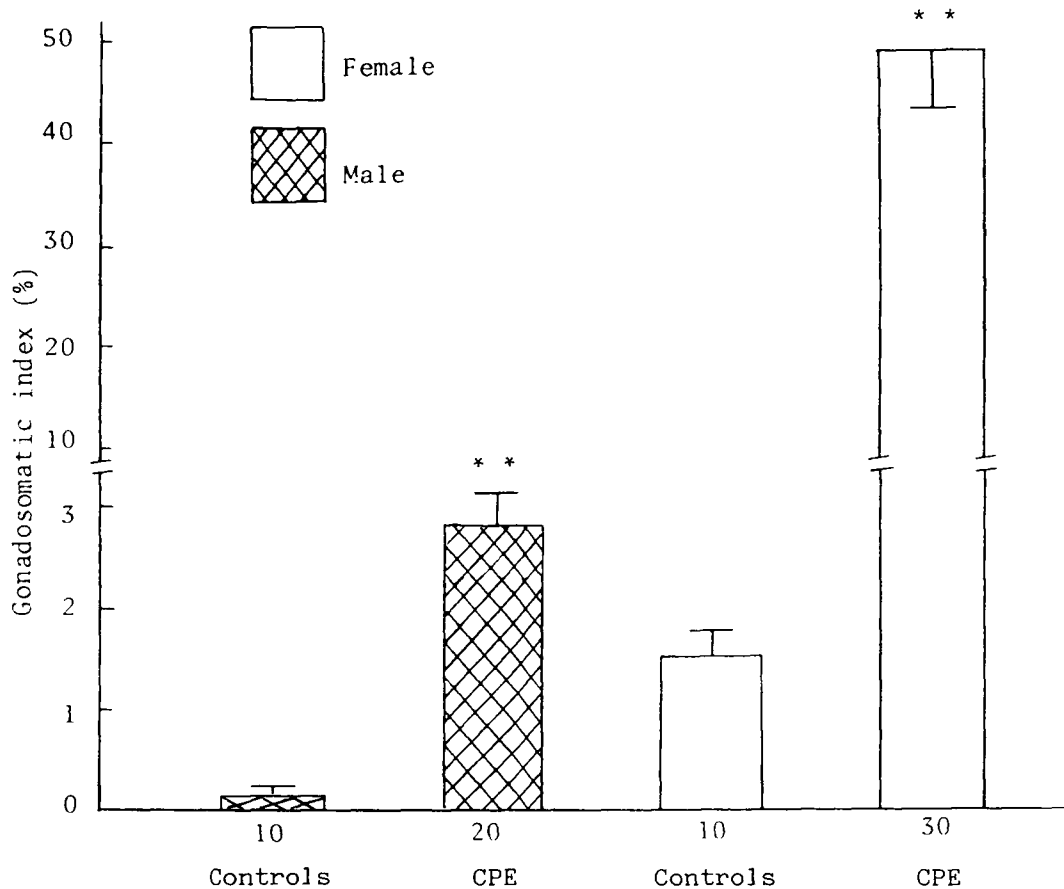
17  $\alpha$ -methyltestosterone (MT) and androstenedione (ADSD) were purchased from Sigma, St Louis, Missouri, U.S.A. ; domperidone (DOM) was purchased from Janssen, Beerse, Belgium ; LHRH-A was purchased from the Ningbo Fish Hormone Factory, Zhejiang Province, China. MT and ADSD were incorporated into silastic and make pellet as described by PANKHURST *et al.* (1986) and LIN *et al.* (1991). Carp pituitaries were collected locally.

The effects of exogeneous GtH and endogeneous GtH on the induction of gonadal maturation were investigated. For the effects of exogeneous GtH, silver eels were injected intramuscularly with carp pituitary extract (CPE) (5 pituitary / female eel and 1–2 pituitary / male eel). Injections were given at the start of the experiment, and at 10-day interval. Controls were injected with freshwater teleost physiological saline (PS ; BURNSTOCK, 1958). For the effects of endogeneous GtH, the silastic pellets which contained MT or ADSD were implanted with forceps into the peritoneal cavity of silver eel through an incision (2–4 mm) cut in the body wall. Implants (MT or ADSD 50  $\mu$ g / g body weight) were given at the start of the experiment, and at 15-day interval. Controls received a blank silastic pellet. Blood samples were obtained by puncturing the caudal vasculature with a 25 guage needle attached to a 1 ml syringe. At the end of experiment, the fish were killed by decapitation, and the pituitaries quickly removed and frozen until extraction. The ovaries were removed and weighed to determine gonadosomatic index (GSI, = gonad weight / body weight X 100). Blood samples were allowed to clot on ice for several hours, and the serum was separated by centrifugation and stored at -25 °C until determination of GtH, testosterone (T) or estradiol ( $E_2$ ) by radioimmunoassay (RIA). Crude pituitary extracts were prepared in RIA buffer by grinding with a homogenizer ; the supernatants were kept frozen at -25 °C until RIA. GtH levels of pituitary and serum samples were measured in a heterologous RIA using an antiserum to the  $\beta$ -subunit of carp GtH and carp GtH for the assay standards and tracer, as described by LIN *et al.* (1986, 1991). Gonadotropin releasing hormone (GnRH) levels of brain and pituitary samples were measured by RIA specific for mammalian GnRH (mGnRH), as described by KING *et al.* (1990). Serum samples were extracted with diethyl ether and analysed for T and  $E_2$  content using the protocol described by VAN DER KRAAK *et al.* (1984). Data were analysed by one-way analysis of variance and Duncan's multiple range test.

## RESULTS AND DISCUSSION

### I. Effects of CPE injections on the induction of gonadal maturation

On day 80, 10 days following the eighth injection of CPE, the GSI of female silver eels were significantly increased and much higher than the controls ; histological examination of the ovaries demonstrated that the oocytes were fully matured. On day 40, 10 days following the fourth injection of CPE, the GSI of male silver eels were significantly increased and higher than the controls, the testes were fully matured, milt being extracted by gently squeezing the abdomen of fish (Figure 1).



**Figure 1**

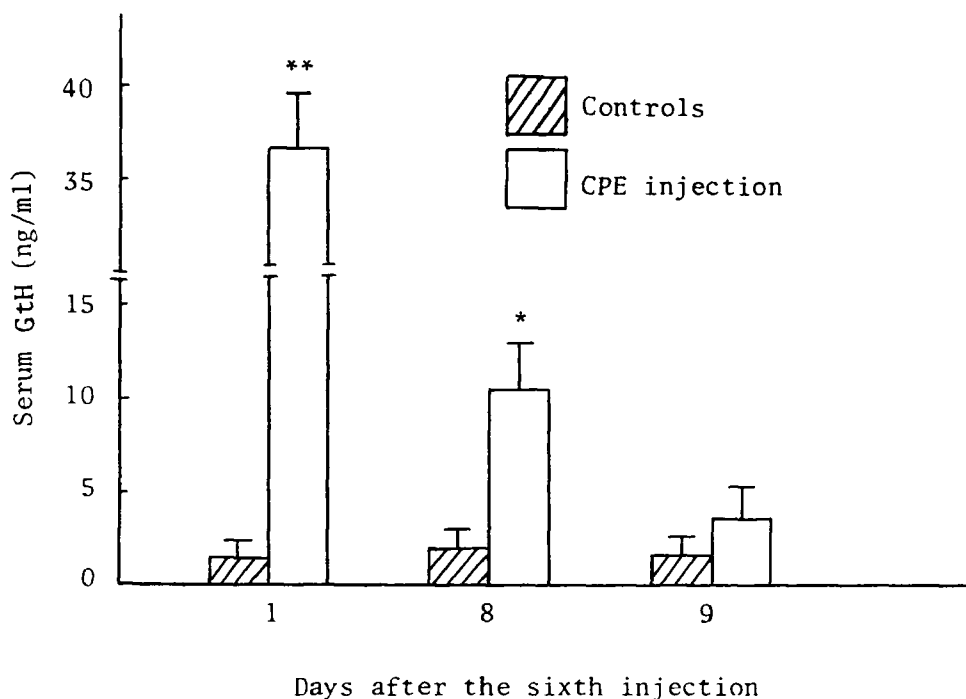
Effects on day 80 of injection every 10 days (injection on day 0, 10, 20, 30, 40, 50, 60, 70) of CPE (5 pituitary / fish) or PS (controls) on the gonadosomatic index (GSI) of female silver eel and on day 40 of injection every 10 days (injection on day 0, 10, 20, 30) of CPE (1 pituitary / fish) or PS (controls) on the GSI of male silver eel. The number of fish in each group is indicated at the base of the columns.

**Figure 1**

Effets, au jour 80, de l'injection tous les 10 jours (injection au jour 0, 10, 20, 30, 40, 50, 60, 70) d'extrait hypophysaire de carpe (CPE) (5 hypophyses / poisson) ou de sérum physiologique (contrôles) sur le rapport gonadosomatique (RGS = Gonadosomatic index) chez l'anguille femelle argentée et, au jour 40, de l'injection tous les 10 jours (injection au jour 0, 10, 20, 30) de CPE (1 hypophyse / poisson) ou de sérum physiologique (contrôles) sur le RGS chez l'anguille mâle argentée. Le nombre de poissons dans chaque groupe est indiqué à la base des colonnes.

The serum GtH contents of female silver eels after six CPE injections were significantly higher than in the controls (Figure 2). On day 51, first day following the sixth injection, the serum GtH levels had increased sharply and averaged  $35.21 \pm 3.52$  ng / ml ; at the 8th day after injection, serum GtH levels were still significantly higher than the controls ; whereas the 9th day after injection, serum GtH levels dropped to  $3.86 \pm 0.78$  ng / ml, very close to the GtH levels of controls ( $1.94 \pm 0.21$  ng / ml). These

results suggest that the high serum GtH level resulting from exogenous CPE injections plays a determinant role in the stimulation of gonadal development and maturation of Japanese silver eel.



**Figure 2**

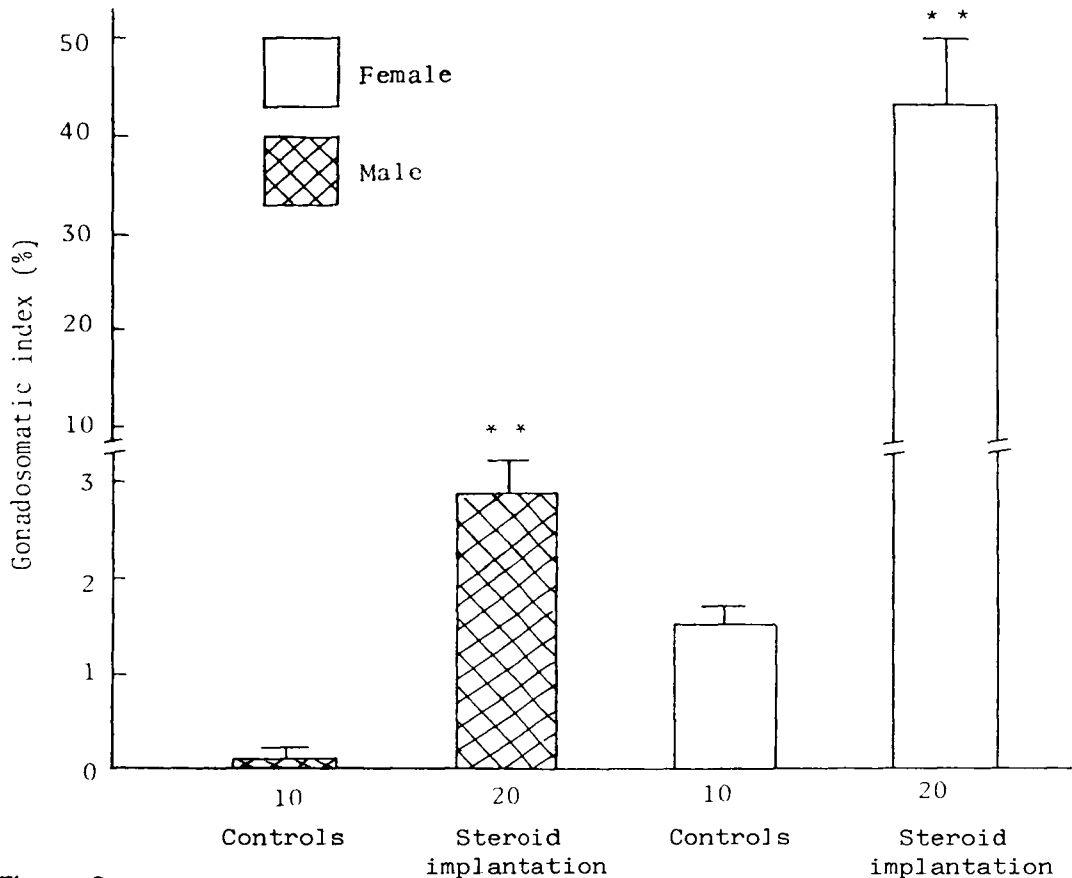
Effects of six injections every 10 days (injection on day 0, 10, 20, 30, 40, 50) of CPE (5 pituitary / fish) or PS (controls) on serum GtH concentrations of female silver eel. Data are represented in mean  $\pm$  SE. Number of fish = 5. CPE injected fish : GSI =  $28.42 \pm 2.56$  % ; controls : GSI =  $1.31 \pm 0.24$  % . \*Significantly higher than the controls ( $p < 0.05$ ) ; \*\*very significantly higher than the controls ( $p < 0.01$ ).

**Figure 2**

Effets de six injections tous les 10 jours (injection au jour 0, 10, 20, 30, 40, 50) de CPE (5 hypophyses / poisson) ou de sérum physiologique (contrôles) sur les taux sériques de gonadotropine (GtH) chez l'anguille femelle argentée. Les données représentent les moyennes  $\pm$  erreur standard ( $n = 5$  poissons / groupe). Poissons injectés avec CPE : RGS =  $28,42 \pm 2,56$  % ; contrôles : RGS =  $1,31 \pm 0,24$  % . \*Significativement plus élevé que les contrôles ( $p < 0,05$ ) ; \*\*très significativement plus élevé que les contrôles ( $p < 0,01$ ).

## II. Effects of MT or ADSD implantations on the induction of gonadal maturation

On day 120, 15 days following the eighth implantation of MT, or ADSD, a precursor of testosterone biosynthesis, the GSI of female silver eels had significantly increased and were much higher than the controls, histological examination of ovaries demonstrated that the oocytes were fully matured. On day 60, 15 days following the fourth implantation of MT or ADSD, the GSI of male silver eels had significantly increased and were higher than the controls, the testes were fully matured (Figure 3).



**Figure 3**

Effects on day 120 of implantation every 15 days (implantation on day 0, 15, 30, 45, 60, 75, 90, 105) of MT or ADSD (50 µg / g body weight), or blank silastic pellet (controls) on the GSI of female silver eel, and on day 60 of implantation every 15 days (implantation on day 0, 15, 30, 45) of MT or ADSD, or blank silastic pellet (controls) on the GSI of male eel. The number of fish in each group is indicated at the base of the columns.

**Figure 3**

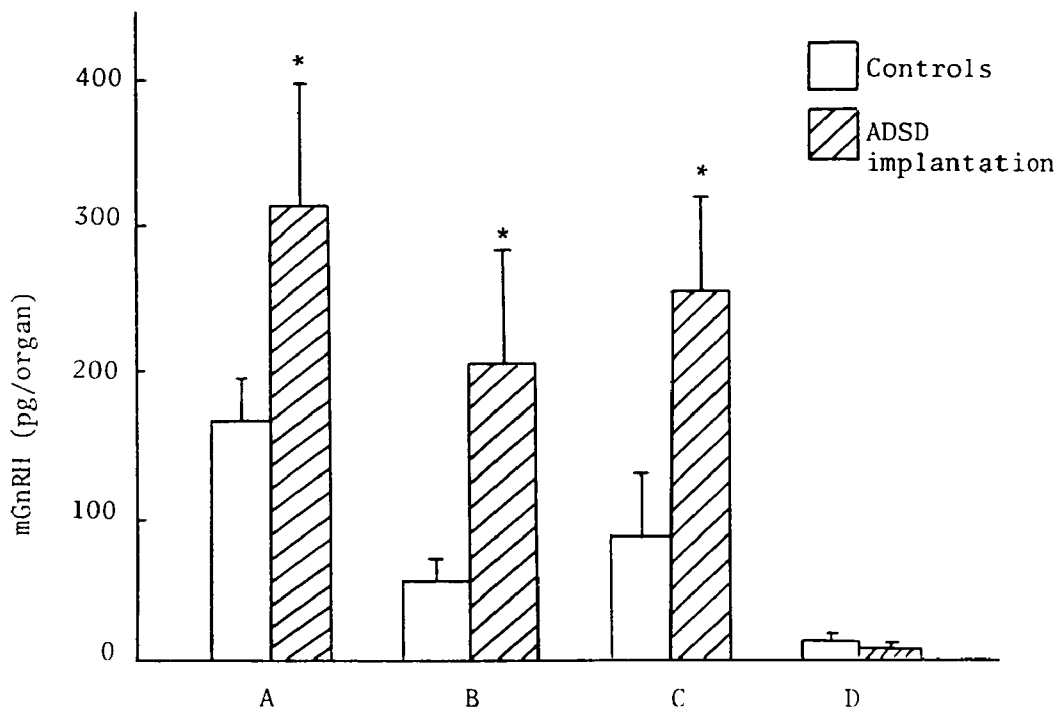
Effets, au jour 120, de l'implantation tous les 15 jours (implantation au jour 0, 15, 30, 45, 60, 75, 90, 105) de méthyltestostérone (MT) ou d'androstènedione (ADSD) (50 µg / g poids du corps), ou de silastique seul (contrôles) sur le RGS (Gonadosomatic index) chez l'anguille femelle argentée et, au jour 60, de l'implantation tous les 15 jours (implantation au jour 0, 15, 30, 45) de MT ou d'ADSD ou de silastique seul (contrôles) chez le mâle. Le nombre de poissons dans chaque groupe est indiqué à la base des colonnes.

### III. Effects of MT or ADSD implantation on the brain (hypothalamus)-pituitary-gonad axis

#### 1. Effects of ADSD implantation on brain and pituitary mGnRH concentrations

Studies on European eel (*Anguilla anguilla*) have demonstrated that two GnRH molecular forms, similar to mammalian GnRH (mGnRH) and to chicken GnRH-II (cGnRH-II) were distributed in the brain and pituitary of silver eel (KING *et al.*, 1990) ; mGnRH is the main form of GnRH involved in the control of GtH release (MONTERO *et al.*, 1995). Therefore, effects of ADSD implantations on brain and pituitary mGnRH concentrations of female silver eel were determined.

In female eels, on day 105, 15 days following the seventh implantation of ADSD, total mGnRH content in the brain (including pituitary) was about twice higher than the blank-implanted controls ; in the olfactory bulbs, telencephalon, optic tectum-thalamus, hypothalamus, and pituitary, mGnRH content was significantly higher than the controls, whereas mGnRH content in cerebellum and medulla oblongata was not different (Figure 4). These results indicated that the positive feedback effects of ADSD presumably effect on the brain (hypothalamus) to stimulate GnRH synthesis and release in the Japanese silver eel.



**Figure 4**

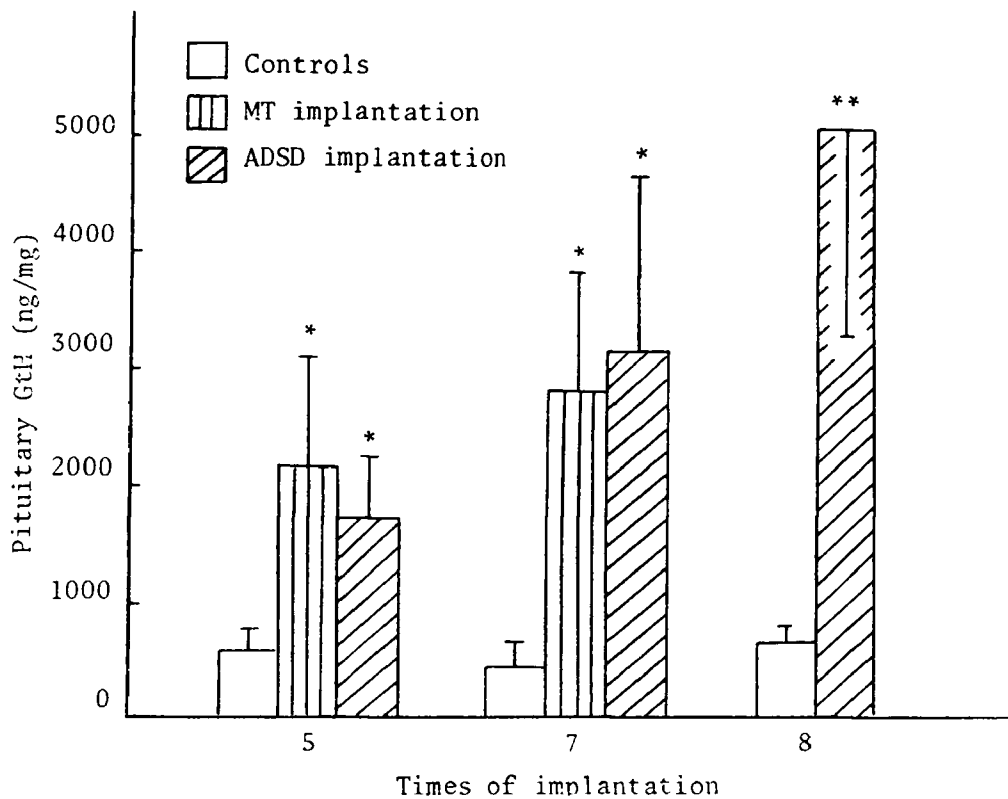
Effects on day 105 of implantation every 15 days (implantation on day 0, 15, 30, 45, 60, 75, 90) of ADSD (50  $\mu\text{g}$  / g body weight) or blank silastic pellet (controls) on mGnRH contents in discrete brain areas and pituitary of female silver eel. A. pituitary ; B. olfactory bulbs and telencephalon ; C. optic tectum-thalamus and hypothalamus ; D. cerebellum and medulla oblongata. Data are represented in mean  $\pm$  SE. Number of fish in each group = 5. ADSD implanted fish : GSI =  $29.08 \pm 5.30$  % ; controls : GSI =  $1.21 \pm 0.36$  %. \*Significantly higher than the controls ( $p < 0.05$ ).

**Figure 4**

Effets, au jour 105, de l'implantation tous les 15 jours (implantation au jour 0, 15, 30, 45, 60, 75, 90) d'ADSD (50  $\mu\text{g}$  / g poids du corps) ou de silastique seul (contrôles) sur le contenu en gonadolibérine de type mammalienne (mGnRH) de l'hypophyse et de différentes régions du cerveau chez l'anguille femelle argentée. A. hypophyse ; B. bulbes olfactifs et télencéphale ; C. toit optique-thalamus et hypothalamus ; D. cervelet et moelle allongée. Les données représentent les moyennes  $\pm$  erreur standard ( $n = 5$  poissons / groupe). Poissons implantés avec ADSD : RGS =  $29,08 \pm 5,30$  % ; contrôles : RGS =  $1,21 \pm 0,36$  %. \*Significativement plus élevé que les contrôles ( $p < 0,05$ ).

## 2. Effects of MT or ADSD implantation on pituitary and serum GtH concentrations

In female eels, on day 75, 105, and 120, during 15 days following the fifth, seventh and eighth implantation of MT or ADSD, GtH content in the pituitary increased gradually and became about 4-7 times higher than the blank-implanted controls (Figure 5). In male eels, on day 60, 15 days following the fourth implantation of MT or ADSD, pituitary GtH content was elevated significantly, about 6-8 times higher than the blank-implanted controls (Figure 6). These results indicated that a positive sex-steroid feedback was active on GtH synthesis in the pituitary of the silver eel.



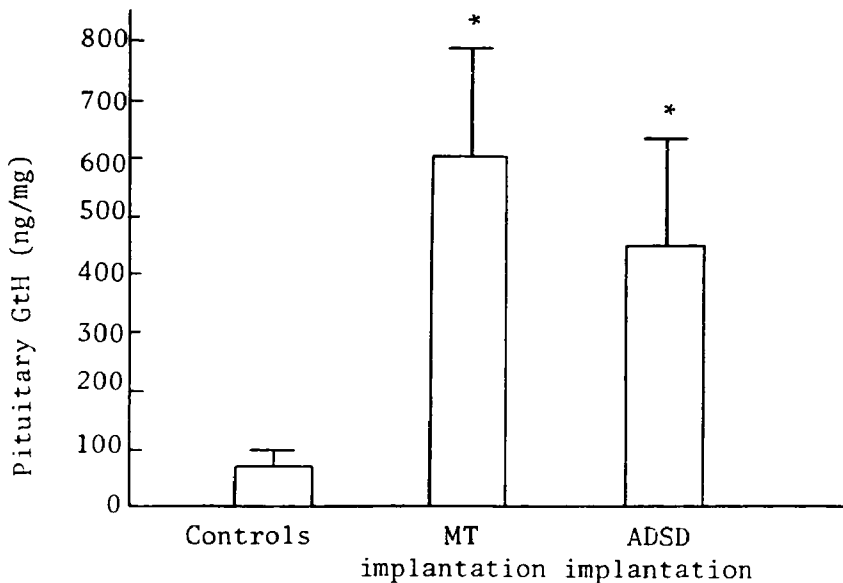
**Figure 5**

Effects of the fifth (on day 75), seventh (on day 105) and eighth (on day 120) (implantation every 15 days) of MT or ADSD, or blank silastic pellet (controls) on GtH contents in pituitary of female silver eel. Data are represented in mean  $\pm$  SE. Number of fish in each group = 6. After the eighth implantation, MT implanted fish : GSI =  $41.38 \pm 2.71$  % ; ADSD implanted fish : GSI =  $43.97 \pm 3.15$  % ; controls : GSI =  $1.37 \pm 0.25$  % . \*Significantly higher than the controls ( $p < 0.05$ ), \*\*very significantly higher than the controls ( $p < 0.01$ ).

**Figure 5**

Effets de la 5<sup>e</sup> implantation (au jour 75), de la 7<sup>e</sup> (au jour 105) et de la 8<sup>e</sup> (au jour 120) (implantation tous les 15 jours) de MT ou d'ADSD ou de silastique seul (contrôles) sur le contenu hypophysaire en gonadotropine (GtH) chez l'anguille femelle argentée. Les données représentent les moyennes  $\pm$  erreur standard ( $n = 6$  poissons / groupe). Après la 8<sup>e</sup> implantation, poissons implantés avec MT : RGS =  $41,38 \pm 2,71$  % ; poissons implantés avec ADSD : RGS =  $43,97 \pm 3,15$  % ; contrôles : RGS =  $1,37 \pm 0,25$  % . \*Significativement plus élevé que les contrôles ( $p < 0,05$ ) ; \*\*très significativement plus élevé que les contrôles ( $p < 0,01$ ).





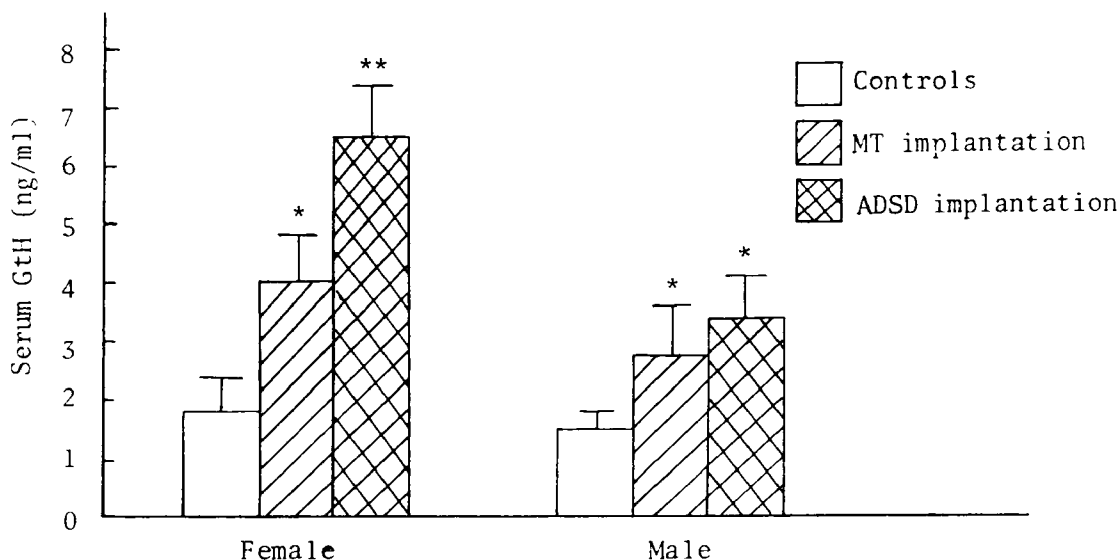
**Figure 6**

Effects of the fourth (on day 60) implantation every 15 days of MT or ADSD, or blank silastic pellet (controls) on GtH contents in pituitary of male silver eel. Data are represented in mean  $\pm$  SE. Number of fish in each group = 5. After the fourth implantation, MT implanted fish : GSI =  $2.54 \pm 0.43$  % ; ADSD implanted fish : GSI =  $2.81 \pm 0.38$  % ; controls : GSI =  $0.25 \pm 0.03$  % . \*Significantly higher than the controls ( $p < 0.05$ ).

**Figure 6**

Effet de la 4<sup>e</sup> implantation (au jour 60) (implantation tous les 15 jours) de MT ou d'ADSD ou de silastique seul (contrôles) sur le contenu en GtH hypophysaire au bout de 60 jours (4 implants) chez l'anguille mâle argentée. Les données représentent les moyennes  $\pm$  erreur standard ( $n = 5$  poissons / groupe). Après la 4<sup>e</sup> implantation, poissons implantés avec MT : RGS =  $2,54 \pm 0,43$  % ; poissons implantés avec ADSD : RGS =  $2,81 \pm 0,38$  % ; contrôles : RGS =  $0,25 \pm 0,03$  % . \*Significativement plus élevé que les contrôles ( $p < 0,05$ ).

In female eels, on day 105, during 15 days following the seventh implantation of MT or ADSD, serum GtH levels increased significantly, becoming about 1-2 times higher than the blank-implanted controls. In male eels, on day 60, 15 days following the fourth implantation of MT or ADSD, serum GtH levels were also increased to a significantly higher level than the controls (Figure 7). The kinetic effects of ADSD implantation on serum GtH levels in female silver eel are shown in Figure 8. On day 91, 1 day after the implantation of ADSD, serum GtH levels were elevated significantly and became higher than the controls ; serum GtH levels were sustainably increased on day 92 till day 97, then decreased moderately ; however, on day 104, 14 days after the seventh implantation, serum GtH levels were still higher than the controls. These results demonstrated that serum GtH levels were significantly and sustainably increased following the MT or ADSD implantations in order to result in the stimulation of gonadal development and maturation.

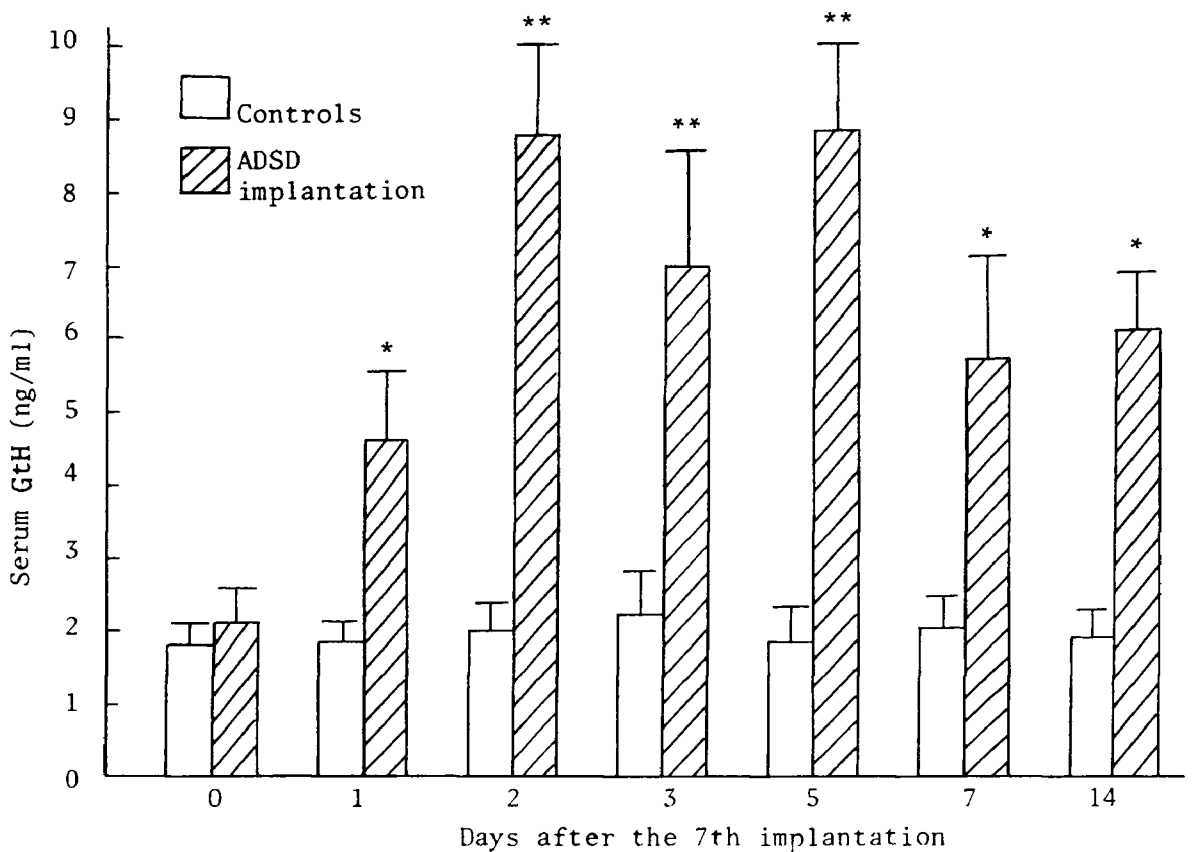


**Figure 7**

Effects of seventh (on day 105) implantation every 15 days of MT or ADSD on serum GtH levels of female silver eel, and fourth (on day 60) implantation every 15 days of MT or ADSD on serum GtH levels of male silver eel. Data are represented in mean  $\pm$  SE. Number of fish in each group = 8. After the seventh implantation, MT implanted female fish : GSI =  $33.15 \pm 3.51$  % ; ADSD implanted female fish : GSI =  $35.27 \pm 6.47$  % ; female controls : GSI =  $1.65 \pm 0.21$  %. After the fourth implantation, MT implanted male fish : GSI =  $2.08 \pm 0.32$  % ; ADSD implanted male fish : GSI =  $2.22 \pm 0.42$  % ; male controls : GSI =  $0.24 \pm 0.04$  % . \*Significantly higher than controls ( $p < 0.05$ ) ; \*\*very significantly higher than controls ( $p < 0.01$ ).

**Figure 7**

Effets de l'administration tous les 15 jours d'implants de MT ou d'ADSD sur les taux de GtH sérique au bout de 105 jours (7 implants) chez l'anguille femelle argentée, et au bout de 60 jours (4 implants) chez l'anguille mâle argentée. Les données représentent les moyennes  $\pm$  erreur standard ( $n = 8$  poissons / groupe). Après la 7<sup>e</sup> implantation, femelles implantées avec MT : RGS =  $33,15 \pm 3,51$  % ; femelles implantées avec ADSD : RGS =  $35,27 \pm 6,47$  % ; femelles contrôles : RGS =  $1,65 \pm 0,21$  %. Après la 4<sup>e</sup> implantation, mâles implantés avec MT : RGS =  $2,08 \pm 0,32$  % ; mâles implantés avec ADSD : RGS =  $2,22 \pm 0,42$  % ; mâles contrôles : RGS =  $0,24 \pm 0,04$  % . \*Significativement plus élevé que les contrôles ( $p < 0,05$ ) ; \*\*très significativement plus élevé que les contrôles ( $p < 0,01$ ).



**Figure 8**

The kinetic effects of the seventh (on day 105) implantation of ASD on serum GtH levels of female silver eel. Data are represented in mean  $\pm$  SE. Number of fish in each group = 6. After the seventh implantation, ASD implanted fish : GSI =  $34.51 \pm 4.82$  % ; controls : GSI =  $2.26 \pm 0.33$  % . \*Significantly higher than controls ( $p < 0.05$ ) ; \*\*very significantly higher than controls ( $p < 0.01$ ).

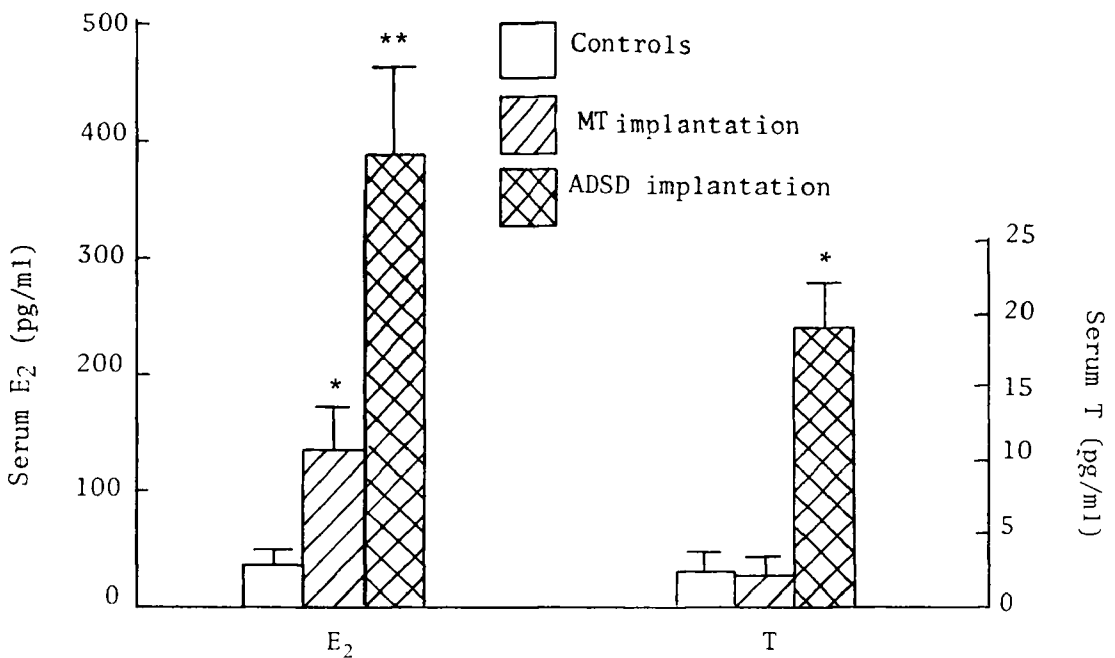
**Figure 8**

Cinétique de l'effet de la 7<sup>e</sup> implantation (au jour 105) d'ASD sur les taux de GtH sérique chez l'anguille femelle argentée. Les données représentent les moyennes  $\pm$  erreur standard ( $n = 6$  poissons / groupe). Après la 7<sup>e</sup> implantation, poissons implantés avec ASD : RGS =  $34,51 \pm 4,82$  % ; contrôles : RGS =  $2,26 \pm 0,33$  % . \*Significativement plus élevé que les contrôles ( $p < 0,05$ ) ; \*\*très significativement plus élevé que les contrôles ( $p < 0,01$ ).

### 3. Effects of MT or ASD implantation on serum $E_2$ and T concentrations

The steroidal responses to MT or ASD implantations in female and male silver eel during gonadal development and maturation are shown in Figures 9 and 10. In females, implantations of MT or ASD both stimulated a significant increase in serum  $E_2$  levels on day 105, 15 days after the seventh treatment ; the serum  $E_2$  levels of MT implanted eels were lower than in fish treated with ASD. ASD implantations also caused a significant

increase in serum T levels on day 105, 15 days after the seventh treatment ; however, MT implantation had no effect on serum T level. After the seventh implantation of MT or ADSD, the ovaries of female were well-developed, GSI = 33.80~34.62 %, serum E<sub>2</sub> levels were much higher than T levels, demonstrating that estradiol plays an important role on the stimulation of ovarian growth and mature. In males, implantations of MT or ADSD both also stimulated a significant increase in serum E<sub>2</sub> and T levels on day 45, 15 days after the third treatment ; the serum T levels of the implanted fish were increased significantly, about 17-40 times higher than in controls, whereas serum E<sub>2</sub> levels of the treated eels were elevated moderately, only 1.6-3.5 times higher than in controls. This high serum T concentration following the third implantation was consistent with the stimulatory effects of testosterone on the development and maturation of testis in the male silver eel.

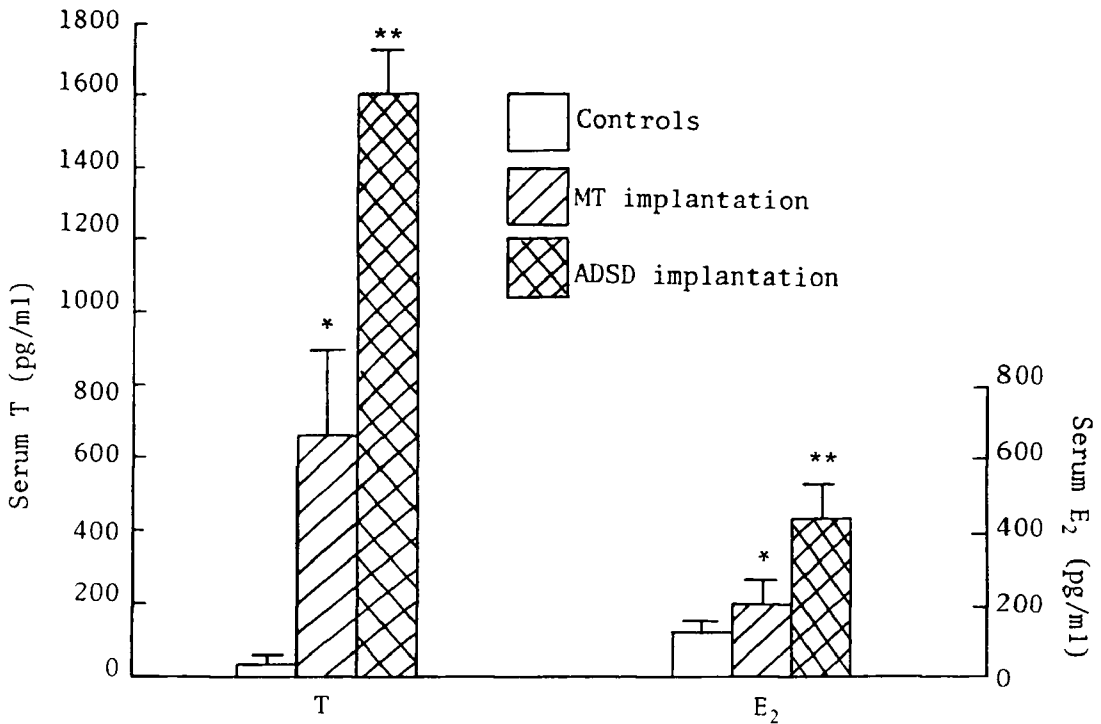


**Figure 9**

Effects of the seventh (on day 105) implantation of MT or ADSD on serum E<sub>2</sub> and T levels of female silver eel. Data are represented in mean  $\pm$  SE. Number of fish in each group = 8. After the seventh implantation, MT implanted fish, GSI = 33.80  $\pm$  4.23 % ; ADSD implanted fish : GSI = 34.62  $\pm$  3.05 % ; controls : GSI = 1.68  $\pm$  0.26 %. \*Significantly higher than controls (p<0.05) ; \*\*very significantly higher than controls (p<0.01).

**Figure 9**

Effets de la 7<sup>e</sup> implantation (au jour 105) de MT ou d'ADSD sur les taux d'estradiol (E<sub>2</sub>) et de testostérone (T) sériques chez l'anguille femelle argentée. Les données représentent les moyennes  $\pm$  erreur standard (n = 8 poissons / groupe). Après la 7<sup>e</sup> implantation, poissons implantés avec MT : RGS = 33,80  $\pm$  4,23 % ; poissons implantés avec ADSD : RGS = 34,62  $\pm$  3,05 % ; contrôles : RGS = 1,68  $\pm$  0,26 %. \*Significativement plus élevé que les contrôles (p<0,05) ; \*\*très significativement plus élevé que les contrôles (p<0,01).



**Figure 10**

Effects of the third (on day 45) implantation of MT or ADSD on serum E<sub>2</sub> and T levels of male silver eel. Data are represented in mean ± SE. Number of fish in each group = 5. After the third implantation, MT implanted fish : GSI = 1.52 ± 0.19 % ; ADSD implanted fish : GSI = 1.62 ± 0.35 % ; controls : GSI = 0.32 ± 0.05 %. \*Significantly higher than controls (p<0.05) ; \*\*very significantly higher than controls (p<0.01).

**Figure 10**

Effets de la 3<sup>e</sup> implantation (au jour 45) de MT ou d'ADSD sur les taux sériques de E<sub>2</sub> et de T chez l'anguille mâle argentée. Les données représentent les moyennes ± erreur standard (n = 5 poissons / groupe). Après la 3<sup>e</sup> implantation, poissons implantés avec MT : RGS = 1,52 ± 0,19 % ; poissons implantés avec ADSD : RGS = 1,62 ± 0,35 % ; contrôles : RGS = 0,32 ± 0,05 %. \*Significativement plus élevé que les contrôles (p<0,05) ; \*\*très significativement plus élevé que les contrôles (p<0,01).

In summary, the present studies provide further evidences that serial implantation of steroids (MT or ADSD) pellets alone can stimulate the brain-pituitary-gonad axis of the Japanese silver eel, to promote GnRH synthesis in brain and / or release in pituitary ; this endogeneous GtH is then released into blood circulation and stimulates the production of sex steroids in the gonads, and then results in the stimulation of gonadal development and maturation.

#### IV. Induced ovulation and spawning of the matured silver eel

In the matured female eels with oocytes at the migratory nucleus stage (GSI = 48~52 %) after 8 injections of CPE (every 10 days), or, 8 implantations of MT or ADSD (every 15 days), ovulation and spawning can be induced 18-22 hours (in April-May, 22-25 °C) following an injection of CPE (5~6 pituitary / fish) alone or in combination with LHRH-A (50 µg / kg body weight) plus DOM (10 mg / kg body weight). The matured male eels were injected with HCG (200~400 I.U. / fish) in order to increase milt volume. 19 of 35 females ovulated in the experiments in 1996 and 22 of 40 females ovulated in the experiments in 1997, the rates of ovulation were 54.3 % and 55.0 %, respectively. Among the ovulated female eels, eleven spawned spontaneously at dawn in the concrete tank (2 X 3 m, with 1.5 m depth) about 18 hours following hormonal treatments and mated with matured male eels. In most cases, artificial fertilization were done immediately after ovulation by using dry method.

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