GENE EXPRESSION REGULATION OF STEROIDOGENIC ENZYMES IN EUROPEAN EEL (*ANGUILLA ANGUILLA*, L.) MALES DURING INDUCED SEXUAL MATURATION UNDER THREE THERMAL REGIMES, AND RELATIONSHIP WITH SPERM QUALITY PARAMETERS

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Introduction

Steroids are derived from cholesterol and depending on species, sex, and reproductive stage, gonadal cells express different genes encoding steroidogenic enzymes (Young *et al*., 2005).
European eel (*A. anguilla*) is a highly valued species mainly demanded by European and Asian markets, but reproduction in captivity has not been got yet and, due to overfishing and environmental conditions, eel populations are decreasing.
The influence of temperature on eel maturation process has been evidenced during the last years by the use of different thermal regimes during hormonal inducing treatments (Sato *et al*., 2006; Peñaranda *et al*., 2007). This study tried to evaluate the influence of temperature on the dynamics of testis steroidogenic enzymes gene expression.

Material and methods

317 males (b.w. 100±2 g; length 40±5 cm) were distributed in six 200-L aquaria and submitted to three thermal regimes: T10, 10 ºC (first 6 weeks), 15 ºC (next 3 weeks) and 20 ºC (last 6 weeks); T15, 15 ºC (first 6 weeks) and 20 ºC (last 9 weeks); and T20, 20 ºC during the whole experimental period. Males were treated for the induction of maturation and spermiation with weekly i.p. injections of hCG (1.5 IU g^{-1} fish).
Once a week, percentage of spermiating males and sperm volume, density and motility parameters (using ISAS® system) were recorded, and 5-8 males/thermal regime were sacrificed to obtain testis samples. Total RNA was extracted using traditional phenol/chloroform method. First-strand cDNA was synthesized (tRNA: 2µg) using random primers. In order to monitor gene expression of steroidogenic enzymes (P450 aromatase, 11β-HSD, 20β-HSD), qrtPCR analyses were performed using an Applied Biosystems 7500 Real-Time PCR Systems with SYBR Green I sequence-unspecific detection (Applied Biosystem, Foster City, USA). The quantification of the results was using a relative standard curve method, and the reference gene used was acidic ribosomal phosphoprotein P0 (ARP).

Results

T20 males began spermiating at the 5^{th} week, whereas those from T15 and T10 began later (6^{th} and 10^{th} week, respectively; Fig. 1A). The delay to initiate the spermiation and the lower spermiating percentage showed by T15 and T10 males are a result of a late gonad development caused by the lower temperatures, as occur in European eel females (Pérez et al., unpublished). In most species, the success in fertilization is guaranteed mainly with sperm of a reasonable volume and concentration, and a high percentage of motile spermatozoa. In this study all treatments caused, with different timings, a progressive increase of sperm volume that resulted significantly higher in some weeks for T20 males, whose produced high volume during a longer period (Fig. 1B). Sperm from T20 fish showed the highest spermatozoa concentration during almost all the

experimental period (Fig. 1C). Sperm motility is the most used parameter to evaluate sperm quality. In this sense, T20 fish showed higher motilities than T15 and T10 males in most of the sampling, with significant differences between 8-11th weeks (Fig. 1D).

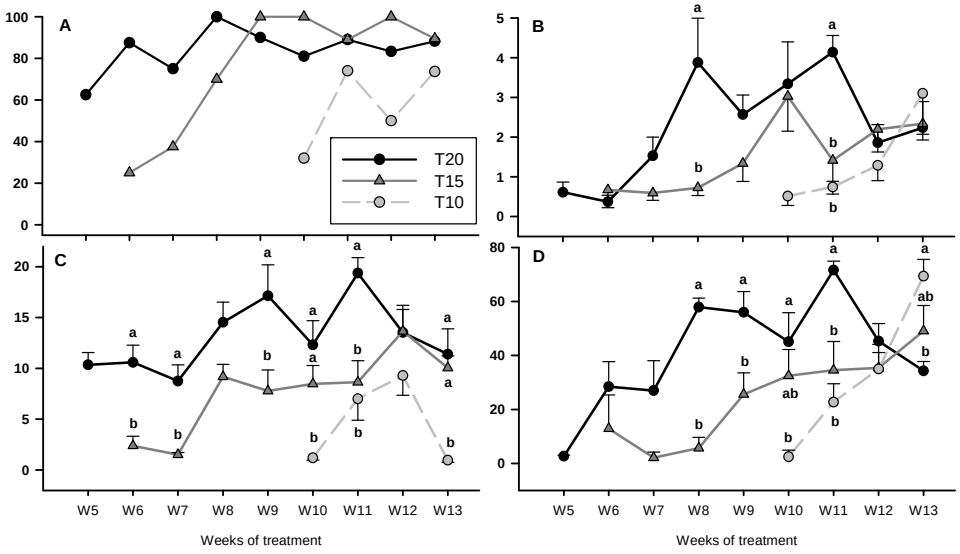

Figure 1. Sperm quality parameters of European eels maintained under different water temperature regimes: A) Percentage of spermiating males (%), B) Sperm volume (ml/100 g^{-1} fish), C) Sperm concentration (10^9 cells ml^{-1}), and D) Sperm motility (% motile cells). Data are expressed as mean ± SEM and different letters indicate significant differences between treatments.

Discussion and Conclusion

The results of this work indicate that thermal regime affects the spermiating process, its timing, as well as the sperm quantity and quality of European eel, getting the best results when the males are maintained in water at 20 ºC during the whole hormonal induction, and coinciding with similar results obtained in previous studies with European eel males hCG-treated at this temperature (i.e.: Asturiano *et al*., 2005).

The role of testis steroidogenic enzymes will be evaluated, studying the expression of some key genes through the hormonal treatment under the different thermal regimes. In the case of sacrificed males (6/week) the relation between genes expression and sperm quality parameters will be analyzed.

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