THE MATERNAL-FETAL RELATIONSHIP

IN THE EELPOUT *ZOARCES VIVIPARUS*:

IMPACTS FROM THE ENVIRONMENT

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Abstract

Pregnant females of the eelpout *Zoarces viviparus* were exposed to different environmental factors to investigate the impact on metabolites which may elucidate physiological interrelations between the embryos and the pregnant motherfish. The maternal organism was exposed to different salinities, elevated levels of nitrogenous endproducts and to different concentrations of the xenobiotic compound octylphenol, respectively. The concentrations of ammonia, urea, low molecular compounds, chloride, and calcium were measured in the maternal plasma and the ovarian fluid, the ambient medium of the embryos. Ammonia increased in the maternal plasma as well as in the ovarian fluid by the highest salinity (28ppt). No change could be observed in the level of urea or chloride by increased salinities. By exposing the embryos to increased levels of urea and ammonia in their ambient medium, the ovarian fluid, it was observed that a steady flow of the nitrogenous compounds occurred from the ovarian fluid to the maternal plasma. In the fish exposed to octylphenol the calcium concentration in the ovarian fluid decreased in a dose-dependent manner by increasing concentrations of the estrogenic compounds. In contrast significant increased levels of total calcium were observed in the plasma of the exposed motherfish.

Introduction

The eelpout *Zoarces viviparus* is an euryhaline viviparous teleost living in near-coastal waters of the North Sea. The viviparous female fish has a complex pattern of reproductive activity. Fertilization follows a summer period of intensive final vitellogenesis and the subsequent gestation occurs in the ovarian lumen of a single ovary. The embryonic development, lasting approximately 5 months, may be divided in three different stages: a prehatch stage, a posthatch yolksac stage followed by a lengthly post-yolksac developmental stage. The last two stages are characterized by an intensive maternal-fetal trophic relationship (Korsgaard and Andersen, 1985; Korsgaard, 1986). The ovarian fluid constitutes the ambient environment of the embryos in the ovary. This fluid is shown to undergo rapid turnover of metabolites. Studies with radiolabeled compounds indicate that the embryos obtain energy from catabolism of low molecular metabolites such as free amino acids (Korsgaard, 1992). As the embryos constitute a very dense population within the ovarian lumen, their survival and growth are dependent on the maternal capability to provide nutrients and oxygen sufficiently and to remove waste and potential toxic products effectively (Korsgaard *et al.* 1995) as well as securing a steady osmotic environment. Environmental impacts on the maternal-fetal relationship *in vivo* were investigated by exposing the fish to elevated levels of urea and ammonia, to different salinities and to the xenobiotic compound octylphenol measuring the subsequent time-course changes in the level of different ions, metabolites and nitrogenous endproducts in the maternal plasma and the ovarian fluid, the latter constituting the ambient embryonic medium.

Material and methods

In the salinity experiment pregnant fish were exposed to 5, 15 and 28 ppt seawater and samples were taken of plasma and ovarian fluid after 24 h.
In the experiment elucidating the effect of elevated levels of urea and ammonia, urea (500µmol urea-N/100g BW) and ammonia (30µmol ammonia-N/100g BW) were injected into the ovarian fluid and the level of the two nitrogenous components were followed during a 24 h time-course.
In the xenobiotic experiment pregnant fish were exposed to different concentrations, 25 and 100 µg/L of 4-t-octylphenol in the ambient seawater using an estrogen-exposed (0.5 µg/L) and a control group, respectively, as reference groups. The fish were sampled after an exposure period of 5 weeks.

Results and Discussion

No significant changes were observed in the chloride concentration in the plasma or ovarian fluid, respectively, in the different salinity groups. However, the chloride concentrations were observed at a lower level in the ovarian fluid when compared to the plasma levels at all three salinities (Figure 1).

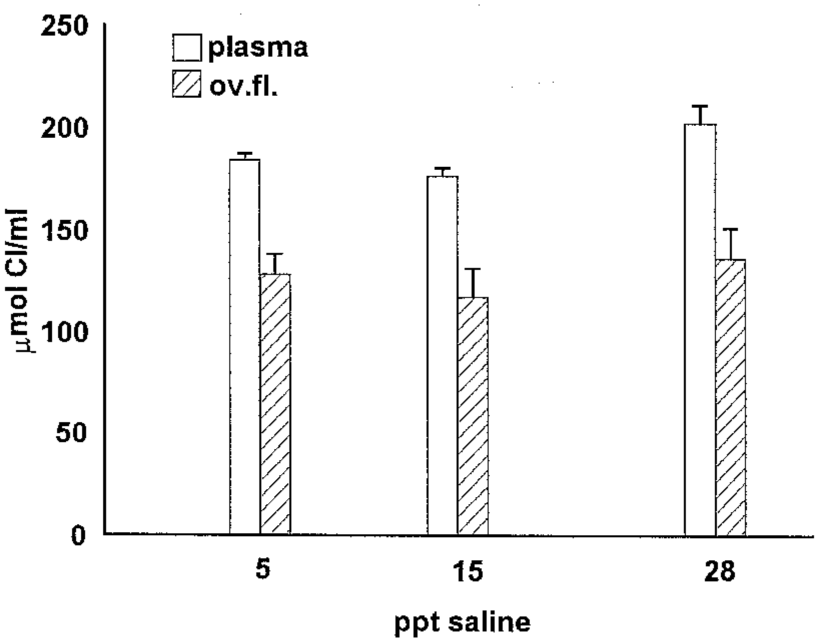

Fig. 1. The concentration of chloride in maternal plasma and ovarian fluid at three different salinities after 24 h acclimation.

Only a few data exist regarding salinity tolerance in viviparous fish. In newborn guppies (*Poecilia reticulata*) the salinity tolerance increased within 5 days after birth concomitant with an increase in size of the chloride cells (Shikano and Fujio,1999). The present study indicates that the embryos are protected from increased levels of chloride by the constant low levels of chloride in the ovarian fluid. No increase could be observed in the concentration of urea in the ovarian fluid or in the plasma when comparing the fish by the different salinities.

In the guppy *Poecilia reticulata* urea was found to be increased in embryos during later development indicating a role of urea as an osmotic component during development in a viviparous fish (Depeche, 1975).

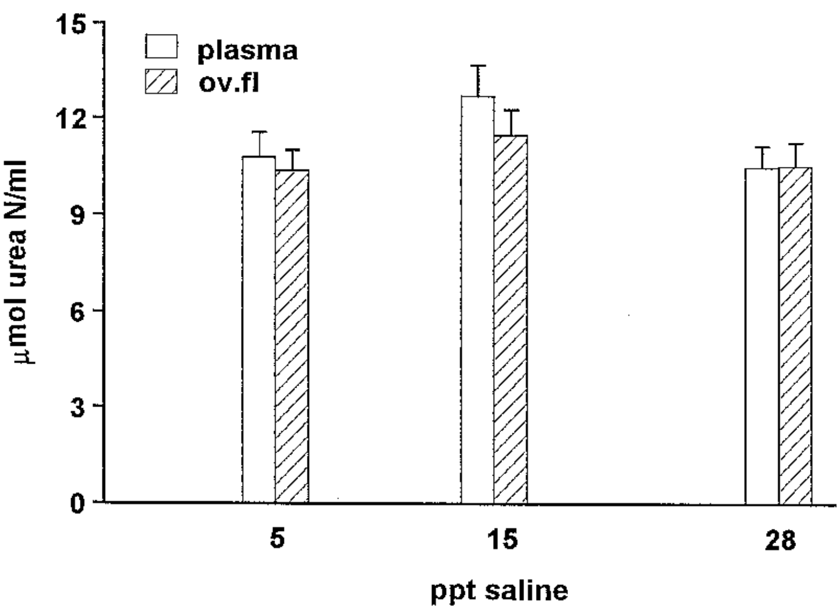

Fig. 2. The concentration of urea in the maternal plasma and ovarian fluid at 3 different salinities after 24 h acclimation.

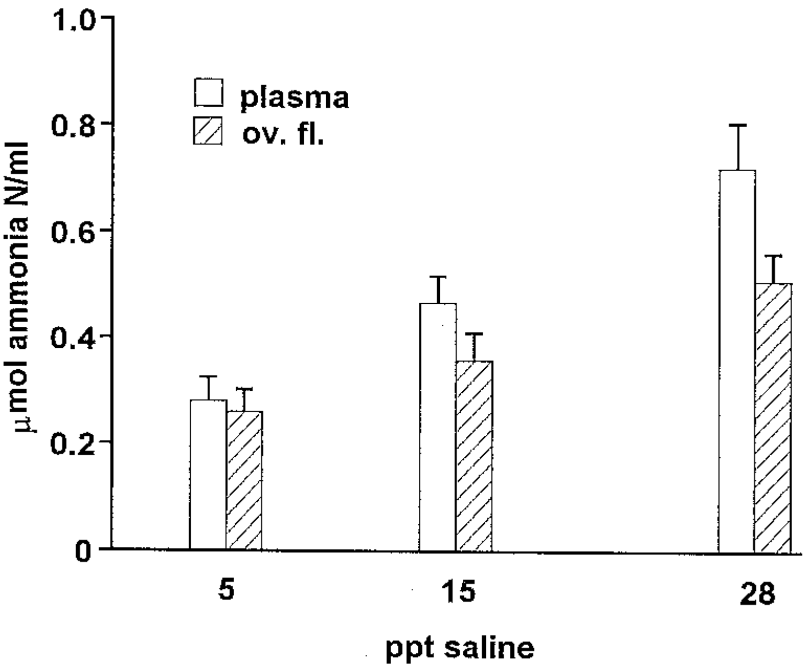

Fig. 3. The concentration of ammonia in maternal plasma and ovarian fluid at three different salinities after 24 h.

The concentrations of ammonia were increased in the plasma as well as in the ovarian fluid by increased salinities (Fig. 3). The results from the present study indicate that urea is not an osmoregulatory component in the maternal-fetal relationship. Time-course studies, however, are needed to elucidate the role of urea and ammonia in relation to increased salinities.

Figure 4 depicts a steady decrease in urea in the ovarian fluid during the 24 h time-course after an intraovarian loading and a concomitant increase in the plasma level. A similar pattern occurred by loading with ammonia.

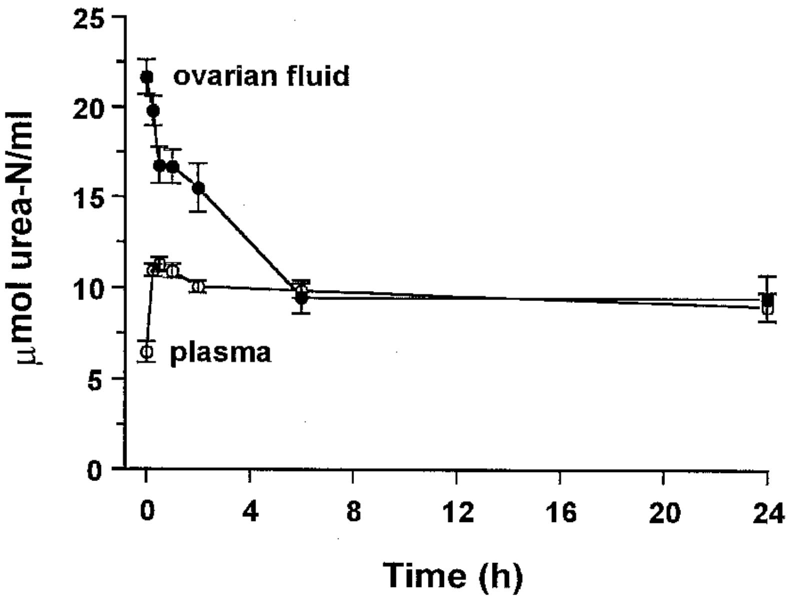

Fig. 4. Time-course levels of urea in the maternal plasma and ovarian fluid after an intraovarian loading with urea at 0 h.

The results indicate a steady flow of nitrogenous compounds from the pregnant ovary into the maternal circulation, a system which may protect the embryos from being exposed to high levels of the nitrogenous end products under the natural crowded conditions in the pregnant ovary (Korsgaard *et al.* 1995).

In the experiment elucidating the effect of the estrogenic compound octylphenol no significant changes could be observed in the concentration of chloride in the ovarian fluid or of ammonia and urea in either of the exposed groups when compared to the controls. In this experiment the concentration of calcium was measured, as calcium is a crucial component for the synthesis of the yolk-precursor protein vitellogenin, which is induced by xenoestrogenic compounds. That vitellogenin was synthesized by the motherfish as a result of the estrogenic exposure by octylphenol and estradiol was confirmed by significantly increased levels of circulating vitellogenin measured by ELISA. Interestingly a significant effect of the exposure could be observed in the concentration of total calcium in both plasma and ovarian fluid as shown in Table 1.

Table 1. The effect of exposure to xenoestrogens on metabolites in plasma and ovarian fluid of pregnant eelpout.

	Urea mol N/ml	Calcium mg/100ml	Glucose mg/100ml	NPS mg/100ml
Plasma				
Control	4.65 (0.36)	11.03 (0.50)	26.66 (1.56)	15.5 (0.60)
OP25	5.28 (0.69)	27.43 (3.06)**	26.45(1.34)	13.6 (0.10)
OP100	3.26 (0.88)	61.44 (1.35)**	13.46 (3.13)*	9.4 (0.55)**
Estradiol	5.08 (0.25)	52.65 (6.48)**	28.77 (4.51)	7.3 (0.11)**
Ov. Fluid				
Control	5.18(0.45)	7.7(0.3)	7.0(0.9)	0.08(0.007)
OP25	5.41(0.33)	5.5(0.8)	9.3(0.7)	0.07(0.005)
OP100	4.27(0.36)	2.3(0.28)**	10.4(1.3)	0.29(0.18)
Estradiol	6.46(0.33)	1.8(0.35)**	10.4(1.5)	0.11(0.02)

Mean ± SEM * P<0.001 compared to controls **P<0.0001 N=7-8

The concentration of calcium in the ovarian fluid decreased in a dose-dependent manner in the octylphenol exposed groups and was also observed to be significantly decreased in the ovarian fluid of the pregnant fish exposed to estradiol, when compared to the control level. In contrast the corresponding level of calcium in the plasma showed a marked increase in all treated groups, most likely due to the increase in vitellogenin-bound calcium. The observed decrease in the level of calcium in the ovarian fluid as well as the decreased level of amino acids (NPS) in the maternal plasma may indicate that these compunds are mobilized in increasing amounts for the hepatic synthesis of vitellogenin in the exposed fish. This may occur at the cost of the normal embryonic development. Thus a negative impact on the calcification process was indicated by deformities in the embryonic skeleton observed in the embryos from motherfish exposed to the estrogenic compound.

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