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Transfer of Material Immunity and Development of Immune Response in Some Fishes

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Transfer of maternal immunity and development of the immune response in some fishes

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B. BRIEF DESCRIPTION OF RESEARCH RESULTS

Bar-Ilan University

First year

1. Purification of tilapia immunoglobulin (TIg) by gradient centrifugation.
2. Immunization of rabbits and mice for the preparation of specific anti TIg antisera.
3. Set up of immunological tests (IEA and immunofluorescence) for direct detection of TIg in serum, in eggs and embryo extracts.
4. Processing of tilapia eggs and embryos for preparation of TIg containing extracts using propanediol and ammonium sulfate precipitations.
5. Set up of ELISA test for the quantification of TIg in serum and in extracts.

Second year

6. Production of monoclonal anti TIg antibodies and performance of ELISA and immunofluorescence tests.
7. Preparation of embryo extracts SE (PBS soluble extract) and YE (yolk extract).
8. preparation of tilapia-serum propanediol precipitate (SPP).
9. Cross antigenicity tests of the embryo extracts SE and YE using immunoprecipitation (IEA and IECA)..
10. Immunoprecipitation tests for detection of TIg in SE and YE in comparison to SPP and TTS.
11. Immunization of mothers against well defined protein antigens, such as porcine serum albumin (PSA) dog serum albumin (DSA) and bovine gamma globulin (BGG).
12. Passive hemagglutination tests for titration of antibody activity in SE and YE as compared with SPP and TTS.

Third Year

13. Qualibration of the ELISA technique for quantification of TIg.
14. Determination of standard ELISA curve for titration of antibody activity.
15. Relationship between the degree of mother immunization and level of transferred immunity.
16. Determination of TIg level in early developing embryos at different intervals of time following mother immunization against PSA.
17. Determination of antibody activity in early developing embryos at different intervals of time following mother immunization against PSA, DSA or BGG and performing cross specificity tests.
18. Follow-up of Ig level and specific antibody activity in embryos during ontogenesis, and in fries.
19. Immunization of mothers against selected pathogens, antibody titration and challenge for embryo resistance (not completed)..

Auburn University

First year

1. Creation of additional facilities for holding and breeding fish.
2. Purification of channel catfish Ig by gradient centrifugation.
3. Immunization of rabbits against channel catfish Ig for the preparation of specific antibodies, tested using IEA and double diffusion in agar.

Second year

5. Immunization of channel catfish females against bovine serum albumin (BSA).
6. Collection of eggs from immunized females.
7. Preparation of egg extracts by propanediol precipitation.
8. Detection of immunoglobulin in the egg extracts using IEA and in histological frozen sections using immunofluorescence staining.

Third Year

9. Preparation of mouse and rabbit antisera against purified channel catfish Ig.
10. Radioimmunoassay, Qualibration the technique for quantification of channel catfish Ig and for antibody titration and determination of standard curves for quantification and titration of channel catfish Ig and antibody activity.
11. Immunization of mothers against a channel catfish virus (CCV) envelop vaccine.
12. Acquired resistance to fingerlings following bath immunization with CCV vaccine.
13. Challenge of embryos from mothers immunized with CCV not completed).
14. Relationship between the degree of mother immunization and level of transferred immunity (To be continued during next spawning season).

List of abbreviations:

BGG, bovine gamma globulin; BSA, bovine serum albumin; CC, channel catfish; CCV, channel catfish virus; CCIg, channel catfish Ig; CPL, cathodic precipitin line; DSA, dog serum albumin; ELISA, enzyme linked immunosorbent assay; Ig, Immunoglobulin; IEA, immunoelectrophoretic analysis; IECA, immunoelectrophoretic cross analysis; IIF, indirect immunofluorescence; IPH, Indirect passive hemagglutination; mAb, monoclonal antibody; PBS, phosphate buffered saline; PH, passive hemagglutination; PSA, porcine serum albumin; Pen, penicilloyl group; RaTIg, rabbit anti TIg; RaCCIg, rabbit anti channel catfish Ig; RaCCS, rabbit anti channel catfish serum; RIA, radioimmunoassay; SaTTS, sheep anti TTS; SE, water soluble extract; SG, sucrose gradient; SPP, serum propanediol precipitate; SRBC, sheep red blood cells; TIg, tilapia immunoglobulin; TS, total serum; TTS, total tilapia serum; YE, yolk extract.

C. EVALUATION OF RESEARCH ACHIEVEMENTS

Bar-Ilan University and Auburn University.

First year

During the first year we concentrated our efforts to develop reagents and methodology needed for the study of the problem of transfer of maternal immunity in tilapias and in catfish and for setting up additional facilities for maintaining and breeding these fishes. We have also made some efforts to provide direct evidence for the occurrence of immunoglobulins in the egg and early embryos of tilapias (Bar-Ilan University) and channel catfish (Auburn University).

Second year

Tilapia

Tilapia immunoglobulins (TIg) purified from TTS by a simple two stages technique (AS precipitation and sucrose gradient centrifugation, SG), gave a single cathodic precipitin line (CPL). Similarly single CPLs were found in the embryo extracts YE (obtained by precipitation with propanediol) and SE (PBS soluble).

A new technique IECA (immunoelectrophoretic cross analysis), that was developed to test antigenic identity of extracts with TIg, showed cross antigenicity between CPLs of extracts. However no antigenic identity between extracts and TIg nor between them and any of the 3 CPLs of TTS. In contrast, significant anti PSA antibody activity, located at the low sucrose density peaks (27%), was found in YE mainly by using the indirect passive hemagglutination (IPH) test. Since, hapten specific rabbit-Ig was located at the same peak, it was suggested that an Ig monomeric fraction, or perhaps a light type of TIg (undetectable by immunoprecipitation but found specifically active by IPH test only) is transferred in small quantities, together with minute amounts of TIg, from mother to egg.

These results strongly indicate that an immune transfer of immunity do occur in tilapias.

Channel catfish

Two out of 20 catfish females, involved in the spawning program, were immunized against BSA (bovine serum albumin). Embryo extracts were prepared from the egg masses using the propanediol extraction technique.

These extracts showed no cross reactivity with catfish Ig (CIg) nor had detectable antibody activity. Similarly, frozen, paraffin and methyl metacrylate sections of fish eggs were found negative for CIg when stained by specific immunofluorescence.

Because, no evidence for the occurrence of maternal transfer of detectable quantities of immunoglobulins in channel catfish, an immunoradioassay test was considered in order to detect minute quantities of Ig or antibody activity in the extracts.

Third Year

Tilapia

Quantification of the transferred immunoglobulin from mother to embryo.

Immunoglobulin of determined specific activity could be detected and measured in extracts of eggs spawned by these fish using the ELISA technique.

The level of immunoglobulins was considerably higher (by 3 log scales) in SE of embryos collected from immunized mothers, than in the same fraction prepared from embryos of non-immunized females. The maximal level of TIg was observed on days 14-37 after mother immunization, followed by a decrease as the interval of time between mother immunization and spawning was higher.

The serum immunoglobulin level (log base 2 titer= 22-27) was higher than in SE by 10-12 Log scales (Tables 2 and 8).

Transfer of antibody activity.

Antibody activity, specific to the antigens (PSA, DSA and BGG) which served for immunization of mothers, could be measured in extracts of eggs spawned by these fish using the ELISA technique.

The level of antibody activity in eggs varied according to the interval of time between mother immunization and spawning. This activity reached its maximum levels on days 14-37 after secondary antigenic stimulation then it undergoes a significant gradual decrease (Tables 3,4 and 5). The specificity of the extract activity was tested by cross adsorptions and could be removed by the specific antigens only (Table 6)

Decrease in maternal Ig and antibody activity during embryonic development

Both transferred Ig and antibody activity were found to decreased gradually with the embryo age. The highest levels of immunoglobulins and antibody activity in egg extracts were found at day 0. A gradual decrease in antibody activity, up to the embryo age of 14 days when the yolk sac reached its complete absorption, was observed. However, the decrease in the curve of Ig was considerably slower (Tables 7-8).

Protection of channel catfish-eggs exposed to CCV

Significant protection of channel catfish fingerlings was obtained following exposure of Eggs or fingerlings to a crude CCV (channel catfish virus) envelope vaccine. Some few mothers have been immunized with this vaccine. The specific transfer of immunity to eggs and offspring would be checked during next spawning season (April 1989).

D. DESCRIPTION AND RESULTS OF COOPERATION

First year. (During the first year the necessary facilities, reagents and techniques were developed)

Purification of Tilapia and Channel catfish immunoglobulins.

TIg and CIg were purified from the total serum (TS) by a two stage method: (1) precipitation from hyperimmune TS in 37% ammonium sulfate (AS) and (2) sucrose gradient centrifugation as detailed in Mor and Avtalion, 1988.

Analysis of purity of fractionated Immunoglobulins.

Poly acrylamide gel electrophoresis. PAGE, used to monitor the Ig purity was carried out in 6 and 7% gel as previously reported (Avtalion et al., 1971; Avtalion et al., 1984).

Immunization and tests for detecting antibody activity.

Preparation of Tilapia and Catfish antibodies. Tilapia and catfish were immunized against different albumin antigens (1mg in emulsion with complete Freund's adjuvant injected 3 times, every two weeks).

Preparation of anti-tilapia and anti catfish antibodies. The following antibodies were prepared by hyperimmunization of rabbits, mice and sheep:

- a. Sheep anti-total Tilapia serum (SaTTS).
- b. Mouse anti-Tilapia Ig (MaTIg).
- c. Rabbit anti-Tilapia Ig (RaTIg).
- d. Rabbit anti total channel catfish serum (RaCCS).
- e. Rabbit anti CC Ig (RaCCIg).

Passive hemagglutination (PH) . The micro passive hemagglutination test was used for titration of antibodies in embryo extracts and to follow this activity in the sucrose gradient fraction. A relatively high titer ($\text{Log}_2, 15$) was obtained in the initial serum. The whole antibody activity ($\text{Log}_2, 13$) was concentrated in the first IgM peak. For passive hemagglutination (PH) we used the glutaraldehyde coupling technique (Avrameas et al., 1969) to coat sheep red blood cells (SRBC) with the antigens. Double dilutions of the tested fractions in 25 μl PBS (PBS contains 10 mg/100 ml, W/V, rabbit serum albumin, RSA) were prepared in U-bottom microhemagglutination plates (polystyrene, A/S Nunc, Denmark). 25 μl of 0.5% PSA-SRBC were added and the plates were incubated for 1 hr at 37°C. The hemagglutination titer was then read.

Indirect passive hemagglutination (IPH). After reading the PH titers, microplates were centrifuged and supernatants were removed from the wells. Then RBC were resuspended after the addition of 25 ul of sheep anti TIg, diluted 1/40 in PBS-RA, were added to each well. Microplates were incubated for 1 hr. at 37 C.

Immuno-electrophoretic analysis (IEA). IEA was carried out on microscope slides as previously reported (Avtalion et al., 1971), and sheep anti-TTS hyperimmune serum (SaTTS), unless otherwise specified, was added to the trough.

Immuno-electrophoretic cross analysis (IECA). IECA is a modified IEA technique which was developed in this work. In this technique, a small trough of 6-12 mm, perpendicular to the main groove, is added at one of the poles of the immuno-electropherogram. This trough was filled with the antigenic fraction to be analyzed immediately after electrophoresis and addition of antiserum to the main groove. The location of the trough at the cathodic or anodic poles is determined depending on the electrophoretic mobility of the precipitin line of the antigen to be identified in order to permit its direct cross reaction with the corresponding fraction in an heterogeneous antigenic solution (detailed in Avtalion and Mor, submitted).

Preparation of embryo extracts. Eggs were homogenized 1:4 (V/V) in PBS-tween (0.13 ml tween in 250 ml phosphate buffered saline 0.15 M pH 7.2) then centrifuged at 9750 g for 20 min. Three different layers were obtained (Figure 1): upper fatty layer (yolk), soluble fraction (SE) and pellet. A protein fraction (YE) was extracted from the yolk layer by propanediol as it has been reported for the extraction of antibodies from hen egg yolk (Bade and Stegemann, 1984) as follows: Three volumes of propanediol (kept at -20 C) were added to 1 volume of the yolk fraction. After vigorous mixing the suspension was incubated at 4 C for 20 min, then centrifuged at 9750 g for 20 min and washed twice with the same volume of cooled propanediol. The precipitate was then solubilized in a volume of PBS (0.01M pH 7.5) equal to half of the initial volume of the yolk.

Second year.

Preparation of Hybridoma cell culture for monoclonal antibodies. Four ug of purified TIg, in emulsion with CFA, were intramuscularly (IM) injected to Balb/c mice. Fourteen and 24 days later, the mice received a booster dose of 4 mug in IFA and 10 days later, 3 or 4 days before being sacrificed, they were injected into the caudal vein with 10 ug of soluble TIg. Their spleen cells were used for the production of hybridoma cells following their fusion with a myeloma cell line polyethylen-glycol (Hybridoma cell lines were cultured for the production of monoclonal antibodies (mAb) as detailed in the literature.

Production of monoclonal antibodies (mAb) against TIg.

Nine different Tilapia-Ig specific hybridomas (364/4, 528/0, 364/0, 665/2, 364/2, 665/0, 665/1, 629/0 and 639/0) were produced. These hybridomas were cultured in the appropriate tissue culture medium different mAb were produced.

ELISA tests of anti TIg mAb.

Anti TIg monoclonal antibodies produced by culture expansion of these hybridomas were tested (1) against TIg using the ELISA test (2) for their ability to stain cytoplasmic Ig of pronephros cells, using the indirect immunofluorescence technique (IIF). ELISA tests showed that some of these MAB (364/4, 528/0 & 364/0) were positive to TIg of all species of tilapia tested where as the others were positive mainly to O.aureus and O.niloticus. (Table 1)

Immunofluorescence tests of anti TIg mAb.

IIF staining of pronephros cell of O.aureus x O. niloticus F1-hybrids showed that some of the MAB were negative whereas others could stain from 1 to 5% of the cells. Each MAB had its characteristic staining intensity (Table 1).

Immunofluorescence tests with polyclonal antibodies.

Tilapia. RaTIg and MaTIg were tested using the indirect immunofluorescence staining technique (IIF) for their ability to stain specifically Tilapia lymphocytes which were obtained from the lymphoid organs of the fish. Both antibodies showed high specificity for cytoplasmic staining of plasma cells and for cell surface staining of other lymphocytes (not shown).

Catfish. This technique was also used for the detection of immunoglobulins in channel catfish egg sections, obtained following deep freezing, and paraffin or methyl metacrylate embedding as detailed in Brinn and Piekett, This last method gave good details of egg sections but fluorescent staining specific to antibodies has not been successful.

Immuno-electrophoretic analysis (IEA).

Tilapia. IEA was carried out on microscopic slides and sheep anti total tilapia serum (TTS) was used to compare SE and YE with TTS. Results revealed the presence of a single cathodic precipitin line (CPL) in both SE and YE, in contrast to 3 CPLs found in TTS (Fig. 1 a&b).

Catfish. Egg masses from channel catfish mothers, immunized against BSA, were ground in a glass-on-glass tissue grinder and diluted 1:2 with phosphate buffered saline. Extraction of Immunoglobulin

followed the procedure of Bade and Stegemann. IEA results yielded no cross-reactive precipitin lines nor was specific antibody activity (anti BSA) detectable.

Immuno-electrophoretic cross analysis (IECA).

We first used this method to test cross antigenicity between TIg and TTS cathodic precipitin lines and to compare SE with YE, and to check for possible cross antigenicity of these extracts with TIg and TTS.

Results showed that:

- 1) Purified TIg cross react with the proximal (to antigen well) CPL of TTS only (Fig. 3a)
- 2) CPLs of YE and SE cross react one with the other assuming that antigenic identity exists between CPLs of SE and YE (Fig. 2a);
- 3) No antigenic identity exists between these extracts and TIg (Fig. 2b)
- 4) No cross reactivity could be found between YE or SE with the CPLs of TTS (Fig. 3b&c). However, because our sheep anti tilapia serum reacted positively with YE and SE, a component of similar antigenicity should exist in TTS (used for sheep immunization) in small amounts undetectable by immunoprecipitation.

These results lead to the conclusion that CPLs of embryo extracts are not antigenically identical with TIg. Therefore, it could be suggested that:

- 1) The above methods of extraction are not suitable for the isolation of TIg.
- 2) Another type of immunoglobulin, antigenically different from TIg, is transferred to eggs.
- 3) TIg is transferred to eggs, but in minute quantities not discernible with immunodiffusion.
- 4) TIg molecules are not transferred to embryos.

In order to test which of these suggestions is close to reality, we first checked if propanediol precipitate Tilapia Ig as it was shown for hen Ig (Bade and Stegemann, 1984).

Propanediol precipitation of TTS. One volume of TTS was added to 3 volumes of precooled (-20) propanediol, and the serum propanediol precipitate (SPP) of TTS was prepared as detailed above for YE extraction. SPP was tested using IECA in comparison to TIg and TTS.

Results showed that this precipitate includes 2 cathodic precipitin lines which cross react with the 2 proximal cathodic fractions of TTS but not with TIg nor with YE or SE (not shown).

Antibody activity of SPP. SPP was prepared from sera of tilapias immunized against PSA showed very low anti PSA PH titer (Log base 2 =2). However, using the indirect (Coombs-like) passive hemagglutination tests (IPH), the titer increased to 8 (Log2). The antibody activity revealed by each of the above tests, could be separated by sucrose gradient showing that the passive hemagglutination activity was located in the high density peak whereas the IPH activity was located in the light density peak (not shown).

This result might suggest the occurrence of a light type of Ig in tilapias.

IEA of TIg and of SE and YE. The immuno-electrophoretic analysis of the PBS soluble extract, the yolk extract and the total tilapia serum, performed with sheep anti-TTS, were compared. Results revealed the presence of a single cathodic precipitin line in each of the extracts. In contrast, three lines were found in TTS (Figs. 1a&b, and 3a).

Comparison of the precipitin lines of TIg and of SE and YE with those of TTS using IECA.

IECA of the soluble and yolk extracts showed that their precipitin lines cross react with one another (Fig. 32a). However, no cross antigenicity could be found between tilapia immunoglobulin and either the soluble or the yolk extracts (Fig. 2b). On the other hand, one (proximal to the antigen well) of the three cathodic lines of the total serum showed cross antigenicity with TIg (Fig. 3a), but none of them showed antigenic similarity with cathodic precipitin line of the yolk and soluble extracts (Figs. 3b and c).

Antibody activity in sucrose gradient fractions of TTS. Total serum taken from mothers immunized against PSA showed high direct and indirect passive hemagglutination anti-PSA activities (Log base 2=8 and 10, respectively), mainly at the 37% sucrose gradient peak (TIg). The anti-penicilloyl activity, due to specific rabbit IgG added to the total serum, was located at the 27% sucrose peak together with lower anti-PSA activity. A significant anti-PSA activity (log base 2, titer=5 and 7 for direct and indirect hemagglutination, respectively) was found at the 5% sucrose density peak (Fig. 4).

Antibody activity in sucrose gradient fractions of SE and YE. The antibody activity of yolk extract was mainly localized at the protein peak which sediment at the 27% sucrose area of the gradient. This activity (log base 2= 5) could be detected by indirect hemagglutination only (Fig. 6a). Similarly, indirect hemagglutination activity located in the same area of the gradient (Log base 2 = 4) was found in the soluble extract (Fig. 6b). However, a low direct hemagglutination antibody activity (Log base 2 = 3) was shown in this extract at the 37% sucrose fraction which corresponded to TIg. As in the total serum, the lightest fraction of the gradient (5% sucrose) showed specific activity (Log base 2 =2 and 3 for direct and indirect hemagglutination, respectively). It is noteworthy that extracts of eggs or embryos from non-immune mothers were negative for PSA (Fig. 5).

Third Year

Tilapia

ELISA for the study of the maternal transfer of immunity.

Immunoglobulins in embryo extracts. SE of embryos (up to 4 days old), collected from mothers immunized with PSA, showed relatively higher levels of immunoglobulins (13, Log 2) in comparison to 10 (Log 2) found in the same fractions prepared from embryos of non-immunized mothers. Maximal levels of Ig (13, Log2) were observed on days 14- 37 after mother immunization, and a decrease to 10 (Log2) was shown on days 47-51 (Table 2).

YE fractions, prepared either from eggs of by immunized or non-immunized mothers, contained lower Ig levels (3, Log 10) in comparison to those of SE (4-5, Log 10). Serum immunoglobulin levels were found higher (6-7, Log 10) than those which were detected in eggs extracts (fig 6).

Antibody activity in SE and YE. The ELISA technique was used for titration of specific antibody activity in the embryo extracts SE and YE. This extracts were prepared from less than 4 days old embryos of mothers separately immunized mothers against the protein antigens PSA, DSA and BGG (Tables 3, 4 and 5).

The level of antibody activity in the egg varied according to the interval of time between mother immunization and spawning. This activity could be detected only from the 10th day after secondary immunization of mothers, and maximal levels were obtained on days 14 to 37 in SE (11-15, Log2) and on day 17 in YE (6, Log2,). These antibody titers decreased gradually to 3 (log2) at days 69 and 37 in SE and YE, respectively (Tables 3 and 4). Similarly, maximal anti BGG antibody titers (9-11, Log 2) were found in SE on days 15-36 following mothers secondary immunization against this antigen (Table 5).

In all cases antibody titers in the sera of the mothers were found to be higher in comparison to those detected in embryo extracts. An other difference between serum and extract was reflected by the profile of the ELISA curve. As a result of dilutions, a sharp slope of the antibody activity curve was obtained with SE, in contrast to a moderate decline of the curve obtained when the mother serum was tested for specific antibody activity. It is noteworthy that the profile of the SE curve is similar to profile of the curve obtained using monoclonal antibodies (Figs.7 a&b).

The antibody activity in embryo extracts was found to be specific to the antigen used for mother immunization; e.g. negative results were observed in extracts prepared from offspring of mothers immunized with PSA and tested against DSA or vice versa. Furthermore, following solid phase adsorptions, the antibody titers of sera of the 2 mothers immunized against PSA or DSA (log2, 27, for both antigens) decreased by 7 log scales each. Similarly the antibody titers of SEs

extracted from offspring of these mothers (Log2, 7 for PSA and 10 for DSA) decreased in their antibody activity by 4 and 3 log scales, respectively following solid phase adsorption (Table 6).

Decrease of antibody activity and TIg with embryo age. Both antibody activity and Ig level were found to decreased gradually with the embryo age. The highest levels of immunoglobulins and antibody activity in egg extracts were found at day 0. A gradual decrease in antibody activity, up to the embryo age of 14 days when the yolk sac reached its complete absorption, was observed. However, the decrease in the curve of Ig was considerably slower (Tables 7 and 8).

Channel Catfish

RIA (radioimmunoassay) for the quantification of CCIg and antibody. Qualibration of the technique was carried out for Channel catfish immunoglobulin and antibody activity titration in serum and embryo extracts. Standard curves were determined. Preliminary work done with egg extracts seems to be promising. (this part needs to be completed).

Protection of channel catfish-eggs exposed to CCV. Significant protection of channel catfish fingerlings was obtained following exposure of eggs and fingerlings to a crude CCV (channel catfish virus) envelope vaccine. Some few mothers have been immunized with this vaccine. The specific transfer of immunity to eggs and offspring would be checked during next spawning season (April 1989).

E. PUBLICATIONS (BARD ECKNOWLEDGED)

1. A. Mor and R.R. Avtalion, (1988) Evidence for transfer of immunoglobulin from mother to eggs in tilapias. Israel J. Aquacult. 40, 22-28.
2. J. Don, M. Koiller, O. Yeheskel, K.E. Nussbaum and R.R. Avtalion. (1987). Increased Tilapia embryo viability using ultraviolet irradiation in a closed circulating Zuger-bottle system. Aquacult. Engin. 6, 69-74.
3. R.R. Avtalion and A. Mor (198). Immuno-electrophoretic cross analysis (IECA) - A method for cross analysis of individual antigens in multi component antigenic systems, (submitted).
4. A. Mor and R.R. Avtalion (198). Transfer of antibody activity from immunized mother to eggs in tilapias (in preparation).
5. R.R. Avtalion and A. Mor (198) Quantification of the immunoglobulin transfer from mother to eggs in tilapias (in preparation).

6. A.Mor and R.R. Avtalion (198) Transfer of immunoglobulin and specific antibody activity from immunized mothers to eggs in tilapias (in preparation).

Relevant Literature

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Table 1. Antibody activity (ELISA and immunofluorescence) and species specificities of anti Tilapia Ig MAB.

Hybridoma number	Ab. Titer* (Log base 10)					Immunofluorescence**	
	A	N	M	G	Z	% positive cells	staining strength
364/4	4	4	4	4	4	5	++
528/0	2	2	2	2	2	1.5	++
364/0	3	3	3	3	3	-	±
665/2	3	3	1	2	2	-	-
364/2	4	4	1	2	2	-	-
665/0	3	3	0	1	1	-	-
665/1	2	2	0	1	1	-	-
629/0	3	3	0	1	1	-	-
639/0	4	4	0	1	1	2.5	++

*, Tested with pronephros cells of *O. aureus* x *O. niloticus* F1-hybrids. **, Determined by ELISA in microplates coated with total serum of *O. aureus* (A), *O. niloticus* (N), *O. mossambicus* (M), *S. galilaeus* (G) or *T. zillii* (Z).

Table 2. Occurrence of TIg in SE extracts at different intervals of time following mother immunization against PSA.

Fish No	Days after mother immunization	Embryo age (days)	Ig titer (Log12)	
			In embryo (SE)	In mother serum
72	0	3	10	nd
34	9	3	10	nd
52	14	4	13	22
52	17	3	13	nd
34	30	1	13	23
52	37	4	13	27
27	47	3	10	nd
52	51	3	10	nd

nd, not done; h, hours; d, days.

Table 3. Antibody activity to PSA in SE at different intervals of time following secondary stimulation of mother.

Female No.	Days after mother immunization	Embryo age (days)	Antibody titer (Log12)	
			In embryo (SE)	In mother serum
72	0	3	0	nd
34	10	9	0	13
48	11	8	4	230a
52	14	1	11	27
110	28	0	14	23
48	29	0	15	27
10	35	1	13	22
52	37	4	11	27
56	45	1	6	nd
56	69	3	3	nd
52	82	1	3	nd

a, blood for serum preparation was wth19 days following mother immunization.

Table 4. Anti PSA antibody activity in YE at different intervals of time following secondary stimulation of mothers.

Fish No	Days after mother immunization	Embryo age (days)	Antibody titer (Log12)	
			In embryo (YE)	In mother serum
72	0	3	0	nd
52	14	4	0	23
25	17	1	6	nd
52	37	4	3	16
52	68	1	3	nd
56	82	3	3	nd

nd, not done.

Table 5. Antibody activity to BGG in SE at different intervals of time following secondary stimulation of mother.

Fish No	Days after mother immunization	Embryo age (days)	Antibody titer (LogS12)	
			In embryo (SE)	In mother serum
11	11	2	8	23
16	15	1	9-10	23
23	15	1	10-11	20
23	36	2	9-10	23
16	40	3	6	20

Table 6. Antibody titers (Log12) in serum and egg extracts before and after adsorption with specific and non specific antigen

Antisera	Female 38		Female 52	
	SE anti DSA	Serum anti DSA	SE anti PSA	Serum anti]
Non adsorbed	10	27	7	27
Adsorbed with PSA	10	27	3	20
Adsorbed with DSA	7	20	7	27

Female 38 was immunized against DSA and female 52 against PSA.

Table 7. Antibody activity to PSA and BGG in SE extracts of sibling embryos of different ages.

	Female No	Days after mother immunization	Embryo age (days)	Ab titer (Log12)	
				In embryo (SE)	In mother serum
PSA	110	28	0	14	23
			5	13-14	
			14	8	
	48	29	0	15	27
			8	15	
			11	11-12	
	10	35	1	13	22
			5	12	
			12	10	
BGG	23	15	1	10-11	20
			3	10-11	
			7	9	
			12	0	
	16	40	3	6	20
			5	6	
			8	<60a	
			12	0	
			15	0	

a, minimal value of sample dilution was 20-6.

Table 8. Immunoglobulin levels in SEs of sibling embryos of different ages, spawned by different mothers immunized against PSA..

Female No	Days after mother immunization	Embryo age (days)	<u>Ig titer (Log12)</u>	
			In embryo (SE)	In mother serum
110	28	0	14	23
		5	13-14	
		14	8	
48	29	0	15	27
		8	15	
		11	11-12	
10	35	1	13	22
		5	12	
		12	10

Fig. 1. IEA of YE (a) and SE (b) extracts as compared with total tilapia serum (TTS). In the groove, sheep anti TTS (SaTTS).

Fig. 2. IECA of SE with YE (a) or of both SE & YE with TIG (b). SaTTS was added to the main groove.

Fig. 3. IECA of TIG (a), SE (b) and YE (c) with TTS. SaTTS was added to the main groove.

Fig. 4. Sucrose gradient (linear, 5-42%) separation of TTS supplemented with rabbit anti pen IgG. Columns show rabbit-IgG anti pen titers ($\square$) and anti PSA activity as detected by PH ($\square$) or by IPH ($\blacksquare$) tests.

Fig. 5. Sucrose gradient (linear, 5-42%) separation of YE (a) and SE (b) and their anti PSA titers (columns), as detected by PH ($\square$) or by IPH ($\blacksquare$) tests.

Fig.6. Determination of the immunoglobulin level in TTS (●), SE (○) and YE (◇) at different intervals of time (a, 17 days; b, 14 days; c, 37 days) after mother immunization.

Fig.7. Anti PSA activity profiles of immune TTS (◇) and SE (●) taken from mothers on 44 after their immunization with PSA, in comparison to the profile obtained with mouse monoclonal antibody against TIg (○).