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Apoptosis phenomenon of gonadal cells during sex-reversal in the bony fish *Rhabdosargus haffara.*

Amal M. Ramadan
National Institute of Oceanography and Fisheries,
Red Sea and Suez & Aqaba Gulfs Branch
P. O. Box 182, Suez, Egypt
amalr66@yahoo.com

ABSTRACT

The purpose of this study was to demonstrate the normal occurrence of programmed cell death (apoptosis) in the gonads (testes and ovaries) of the fish *Rhabdosargus haffara* during sex-reversal process. Histological evidence marked apoptosis in function male during transformation of gonads from male to female, where there was decrease in size of testis with condensed spermatogonia and closed spermatic ducts with residual sperm. On the other hand, oocyte apoptosis in *R. haffara* occurs in three common stages, which start at the wall of oocytes. The first stage includes shrinkage of oocyte size. Theca and granulosa of apoptotic oocyte transformed to phagocytic cells. In the second type protrusions appear in the oocyte wall until the oocyte looses its identity and displayed amoeboid shape with liquefied content. The third stage depends on the breaking down of oocytes wall into small fragments. Finally, all types of apoptosis lead to lysis and loss of oocyte content. The importance of this phenomenon with sex-reversal may lead to social interactions and the natural balance in the *R. haffara* population.

Key words: apoptosis, sex - reversal, *Rhabdosargus haffara*, gonadal cells

INTRODUCTION

Hermaphroditism is expressed in 15 % of teleost families (Lau and Sadovy, 2001). The term apoptosis was illustrated as programmed cell death during sex-reversal or sex differentiation of fish (Uchida *et al.*, 2002).

Apoptosis is a form of cell death that plays a major role during the normal development and homeostasis of multicellular organisms (Sinha Hikim and Swerdloff, 1999). Germ cell apoptosis occurs during spermatogenesis of various mammalian species, and plays an important role in determining sperm output (Sharpe, 1994). In mammalian spermatogenesis, apoptosis occurs mainly at the level of spermatogonia (Dunkel *et al.*, 1997; Rodriguez *et al.*, 1997) or spermatocytes (Young *et al.*, 1999; Strbenc *et al.*, 2003). Proliferation and apoptosis of male germ cells have been observed in different seasonally bred mammals (Blottner *et al.*, 1995; Young and Nelson, 2001; Strbenc *et al.*, 2003).

Scott *et al.* (1980), Billard *et al.* (1982), Kime and Manning (1982), Borg (1994) and Weltzien *et al.* (2002) studied the role of apoptosis in the regulation of reproduction of teleosts. Few investigations have been carried out on cartilaginous fishes such as the shark *Squalus achantias* L., where apoptosis has been documented at the level of spermatogonia and preleptotene spermatocytes (Callard *et al.*, 1995).

Rhabdosargus haffara is one of the most abundant fish species in Suez Bay. Previous publications have described the gonads of these fish as having two distinct forms in the beginning of their life; a) protandrous hermaphroditism in the majority and b) ovaries only do not have any hermaphroditic structure (EL-Halfawy, 2001). Fish oocytes generally have three distinct layers, namely, an outermost follicular layer, a median zona radiata (ZR) and an inner oolemma or oocyte plasma membrane. The follicular layer consists of an outer theca and an inner granulosa layer.

Apoptosis phenomenon in this fish has not so far been studied during the gonad transformation, from testis to ovary or from ovary to testis. The present study is therefore, focused on this phenomenon and its role in sex-reversal.

MATERIALS AND METHODS

Specimens of the fish *R. haffara* were collected from commercial catch at Ataqa harbor Suez Bay. Fish were dissected and gonads were taken out to be fixed in Bouin's solution. The fixed gonad samples were dehydrated in ethanol and embedded in paraffin wax. The gonads were sectioned at 7 µm in thickness. The sections were de-paraffinized in xylene and stained with haematoxylin - eosin and Milligan trichrome stains . The stained sections were examined under Nikon microscope. Photomicrographs were taken to illustrate apoptosis.

RESULTS

The present study showed that *Rhabdosargus hafara* starts its life as female only or hermaphrodite male. After the first spawning season, groups of the functional male are transformed into functional female. During this process, apoptosis helps in the transformation steps as follows:

A- Apoptosis in the normal testis cells:-

The transformation of functional male to female (in this case the testes in spent stage and the ovaries cells are arrested on perinucleolus stage). At the beginning of this process, ovarian size increases and testicular size decreases. The spermatogonia, which line the wall of spermatic lobules in addition to the residuals of spermatozoa are scattered in the gonadal stroma (Fig. 1). Then, the lobule lumens are closed and the spermatic duct appears coalesced (Fig. 2). Finally, the testicular lobe appears reduced in size and attached to the active ovary wall (Fig. 3).

B- Apoptosis in the normal ovaries:-

Ovaries of *R. haffara* demonstrated apoptosis of oocytes in females at spent stage or at the onset of ovary transformation, where fish behaviour is function female with rudimentary testes. Histological investigation of ovaries showed the manner of transformation from ovary to function male; it is correlated with inactive ovary, which is reduced in size where the oocytes start the mechanisms of death in major three types and the testis began to be active as follows:

I- First type:-

The apoptotic signs in this type are:-

I a- The oocyte wall becomes more thickened than the normal one (Fig. 4).

I b- The oocyte wall thickens more and more and the oocyte size is reduced (Fig. 5).

I c- The oocyte looses its identity and it's wall is converted into phagocytic cells (Fig. 6).

II- Second type:-

The apoptotic signs are as follow:-

II a- The oocyte wall convoluted (Fig. 7).

II b-The oocyte contents (yolk, cytoplasm and nucleus) liquefied and .become irregular forming an amoeboid shape (Fig. 8).

III- The third type:-

III a- The oocyte wall became slightly thickened than normal and the oocyte contents liquefied. The follicular layer consists of an outer theca and an inner granulosa layer, where they display destruction (Fig. 9).

III b- The follicular layer fragments increase in number and loose their identity (Fig. 10).

Finally, the apoptotic oocytes lysed and lost their ooplasm contents in the three mentioned types (Figure 11).

DISCUSSION

Apoptosis induced by genetic sex determination, environmental factors such as heat, stress, low pH, salinity, nutrition and exogenous estrogen and androgen levels are known to influence the determination of phenotypic sex in fish (Shapiro, 1990; Korpelaninen, 1990; Kitano *et al.*, 1999). Kurita *et al.* (2003) stated that atresia is associated with variable energy resources and environmental conditions. This phenomenon is found in *Rhabdosargus haffara* during sex-reversal as shown in the present results in both male and female. So, apoptosis in *Rhabdosargus haffara* is correlated with hermaphroditism and sex reversal in testes and ovaries and these results agree with Wang *et al.* (2007) who mentioned that apoptosis occurred in sex differentiation and sex change in adult teleosts. The testes undergo a remarkable atrophy due both to reduction of cell

size and to cell death (Young and Nelson, 2001). Testis enters a drastic formation process, which initiates the conversion of such testis into ovary.

Corrieroa *et al.*(2007) stated that apoptosis occurs throughout the reproductive season although it's maximal is at the onset of spermatogenesis. Our results illustrated that apoptotic male is similar to spent stage. During sex reversal testes and spermatic lobules became reduced in size with scattered residuals of sperms, but the spermatic ducts of the testis is coalesced. These observations tempt us to suggest that spontaneous degeneration of spermatogenesis is demonstrated at all the developmental stages. These observations on transformed male agree with Uchida *et al.* (2002); they mentioned that apoptotic spermatogonia were observed in sex differentiation. Apoptotic spermatogonia, spermatocytes and condensed cells were located in the same lobule beside large number of apoptotic spermatids. During differentiation, the gonads of male zebra fish go through a process similar to that of sequential hermaphrodite teleost that changes sex from female to male during adulthood (Frisch, 2004).

Subsequent histological analysis of another batch of ovaries clears that the wall of the oocyte plays an essential role in the apoptotic process and this result agrees with that obtained by Ramadan *et al.* (1987), who indicated that deformation of the wall of the oocyte was considered as the first step of atresia (rentention or apoptosis) and phagocytosis of oocytes. Our observations agree with the results obtained by Ramadan and EL-Halfawy (2007); they mentioned that about 30 % to 35 % of the ova became atretic in *Rhabdosargus haffara* as a natural phenomenon and there were two types: burasting atresia and non-burasting atresia.

Apoptosis is a form of cell death that plays a major role during development of multicellular organisms (Sinha Hikim and Swerdloff, 1999) These findings suggest that apoptosis takes place in *Rhabdosargus haffara* in three major types that include thickening of the oocyte, fragmentation or protrusion; and finally all the products lyse. So, apoptotic phenomenon may achieve a natural balance to the population and optimum sex-ratio which fish community needs it in the hermaphrodite fishes. Ramadan and EL-Halfawy (2007) mentioned that atresia (apoptosis) may be important as it affects the estimated spawning potential of the stock.

In conclusion our findings suggest that programmed cell death mechanisms occur in *Rhabdosargus haffara* during sex-reversal occurs as a normal phenomenon after first spawning season of this fish. The essential importance of this phenomenon in *Rhabdosargus haffara* may achieve natural balance to the fish population (social interaction).

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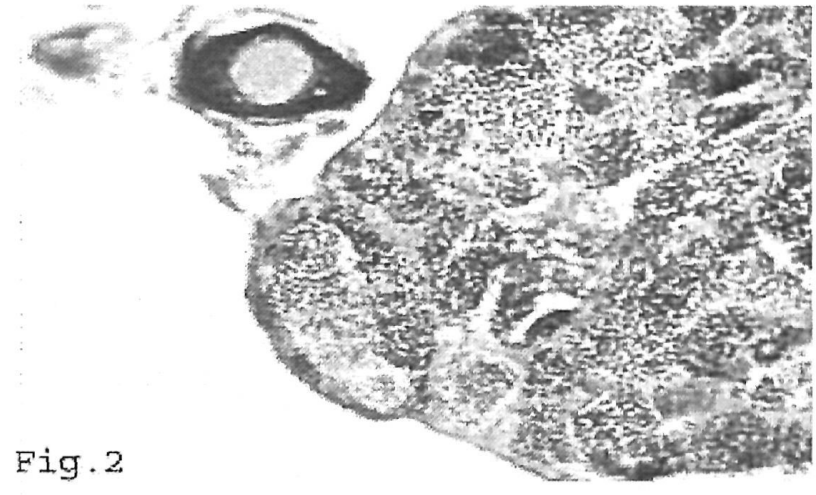
LIST OF FIGURES



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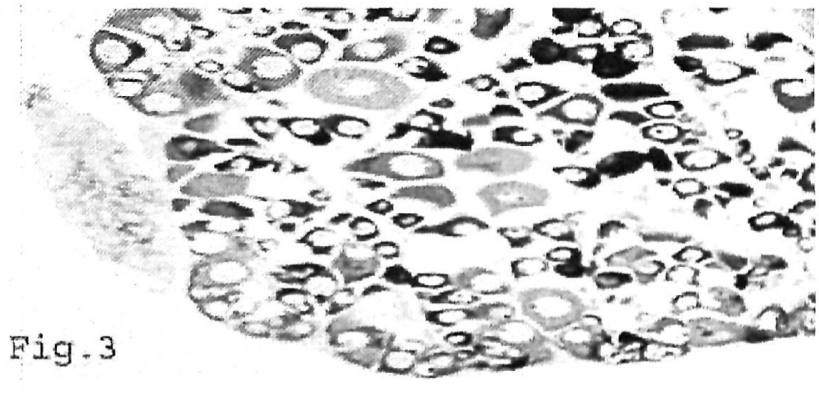

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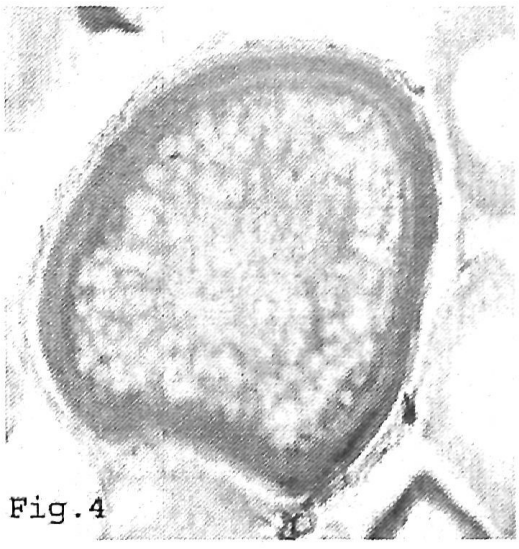

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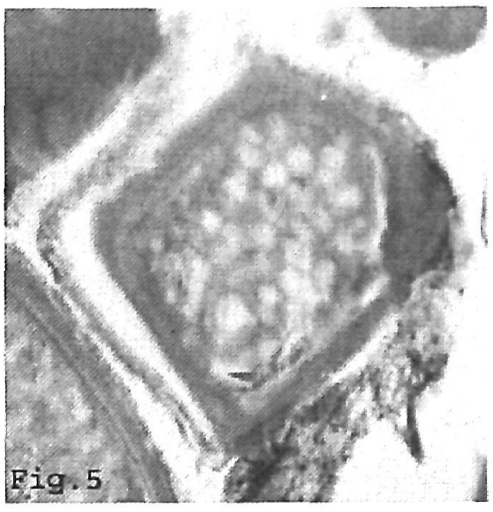

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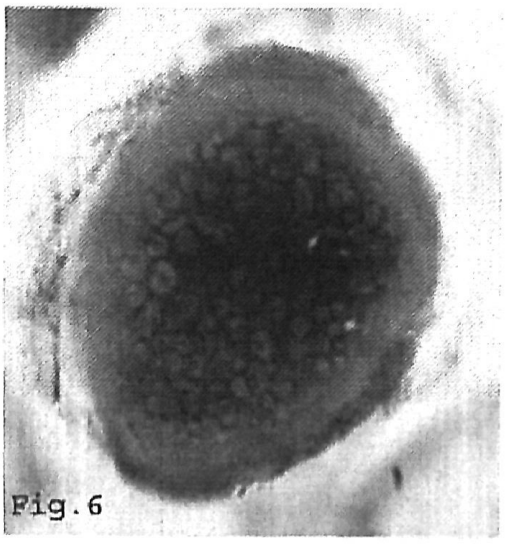

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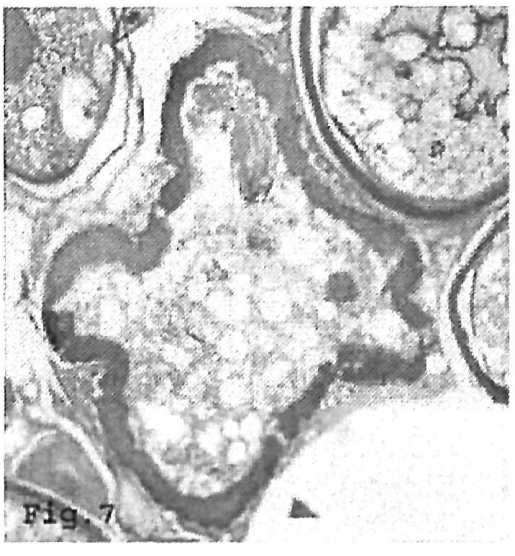

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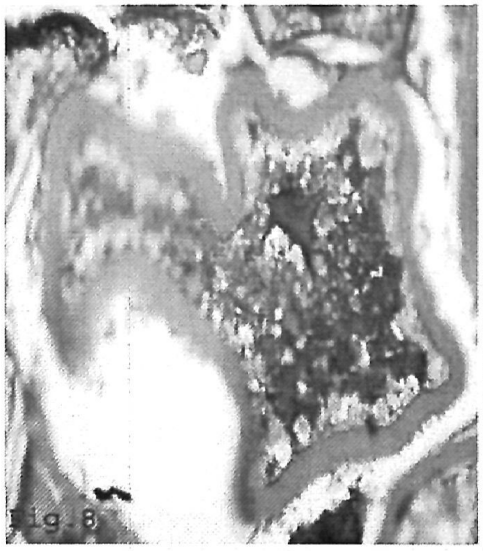

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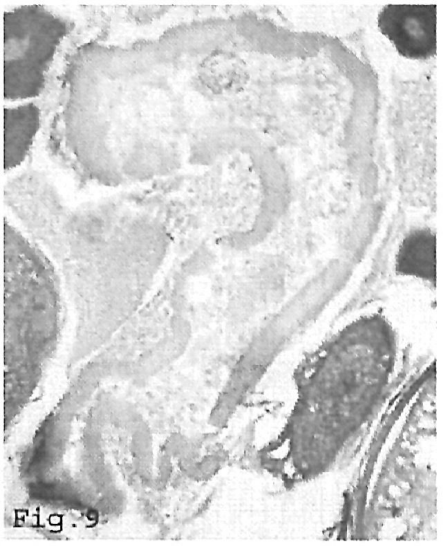

Fig.9

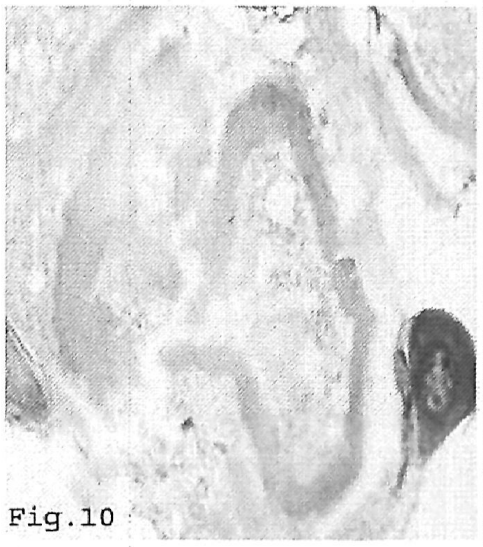

Fig.10

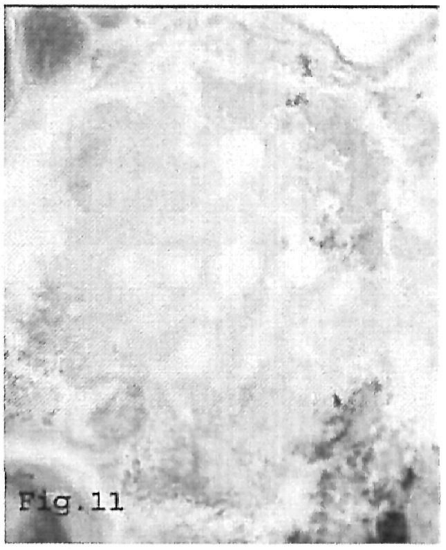

Fig.11