

THE ANDROGENIC GLAND IN CRUSTACEA - WITH EMPHASIS
ON THE CULTURED FRESHWATER PRAWN
MACROBRACHIUM ROSENBERGII - A REVIEW

ד"ר אמיר שגיא
המתקנה לפרוייקט החיים
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Introduction

The androgenic gland plays an important role in crustacean sex determination as well as in the regulation of primary and secondary sexual characteristics. The importance of this gland for aquaculture research lies in the fact that, in some crustaceans, males and females differ in their growth patterns. In *Macrobrachium rosenbergii*, the male growth rate is considerably higher than that of the female (Sagi et al., 1986). However, male growth rates vary greatly (Jimura and Okamoto, 1972; Smith et al., 1978; Brody et al., 1980; Malecha et al., 1984) due to the existence of different phenotypes within the prawn population (Ra'anan and Cohen, 1985; Sagi et al., 1987).

The objective of this review is to assemble literature describing the androgenic gland with a focus on studies of the nature and function of this endocrine gland, providing the general reader and the crustacean aquaculture researcher with an update on androgenic gland research as well as indications of its potential application in crustacean aquaculture.

The review

A. The androgenic gland - anatomy and histology

The androgenic gland (AG) was first described in the crab *Callinectes sapidus* by Cronin (1947), while the first insight into its function was provided by Charniaux-Cotton (1954) in the amphipod *Orchestia*. The glands are usually located at the subterminal portion of the sperm ducts. The cells may be arranged as thin, inconspicuous strands or in a compact, lobed structure (Kleinholz and Keller, 1979).

The ultrastructure of the AG of the crab *Pachygrapsus crassipes* resembles that of a vertebrate protein-producing cell rather than a steroid-producing cell (King, 1964), since it is characterized by a well-developed granular endoplasmic reticulum and abundant mitochondria. The cells contain numerous large multivesicular bodies. These bodies resemble lysosomes, however, acid phosphatase activity has not been observed. The proteinaceous nature of

the secretions was confirmed by Taketomi (1986) who reported the existence of two kinds of AG cells in *Procambarus clarki*, type A which resemble protein-secreting cells and type B which do not. As discussed below, Veith and Malecha (1983) have also observed more than one type of cell in the AG of *Macrobrachium rosenbergii*.

B. The role of the androgenic gland.

Bilateral AG ablation (andrectomy) in *Orchestia gammarella* blocks differentiation of secondary male characteristics and spermatogenesis decreases (Charniaux-Cotton, 1954). When a mature ovary was transplanted into an andrectomized male, it remained unmasculinized. However, when transplanted into an intact male, the ovary transformed into a testis (Charniaux-Cotton, 1955, 1957). Once the external male sexual characteristics are formed, in gonochoristic species, the AG is not needed for their maintenance (Tour, 1977).

Masculinization of external characteristics of female crayfish was observed after the implantation of the AG (Nagamine and Knight, 1987a). In addition to this effect, implantation of the AG into female *Sphaeroma serratum* resulted in conversion of the ovary to a functional testis able to produce spermatozoa, observed after the first or the second post-implantation molt (93-224 days) (Raimond and Juchault, 1982). The AG in *Natantia* inhibits or at least delays previtellogenesis and vitellogenesis (Tour, 1977).

C. The biochemical nature of the androgenic gland's secretion.

The ultrastructure of the AG in the crab *Pachygrapsus crassipes* and the presence of considerable protein in the secretory vesicles of the cytoplasm suggest that the androgenic hormone may be a protein or a polypeptide (King, 1964). Juchault et al. (1978) have extracted a dialyzable water-soluble substance which was still active at 125°C from the AG of intersex *Armadillium vulgare*. A single injection of this substance into female *A. vulgare* induced the appearance of all the external male characteristics, but after two hours of incubation in the proteolytic enzyme pronase, the substance became

inactive and ceased to induce male characteristics. Lipid and steroid extracts of the AG were reported to be inactive. Katakura et al. (1975) extracted an active water-soluble substance from the male reproductive system of *Armadillidium*. The substance, estimated at molecular weight of 15,000 to 17,000 daltons, was inactivated by proteolytic digestion. Injection of the active extract into young females induced masculinization of the external sexual characteristics and transformation of the internal female reproductive organs into testes and sperm ducts (Katakura et al., 1975; Katakura and Hasegawa, 1983). Using the same bioassay, Hasegawa et al. (1987) isolated and characterized two proteinaceous androgenic hormones from the reproductive system of *A. vulgare*. The two proteins, termed AGH-I and AGH-II, consisted of 157 and 166 amino acid residues with molecular weights of 17,000 ± 800 and 18,300 ± 1000 daltons, respectively.

Contrary to the findings reported on *A. vulgare*, Veith and Malecha (1983) found that the AG of *M. rosenbergii* stained positive for lipids. Berreur-Bonnenfant et al. (1973) extracted a lipoidal substance, with a molecular weight of 200 to 250 daltons, from the AG of the crab *Carcinus maenas*. Injection of the substance every second day inhibited vitellogenesis in sexually active female *Orchestia*. Carotenoid pigment on the second antennae, a secondary male characteristic, appeared as early as the sixth day after similar injections in *Talitrus* females. However, spermatogenesis was not induced. The active molecule has been characterized by Ferezou et al. (1978) as farnesylacetone and was shown to be synthesized by the androgenic gland. The action of farnesylacetone at a low concentration is rapid, organ specific (being expressed in the gonads) and did not exhibit any species specificity. The farnesylacetone affects protein and RNA synthesis in its target organs (Berreur-Bonnenfant and Lawrence, 1984).

Gilgan and Idler (1967) reported the conversion of androstenedione to testosterone by *Homarus americanus* testes and AG. The tissues of these organs contain 17

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Beta-hydroxysteroid dehydrogenase (HSD). A comparison of the ability of different tissues to synthesize testosterone indicated that the AG was the most active. 17 Beta-HSD activity was demonstrated in the AG of the blue crab *Callinectes sapidus* (Tcholakian and Eik-Ness, 1971). The AG tissue converted progesterone to hydroxyprogesterone, androstendione, testosterone and deoxycorticosterone. The conversion was demonstrated *in vitro* and *in vivo*. On a weight basis, the AG converted more progesterone than did the posterior sperm duct or the hepatopancreas.

The different molecules mentioned above, i.e. steroids, isoprenoids and proteins, may play complementary roles. For example, in mammals, maleness is induced by both proteins and steroids secreted by testicular cells. Organogenesis of the testis is thought to be initiated by the presence of the H-Y antigen; male primary and secondary sex characteristics are induced by the subsequent stimulation of testosterone. The regression of the Mullerian ducts (the fetal duct from which the female reproductive duct is developed) is induced by a glycoprotein, the anti-Mullerian hormone (AMH) (Josso, 1986). I suggest that the androgenic gland may be involved in the secretion of more than one hormone. These may control different aspects of the wide spectrum of male biological functions including sex determination in postlarval stages, primary and secondary sexual characteristics, morphotypic differentiation and growth rate in the adult males.

D. The androgenic gland in *Macrobrachium rosenbergii*

The androgenic gland of *Macrobrachium rosenbergii* consists of strands of cells forming a pyramidal cluster loosely associated with the posterior portion of the ejaculatory duct. The strands are surrounded by a thin layer of connective tissue and consist of three principal cell types. Cells of type 1 are small with dense cytoplasm, often containing two nuclei. Cells of type 2 are slightly larger cells and vacuolated. Cells of type 3 are large cells in which most of the intracellular space

consists of vacuoles. Cellular degeneration was observed in certain areas of the gland, possibly indicating a holocrine mode of secretion. The AG of *M. rosenbergii* stained positive for lipids. The greatest concentration of lipids was seen in the epithelial cells lining the lumen of the ejaculatory duct. The lipids appeared to be evenly distributed throughout the gland and were not confined to any of the three cell types (Veith and Malecha, 1983).

Andrectomy appears to inhibit but not prevent meiosis. Complete feminization, which includes initiation of oogenesis, development of oviducts and female gonopores, occurred in males which were andrectomized in the earliest maturation stage after the juvenile stage. Males andrectomized in later developmental stages were either partially feminized or not feminized at all (Nagamine et al., 1980a). Reimplantation of the AG into andrectomized *M. rosenbergii* males reversed the effect of the andrectomy.

AG implantation masculinized the female recipients. Females that were sexually immature at implantation did not develop female secondary characteristics. Two of these small immature females were completely masculinized. Effects included the development of the *appendices masculina*, the male gonopore complex and mature masculine chelipeds and the initiation of spermatogenesis in the ovaries. Larger and mature females retained most of their secondary sexual characteristics. Oogenesis in masculinized *M. rosenbergii* females was still present, but the oocytes never completed vitellogenesis. Spermatogenesis did not occur in any of the mature females (Nagamine et al., 1980ab).

The implantation of ovarian tissue into andrectomized males resulted in the development of female secondary sexual characteristics (Nagamine and Knight, 1987b), suggesting that the androgenic gland prevents the appearance of female characteristics only in early stages, via inhibition of differentiation of the larval gonad into an ovary.

Recently Sagi et al. (1988a) demonstrated that androgenic gland ablation affects not only primary and secondary sex

characteristics but also growth rates and morphotypic differentiation into the three distinct adult male morphotypes which coexist in an *M. rosenbergii* population: small males, orange claw males and blue claw males (Ra'anani and Cohen, 1985). The three male morphotypes differ from each other morphologically, anatomically and physiologically and the hierarchy among the three types, is closely associated with social roles and reproductive activities (Ra'anani and Sagi, 1985; Kuris et al., 1987; Sagi and Ra'anani, 1988; Sagi et al., 1988b).

Andrectomy of small males did not prevent transformation into the orange claw morphotype but did prevent further transformation into the blue claw morphotype. However, andrectomy of orange claw males did not prevent transformation into the blue claw morphotype. The growth rates of the andrectomized small and orange claw males were significantly lower than those of the controls (Sagi et al., 1988a). In experiments conducted by Nagamine et al. (1980a) before the male morphotypes were recognized, immature males and two types of mature males with *appendices masculina* were andrectomized. One (Stage I) possessed "immature claws" and the second (Stage II) "mature chelipeds". Kuris et al. (1987) have postulated that these two types of males were small males and orange claw males, respectively. The small males did not develop "mature claws" following the andrectomy while the orange claw males did not lose the "mature claws". The appearance of sperm in the former suggests that when the andrectomy is performed on young males, differentiation into the mature small male morphotype takes place but further differentiation is prevented.

E. Significance of the androgenic gland in aquaculture

The sexual polymorphic growth patterns seen in *Macrobrachium* influence the marketable yield from aquaculture, especially in temperate zone prawn aquaculture where the growout period is shortened by early onset of winter temperatures. The variation between male and female growth rates influences the percentage of individuals which reach

market size by the end of the growout season, affecting the profitability of prawn farming. An examination of the potential for producing prawns in monosex populations revealed that, under the same culturing conditions, marketable yields obtained from all-male populations were significantly higher and more profitable than those produced from the mixed, normal population (Sagi et al., 1986; Hulata et al., 1988). Malecha (1986) concluded that the ability to create monosex populations is "one of the four important new techniques which can overcome all the drawbacks of the traditional culturing system". Since the blue claw and small males grow at a reduced rate (Ra'anani and Cohen, 1985), the choice for aquaculture production should be to raise all-male populations of orange claw males. The experimental demonstration that the transformation from orange to blue claw morphotype can be blocked (Sagi et al., 1988a) suggests that such control could be possible.

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