

Technical Paper

Technique for Making Chronic-Release LHRH-a and 17 α -Methyltestosterone Pellets for Intramuscular Implantation in Fishes

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(Accepted 14 July 1986)

ABSTRACT

Lee, C.-S., Tamaru, C.S. and Kelley, C.D., 1986. Technique for making chronic-release LHRH-a and 17 α -methyltestosterone pellets for intramuscular implantation in fishes. *Aquaculture*, 59: 161-168.

Interest in stimulating vitellogenesis in commercially important fishes has led to the use of implantable chronic-release cholesterol pellets and silastic capsules containing LHRH-a and 17 α -methyltestosterone, respectively. A detailed description of how these implants are made is provided.

INTRODUCTION

For a number of years, there has been interest in developing techniques using hormones to induce or accelerate vitellogenesis in commercially important fish species (reviewed by Lam, 1982). This stage of oocyte maturation can be of considerable duration, in some cases, months, and has been shown to be dependent on elevated pituitary gonadotropin (GtH) and gonadal steroid levels (reviewed by Ng and Idler, 1983). Satisfactory replication or augmentation of these hormone profiles, in order to stimulate vitellogenesis, has proven difficult. This is due in part to their chronic nature. Repetitive injections have caused gonadal atresia and even death in species sensitive to stress (Lam, 1982). Alternative methods for chronic delivery of hormones have been reviewed by Crim (1985). Continuous administration of exogenous GtH may result in an undesirable antibody reaction (Lam, 1982; Crim et al., 1983a). This potential problem can be avoided by using a GtH-releasing hormone such as LHRH or one of its superactive analogues, LHRH-a. However, with its relatively short half-life (Kent et al., 1980), LHRH-a must be introduced frequently into the fish's circulation to effect a chronically elevated GtH profile. LHRH (Windholz et al., 1983), and presumably LHRH-a, are rendered inac-

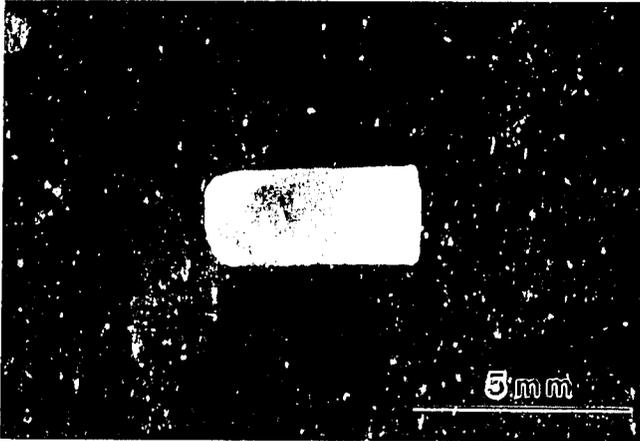


Fig. 1. LHRH-a cholesterol pellet.

tive by chymotrypsin, precluding oral administration. Some steroids, on the other hand, can be administered in the diet, but this route usually requires higher dosages to be effective.

Recently, implantation of a LHRH-a cholesterol pellet either alone (Crim et al., 1983b; Weil and Crim, 1983; Crim and Glebe, 1984) or in combination with a 17α -methyltestosterone (17α -MT) silastic capsule (Lee et al., 1986a,b) stimulated vitellogenesis as well as spermatogenesis in salmonids and chanids. The effectiveness of cholesterol as an excipient for peptide hormones has already been documented (see Parkes, 1942) and chronic-release LHRH-a cholesterol tablets used in mammals were described by Kent et al. (1980). Furthermore, Moore (1981) described the technique for making silastic capsules containing crystalline testosterone. Using formulations provided by L. Crim, Lee et al. (1985) made procedural modifications designed to increase the simplicity and cost-efficiency of producing these implants in order to make this technology transferable to Third World countries. These descriptions, however, do not include the latest modifications, do not provide adequate procedural details, or are not easily obtained. Due to the number of inquiries we have received requesting specific details on how these implants are made, the purpose of this paper is to provide a description detailed enough to serve as a laboratory guide.

LHRH-a CHOLESTEROL PELLETS

This procedure was used to make 15 cylindrical pellets (Fig. 1), each weighing an average 20 mg (range = 19–22 mg) and measuring 2.4 mm in diameter by 5.0 mm in length (range = 4.9–5.1 mm). The average dosage of LHRH-a per pellet was estimated at 200 μ g (range = 188–218 μ g). The shape of the

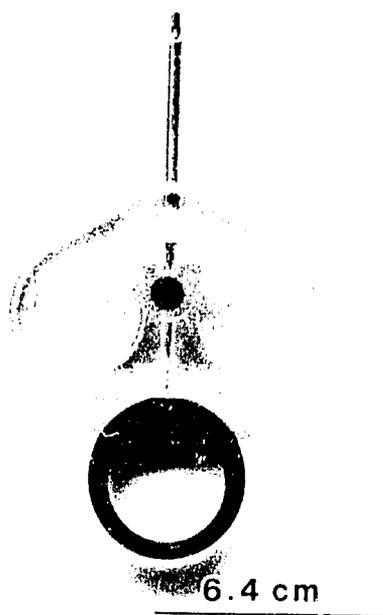


Fig. 2. Implanter used for both LHRH-a pellets and 17α -MT capsules.

pellets was designed to allow their loading into an implanter (Wick and Fry, patent no. 2 502 909) with an internal bore diameter of $3/32$ in. (2.34 mm) (Fig. 2).

The pellet mold

Using a $3/32$ -in. (2.34 mm) drill bit, 15 holes were drilled into a plastic sheet in the pattern illustrated in Fig. 3. This pattern was found to decrease the amount of time involved in making the pellets. Using a $9/64$ -in. (3.52 mm) drill bit, the top edge of each hole was beveled. An undrilled plastic sheet of similar dimensions was used as a base for the mold.

The hormone mixture

Four milligrams of LHRH-a (we used LHRH des Gly¹⁰[D-Ala⁶], Sigma Chemical) were dissolved in 0.4–0.6 ml of 50% punctilious ethanol. The dissolved hormone was then thoroughly mixed with 380 mg of cholesterol and dried for 1 h at 37°C. Twenty milligrams of cocoa butter (two drops, if molten) were thoroughly mixed into the cholesterol mixture upon its removal from the oven until its consistency became somewhat flakey. The LHRH-a-cholesterol-cocoa butter mixture was then ready to load into the pellet mold.



6.4 cm

Fig. 3. LHRH-a cholesterol pellet mold.

The cocoa butter constituted approximately 5% of the final mixture, which amount, according to our experience, gave the best pelleting properties using the method described here. Less than 5% produced "powdery" pellets which easily broke or crumbled, while more than 5% produced a mixture which was too soft when compressed into a pellet. One additional point, the LHRH-a from at least one source (Oriental Scientific Instruments Import and Export) comes combined with a substantial amount of mannitol which must be compensated for by a corresponding decrease in the amount of cholesterol used.

Pelleting the mixture

The pellet mold was placed on top of the base and the mixture was loaded into the block of 12 holes. A flat-ended rod or a nail with the point sawed off was used to pack the mixture in each hole by hand until completely full. The mixture in each hole was then compressed using a hammer and the packing rod or nail. The sequence of loading, handpacking, and compressing was repeated two more times at which point each of the holes was completely filled with the compressed mixture. The finished pellets were then hammered out through the bottom of the mold. The remaining mixture was loaded into the single and double holes to make the last three pellets.

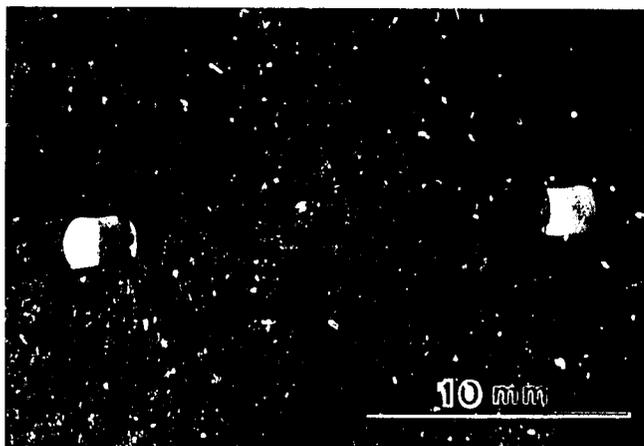


Fig. 4. 17α -MT silastic capsule

Control pellets

Control pellets were made in the same manner as described above except the 4 mg of LHRH-a was omitted. Therefore, before the cocoa butter was added, 0.4–0.6 ml of 50% punctilious ethanol was mixed into 380 mg of cholesterol and the mixture was then dried at 37°C.

17α -METHYLTESTOSTERONE SILASTIC CAPSULES

The following procedure was used to make 30–35 silastic capsules, each containing approximately 250 μ g of 17α -methyltestosterone (Fig. 4). The capsules were designed to fit the same implanter used for the LHRH-a cholesterol pellets.

The hormone mixture

Ten milligrams of 17α -methyltestosterone (Sigma Chemical) were dissolved in 0.1 ml of 100% punctilious ethanol. The dissolved hormone was then thoroughly mixed with 0.9 ml of castor oil.

The capsules

Silastic tubing, having the dimensions of 0.058 in. (1.45 mm) ID \times 0.077 in. (1.925 mm) OD (Dow Corning), was cut into 2-cm lengths. Medical-grade elastomer (Dow Corning) was used to seal one end of each 2-cm tube. In contrast to the method described by Moore (1981), we submerged one end of the tube into the elastomer, wiped off the excess from around the outside, and

placed it upright in a holder to cure. This eliminated considerable time spent trimming the excess elastomer to the point where the tubing would fit into the implanter.

A microdispenser was used to pipet 25 μl of the $17\alpha\text{-MT}$ -castor oil mixture into each open-ended tube. This was accomplished by inserting the tip of the microdispenser all the way to the bottom of the tube, then slowly withdrawing while the mixture was simultaneously evacuated. The open end of the tube was sealed with elastomer in the same manner as described above to produce the finished capsule.

Control capsules

Control capsules were similarly made except the 10 mg of $17\alpha\text{-MT}$ was omitted. Therefore, each pellet contained 25 μl of a mixture of 0.1 ml of 100% ethanol and 0.9 ml of castor oil.

IMPLANTING THE PELLETS

The implanter shown in Fig. 2 was used to implant both the pellets and the capsules into the dorsal musculature of the fish. With the butt end of a scalpel, a single scale was removed from the site of implantation. The exposed skin was pricked with the scalpel and the implanter tip was inserted at the site of the prick. After evacuating the pellet and removing the implanter, pressure was briefly applied with a fingertip to the insertion site to aid in resealing the wound.

FINAL REMARKS

Crim (1985) reviewed the various types of chronic-release hormone delivery systems which have been used in fishes. As he points out "the choice of methodology depends primarily upon economic and convenience considerations while endeavoring to minimize trauma...". The formulation for the pellet described here was originally used by him in pellets that were implanted in the intraperitoneal cavity. We subsequently modified the shape for intramuscular implantation (IM) in order to reduce trauma to the fish. L.W.Crim (personal communication) has recently found these IM pellets to be equally as effective when implanted into the intraperitoneal cavity.

Kent et al. (1980) found that the exposed surface area of the pellet can influence the release rate of the LHRH-a. Using our technique, consistency in the size and weight of each pellet can be obtained after a small amount of practice. Until this skill is acquired, we suggest that not only the weight but the diameter and length of each pellet be recorded. These measurements provide an estimate of dosage, surface area, and packing density, all of which could influence release rate and subsequent biological activity of the LHRH-a. For

this reason, the effectiveness of pellets composed of the same hormone mixture but compressed differently, with a Parr pellet press for example, may differ. At this early stage, comparative tests are lacking.

Silastic capsules containing crystalline testosterone can effectively release hormone for a year or longer (Moore, 1981). To increase the releasing rate of the hormone, L.W. Crim (personal communication) suggested dissolving the 17α -methyltestosterone in castor oil. This modification, as well as the lowered dosage, may have reduced the duration of effectiveness. Lee et al. (1986b) found the capsules described here to be superior to crystalline testosterone capsules when reimplanted every 2-3 months in milkfish. However, we urge researchers to take these differences into account in assessing the potential effectiveness of this pellet for other species.

ACKNOWLEDGEMENTS

This research was supported by the United States Agency for International Development, Grant #DAN-4161-A-00-4055-00. The authors wish to thank Dr. L.W. Crim for his invaluable advice and recent work testing the effectiveness of the LHRH-a pellet described above. We also thank V. Sato for the photography and A. Belanger for preparing the manuscript.

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