Infertility in Female Rabbits Immunized with Lactate Dehydrogenase X

Abstract. *Immunization of female rabbits with the sperm-specific lactate dehydrogenase (LDH-X) resulted in a highly significant reduction of pregnancies compared to nonimmunized controls. This is the first demonstration of immunosuppression of fertility by a crystalline protein shown to be strictly homogeneous by ultracentrifugation, polyacrylamide gel electrophoresis, immunodiffusion, and micro complement fixation.*

Immunization of female rabbits with spermatozoa, semen, or extracts of testes results in both fertilization inhibition and increased embryo mortality (*1–3*). Since spermatozoa contain a unique isozyme of lactate dehydrogenase (LDH-X), which provokes antibody formation in rabbits, it is of interest to determine whether immunized females are also infertile. Goldberg and Lerum showed that pregnancy suppression occurred in mice treated with rabbit antiserum to LDH-X (*4*). The immunosuppression of fertility in female rabbits by LDH-X is now described.

Sexually mature virgin rabbits (New Zealand White) were used. Animals were immunized by multiple intradermal and intramuscular injections of LDH-X emulsified in Freund's complete adjuvant. Each rabbit received 1 mg of crystalline enzyme in 1 ml of adjuvant. The LDH-X from mouse testes was purified and crystallized (*5*) and shown to be strictly homogeneous (*5, 6*). Blood samples were obtained from the ear artery and allowed to clot for serum separation. The serum was tested for antibody titer by assay of enzymic inhibition (*6*) and for specificity by the Ouchterlony diffusion method as recommended by Stollar and Levine (*7*). Statistical analyses were performed by Student's *t*-test.

Immunized females were mated to proven fertile bucks and injected intravenously with 150 international units (I.U.) of human chorionic gonadotropin (HCG) to ensure ovulation. The females were killed on day 10 after mating, and corpora lutea were compared with the number of live, implanted embryos. Noninjected females mated to these same bucks served as controls. Freund's adjuvant alone does not influence conception or pregnancy in rabbits (*1, 3*).

Females were somewhat variable in production of circulating antibody to LDH-X, with peak titer reached between 3 and 8 weeks after antigen injection. The maximum titer also varied with equivalence points ranging from 105 to 210. The equivalence point is defined as the amount of enzymic activity inhibited by 1 ml of serum (*6*). In all cases the specificity of the antibody to LDH-X was confirmed by the appearance of a single precipitin line in immunodiffusion plates.

The number of corpora lutea represented by normal embryos is very significantly ($t = 5.75$; $P < .0001$) reduced in rabbits immunized with LDH-X (Table 1). The difference in corpora lutea between immunized and control animals is not significant ($t = 1.94$; $P > .05$). Of the seven animals tested in these experiments, only two showed a pregnancy rate approaching but still significantly lower than that of the nonimmunized controls. All of the embryos that did implant were alive and appeared to be developing normally. In addition there were no implantation sites from aborted or resorbed embryos visible in uteri from immunized animals.

The pregnancy rate in these animals immunized with LDH-X was similar to that obtained by Menge (*1*) for rabbits immunized against semen or testis extracts. Subsequently, Menge (*2*) showed that isoimmunization with semen resulted in a trend for fewer sperm to be recovered from the oviducts, in significant decreases in motility of sperm from the oviduct, and in progression of sperm recovered from the uterus, and prevention of fertilization. It was also demonstrated (*3*) that a significant increase in embryo mortality occurred both before and after implantation in rabbits immunized with rabbit semen. Metz and Anika (*8*) treated semen with univalent antibodies to whole rabbit semen. Artificial insemination was used to show that relatively few sperm could be recovered from the uteri and oviducts.

By extrapolation from these data and with the present observation that no abnormal implantation sites or embryo resorption sites were detectable, it seems likely that immunosuppression of fertility is due to a reduction in the number of sperm capable of fertilizing all the eggs released from the ovary, although interference with implantation cannot be ruled out.

In agreement with findings on isoimmunization with semen (*1*), there is no apparent correlation between circulating antibody titer and fertility (Table 1). Very probably, cellular and locally produced antibodies would be required to interfere with normal sperm physiology, and on the basis of present knowledge that LDH-X is restricted to the sperm cell, this most likely is where immunosuppression of fertility occurs. The foregoing experiments demonstrate immunologically induced fertility reduction. This defined antigen-antibody system should facilitate studies of the mechanism of infertility and allow a more rational approach to fertility control by immunization.

Table 1. Pregnancy rate in rabbits immunized with LDH-X. Each experiment represents the mating of a single immunized and nonimmunized female with the same male. The antibody titer is the equivalence point of serum from immunized animals at time of mating.

Experiment	Antibody titer	Immunized (No.)		Nonimmunized (No.)	
		Corpora lutea	Embryos	Corpora lutea	Embryos
1	47	8	3	10	10
2	146	10	6	6	6
3	100	13	2	6	5
4	130	11	4	11	10
5	136	14	1	8	8
6	37	9	6	10	7
7	97	10	0	9	7
Total		75	22	60	53
(Embryos/corpora lutea) × 100			29.3		88.3

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Snapping Behavior of the Shrimp Alpheus californiensis

Abstract. *A pair of very smooth disks, located on the claw of the snapping shrimp* Alpheus californiensis, *are temporarily held together by cohesive forces of water. This allows the closer muscle of the claw to generate a large amount of tension before these cohesive forces are overcome, and results in a rapid closing movement.*

Snapping shrimps (family Crangonidae) have one greatly enlarged claw. Rapid closure of this claw, resulting in the expulsion of a jet of water and an audible snap, is a principal component of the defensive and aggressive behavior in these animals. While this behavior has been well known for several years (*1*, *2*), its mechanism has not been adequately described. The work reported here shows that for at least one species, *Alpheus californiensis*, the snapping behavior is made possible by the cohesive forces of water and a modification of the animal's exoskeleton.

Electrophysiological recordings, made with copper wires inserted through the exoskeleton and into the closer muscle of the propus, indicate that the dactyl is temporarily held in the open position even while the closer is being excited (Fig. 1). After the dactyl has been opened, large muscle potentials are observed in the closer muscle, but the dactyl does not move for 0.2 to 0.4 second after these muscle potentials are initiated. By the time the dactyl does move, the closer muscle has developed a large amount of force and snaps the dactyl closed.

The dactyl of an intact animal or an isolated claw can be set manually in the open (cocked) position. Once the dactyl of an amputated claw has been cocked, it will stay open until forcibly closed. By using a calibrated force transducer to close the dactyl, the force that the closer muscle must overcome to move the dactyl from the cocked position has been found to be 2.0×10^5 dynes, with a standard deviation of 0.36×10^5 dynes.

Since a claw that has been isolated from an animal for several hours can still be cocked, the mechanism for holding the dactyl open cannot be muscular action. This is further demonstrated by severing the opener muscle tendon of an isolated claw. Such an operation has no effect on the ability of the dactyl to remain in the cocked position.

Both the propus and the dactyl of the claw possess a hardened exoskeletal disk (Fig. 2a). When the dactyl is open these disks abut each other perfectly. A small, thin scratch on the surface of either of the disks completely disrupts an animal's ability to keep the dactyl open. The disks thus appear to be the structures responsible for holding the dactyl in the cocked position (*3*).

Scanning electron micrographs of the disks show some interesting features. The two disks are almost perfectly matched in size and shape (Fig. 2, b and c). The propus disk appears to have a layer of a soft substance spread over its surface; the substance cracks during preparation for the scanning electron microscope and looks much like dried clay (Fig. 2d). This layer is probably the epicuticle. If so, it is much thicker here than in any other part of the exoskeleton, including the dactyl disk. High-power micrographs of the troughlike rim surrounding the propus disk reveal many pores (Fig. 2d, inset). These pores are seen only in the rim area, not on the main surface of the disk. They may be from tegumental glands, which are thought to function in either the secretion or the maintenance of the epicuticle (*4*). The surfaces of the disks are remarkably flat and smooth (Fig. 2e). Micrographs at a magnification of 2000 reveal nothing to detract from the smoothness of these surfaces, aside from the cracks in the propus disk epicuticle. There are no structures that could be interpreted as minute latching or suction devices.

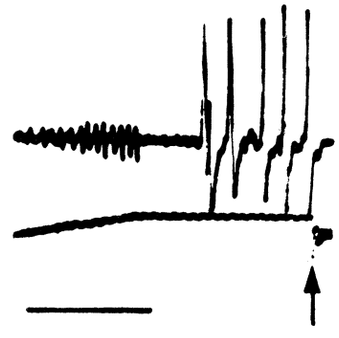

Fig. 1. Extracellular recording of muscle electrical potentials during a snap. The top trace is from the muscles of the propus. The bottom trace is from a movement transducer attached to the dactyl. In the transducer record, up is opening of the dactyl and down is closing. The snap occurs at the sharp downward movement of the transducer record (arrow). The large potentials are from the closer muscle. Note that there is no closing movement until after the last large potential. The small potentials that occur during opening are from the opener muscle, which is approximately 1 cm from the electrodes. Calibration, 200 msec.

There are several ways of explaining how the disks are held together. The soft substance on the propus disk may act as an adhesive. While this possibility cannot be completely eliminated, it is difficult to imagine an adhesive that would become completely ineffective when a small scratch is made on its surface or on the surface it would adhere to. Also, the alignment of the disks seems to be critical. If the articulation between the dactyl and the propus is damaged so that the disks no longer match precisely, the cocking mechanism is completely disrupted. This would not be expected if an adhesive were responsible for this mechanism.

Another possibility is that a vacuum is formed between the two disks, and the soft epicuticle on the propus disk acts as a seal. If this were true the force needed to separate the disks under standard conditions of temperature and pressure should be just enough to overcome a pressure of 1 atm. On the claws used to determine the force required to uncock the dactyl, the area of the disks was 0.7 mm^2. One atmosphere of pressure on an area of 0.7 mm^2 is equal to 0.7×10^4 dynes. Since the force needed to uncock the dactyl has been measured to be 2.0×10^5 dynes, a vacuum mechanism appears to be insufficient to account for the force holding the dactyl open (*5*).

The most reasonable hypothesis seems to be that forces of cohesion of the water between the disks are responsible for keeping the disks together. The amount of force that could theoretically be developed in this manner can be calculated by using the heat of vaporization of water, which is a measure of the amount of energy needed to separate water molecules from one another. Multiplying this figure by the