**Goals/Rationale:** [A few sentences on the goals of the research or teaching protocol in non-technical terms.]

Sjogren’s syndrome (SS) is an autoimmune disease characterized by infiltration of lymphocytes in the bloodstream, and progressive destruction and dysfunction of the lacrimal (tear) glands. Removal of the ovaries has been shown to accelerate autoimmune lesions in tear glands in mouse models of the disease. The objective of this proposal is to shed light on the early events that occur in the tear gland after removal of ovarian hormones by comparing genetically predisposed mice to mice without the condition. These studies will allow us to define novel targets and therapies for the treatment of SS.

**Only if 3 year renewal**

**Previous Work:**

In the past 3 years, we have utilized 350 of the approved 500 mice due to difficulties in breeding appropriate numbers. We have completed the non-surgical experiments, which correspond with Aims 1 & 2 of our grant. We now propose to complete Aims 3-5 of our grant, as outlined below. Based on statistical calculation for appropriate power, we estimate we will need x numbers of mice over the next 3 years.

**Quarantine/stabilization:** Animals will be stabilized for a period of two days. For the purposes of these experiments, the stabilization period of two days instead of three days will be adequate, since we are interested in the levels of estrogen (female hormone) and androgen (male hormone) after the surgery.

**Procedures:**

Female mice will be divided into four groups: Sham (control group where procedure is simulated but no ovaries are removed); those with ovary removed (OVX); those with ovary removed and treated with estrogen; and those with ovary removed treated and with the androgen DHT.

One group of mice will be anesthetized and the ovaries will be removed under aseptic conditions. While they are still under anesthesia, a small incision (~3 mm) will be made in the skin on the back of the neck and a pellet containing either DHT or estrogen will be implanted under the skin of the OVX animals treated with androgen and estrogen, respectively. These pellets will deliver the hormones for the duration of the experiments. A placebo pellet will be implanted in the OVX group.
During surgery, the animal will be monitored by pinching the ear every five minutes to assure that they are still under anesthesia. If the animals start to wake up before the surgery ends, half of the dose of the anesthesia will be given. Survival time following surgery varies within the experimental groups; therefore for animals that will survive more than 14 days, sutures will be removed without anesthesia after seven days. The animals will recover on a warming pad or incubator. They will be turned over every five minutes until completely recovered from anesthesia. Mice will be observed until they are alert and then returned to their home cage. A potential for pain is associated with these procedures, therefore half of the dose of the analgesic buprenorphine will be given as they begin to recover from surgery to eliminate any pain, and the other half after they completely recover. Ibuprofen will be added to the drinking water and given for two days after surgery.

For the sham group, vaginal smears will be collected daily to determine the phase of the estrous cycle. This will be done for 10 to 12 days starting the day after they arrive. For this procedure, a sterile pipette tip will be placed at the vaginal opening no more than 1 mm deep. One drop of sterile water will be gently expelled into the vagina and aspirated back into the tip, and transferred to a microscope slide. During this time, once the estrous cycle is determined for each mouse, they will receive the sham operation (exactly the same procedures as the animals having the ovaries removed, but without removing the ovaries) and used as controls for each strain. A placebo pellet will be implanted into these mice.

The preparation for surgery, the surgical procedures and the recovery monitoring for these mice will be done as described above for the mice having ovaries removed. The experimental times will also be the same as for the mice having ovaries removed; however the time of surgery will depend on the estrous cycle determination. Each mouse should be in the same estrous cycle phase when they are euthanized.

**Euthanasia:**
After the experimental period, half of the mice will be anesthetized and blood will be collected for hormone level measurements. This procedure will result in the death of the animal since all the blood will be removed. The other half of the mice will be euthanized with an overdose of ketamine/xylazine. All the mice will have their extraorbital tear glands removed after euthanasia for analysis.

**Breeding:**
Since a special strain of NOD.B10.H2b mice is required, and they are hard to obtain, we will breed our own mice using a 1 male to 1 female approach. Specific details are provided in Appendix U of the protocol.