

# The Technique of Orthotopic Ovarian Transplantation in the Chicken<sup>1</sup>

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**ABSTRACT** A surgical technique has been established for orthotopic transplantation of ovarian tissue in newly hatched chickens. In these trials survivability of the chicks after surgical manipulation was 100%. The size and orientation of the ovary at 2 wk of age suggested that the

graft was attached and had undergone development. This technique should allow the development of a cryopreservation protocol of chicken ovarian tissue for the conservation of poultry genetic material.

**Key words:** ovarian tissue, transplantation, chicken

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## INTRODUCTION

The number of poultry lines kept by industry and in public institutions is declining at an alarming rate (Miller, 2004). Unfortunately, techniques for conservation of avian genetic material are limited in terms of practicality, efficiency, and success rate (Fulton and Delany, 2003). At present, the only effective method of conserving poultry germplasm is by keeping live animals, which is costly and leaves the line vulnerable to disease outbreaks or natural disasters. There is an urgent need to develop new techniques for avian germplasm conservation.

Orthotopic transplantation of ovarian tissue has been used to maintain lines of transgenic mice (Sztein et al., 1998). In birds, Guthrie (1908) first reported transplantation of ovaries in hens and suggested that the transplanted ovaries had normal function. However, using similar experimental techniques as those of Guthrie (1908), Davenport (1911) demonstrated that the offspring of the transplanted hens were from regenerated host ovary. Until now, research with ovarian transplants performed in chicks older than 3 wk has failed to demonstrate that the grafted ovarian tissue was attached (Grossman and Siegel, 1966). A pilot experiment on chicken skin grafts (unpublished) indicated that skin grafts in 1-d-old chicks were usually accepted and skin grafts in 2-wk-old chicks were usually rejected, suggesting that transplantation of ovarian tissue in newly hatched chicks might be successful. The objective of the present research was to develop the technique of ovarian

tissue transplantation in newly hatched chicks, which should allow conservation of avian germplasm.

## MATERIALS AND METHODS

### *Birds*

Barred Plymouth Rock (BPR) and White Leghorn (WL) chicks, from lines obtained from D. Shaver (20 Berkley Road, Cambridge, ON, Canada N1S 4S8) and maintained at Agassiz, were used as donors and recipients, respectively, in this experiment. The surgical transplantation protocol was approved by Pacific Agri-Food Research Center (Agassiz) Animal Care Committee and followed principles outlined by the Canadian Council of Animal Care (1993).

### *Preparation of Donor Tissue*

Ovaries from 1-d-old BPR chicks were isolated from chicks that had been freshly euthanized by cervical dislocation. Ovaries were cleaned of connective tissue and cut into 2 to 3 pieces per ovary. Ovarian tissue was placed in Dulbecco's Modified Eagle's Medium culture medium (Sigma Chemical Co., St. Louis, MO) on ice and transplanted within 4 h.

### *Ovariectomy*

Newly hatched WL chicks were anesthetized by intramuscular injection of 0.5 mg of ketamine (Ketaset, Ayerst Veterinary Laboratories, Guelph, ON, Canada) and 0.1 mg of xylazine (Rompun, Bayer Inc., Toronto, ON, Canada). The chick was placed on its back on a heated surgical table. The abdominal skin was swabbed with 70% ethanol, and the feathers were removed from the area of the operation. A 2.5- to 3-cm transverse incision was made approximately 1 cm distal to the last rib to expose

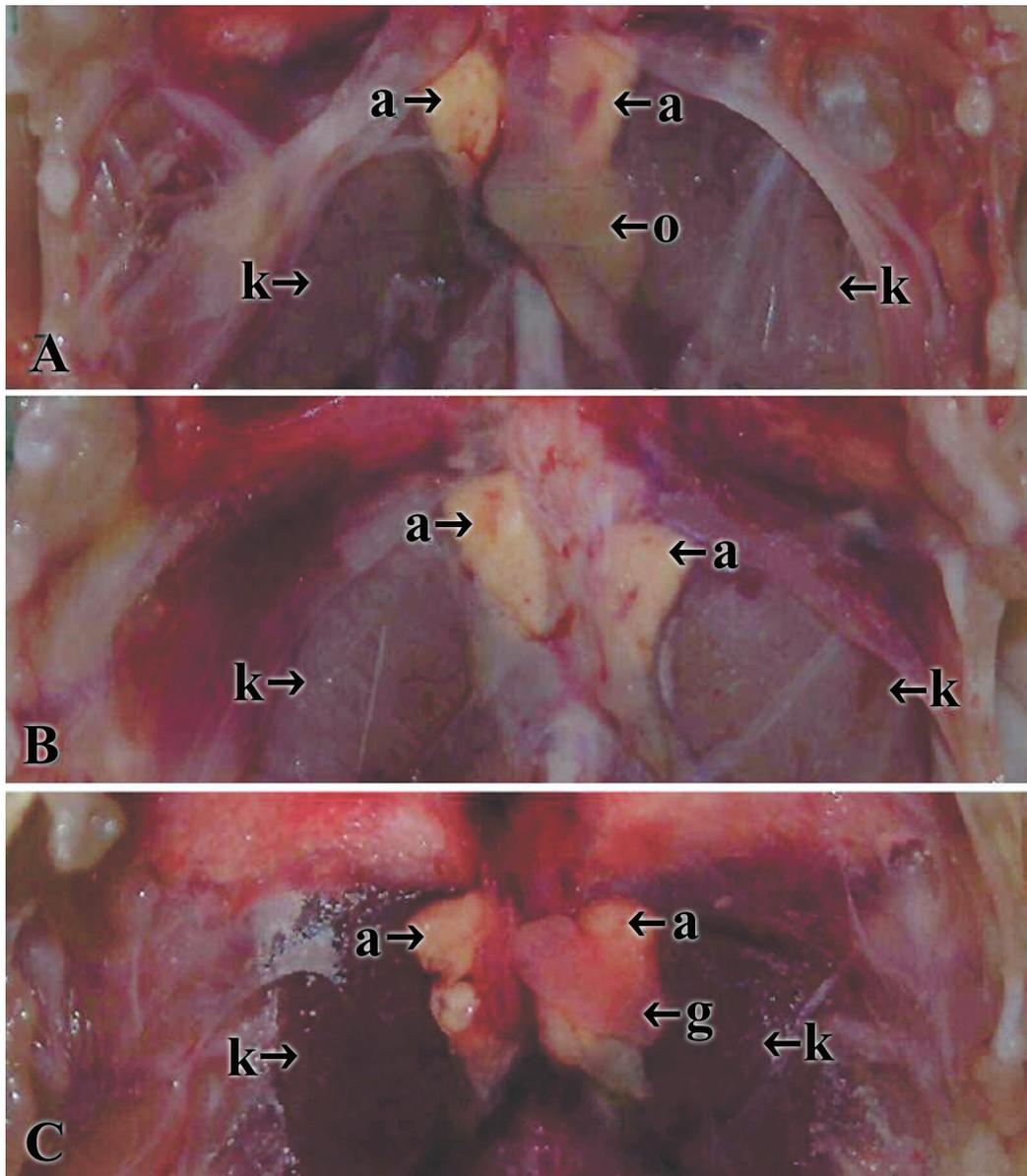
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**Figure 1.** Ovarian tissue in 2-wk-old White Leghorn (WL) chicks. (A) normal ovary; (B) ovariectomized chick; (C) attached Barred Plymouth Rock (BPR) ovarian tissue in WL chick. a = adrenal gland; k = kidney; o = normal ovary; g = grafted ovary.

the left side of the abdominal cavity. The peritoneal membrane was carefully detached from the gizzard, and the yolk was removed after tying the connecting stalk with surgical suture. The gizzard and intestine were carefully displaced, and the ovary was illuminated with a fiber light source. In the 1-d-old chick, the ovary is located in the extreme cranial part of the left abdominal cavity, in a small corner enclosed by the left kidney, the colic mesentery, and the greater abdominal air sac. The greater abdominal air sac was displaced, and the ovary was completely removed with a fine forceps (Dumont Medical #5-45, Outils Dumont SA, Montigney, Switzerland). Removal of the ovary sometimes resulted in massive hemorrhage, in which case the chick was culled by cervical dislocation. After ovariectomy, sterile cotton was used to clean the area of blood and residual ovarian tissue.

### **Transplantation**

Two fresh pieces of BPR ovarian tissue were placed in the position of the removed ovary, and the greater abdominal air sac was torn open and used to cover the transplanted tissue. The abdominal incision was closed by 2 layers of continuous surgical suture. The operated chicks were administered 5 mg of an antibiotic (Excenel, Pharmacia Animal Health, Orangeville, ON, Canada) intramuscularly immediately after surgery and were given a daily oral dose (100 mg/kg) of an immunosuppressant (CellCept, Hoffmann-LaRoche Limited, Mississauga, ON, Canada).

At 2 wk of age, an unoperated chick, an ovariectomized chick, and a transplanted chick were killed by cervical dislocation for verification of the techniques.

## RESULTS AND DISCUSSION

A surgical technique was established for orthotopic transplantation of ovarian tissue in newly hatched chickens. Excessive bleeding was the primary reason for failure in this technique, but survivability after surgery was 100% when the birds with massive hemorrhage (as low as 20% depending on the skill of the surgeon) were culled. Surgical transplantation took approximately 25 min per bird, and manipulated birds recovered within 2 h. Thirty-five birds were produced by transplantation of fresh ovarian tissue from BPR to WL chicks. These results demonstrated that newly hatched chicks are quite resistant to this aggressive surgery.

At 2 wk of age, the ovary was seen in its normal position in the abdominal cavity (Figure 1A), and ovarian tissue was completely absent from the ovariectomized chick (Figure 1B). In the WL host with a transplanted BPR ovary, the piece of ovarian tissue was attached and had undergone development, but its orientation was different from that of the normal ovary (Figure 1C). This technique should allow development of a cryopreservation protocol for chicken ovarian tissue for the conservation of poultry genetic material.

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