

Autologous transplantation of cryopreserved ovary induces the generation of antiovary antibodies in sheep

Ovarian cryopreservation and grafting has resulted in successful ovulation, pregnancy, and live-born animals including mice and sheep (1, 2). The procedure is currently being offered to humans as a new method of assisted reproduction. However, its value frequently has been questioned because of a potential to reintroduce the malignant cells, viral infections, and induction of immune responses against the transplanted ovarian tissue (3, 4).

The objective of this study was to determine the relationship between ovary autotransplantation and antiovary antibody formation in 16 adult female, nonpregnant merino ewes. Surgical procedures were performed at the Cleveland Clinic Foundation Biological Resources Unit in accordance with the facility's Standard Operating Procedures (Institutional Animal Care and Ewe Committee). The institutional animal research committee approved the study protocol.

All animals underwent bilateral laparoscopic oophorectomy. Ovarian cortex fragments (5 mm²) from 6 sheep and whole ovaries from 10 sheep were removed and cryopreserved using a controlled rate freezer (Planer Kryo 10, Series II; TS Scientific, Perkasio, PA). After 7 days of storage in liquid nitrogen (−196°C), the specimens were thawed. All 6 ovarian cortex fragments and 10 whole ovaries were transplanted back into their original donors. The grafts were removed 7 days later for histological evaluations. Blood samples were withdrawn preoperatively, at transplantation, and at transplant removal. Positive sera was treated for possible heterophilic interference using heterophilic blocking tube (Scantibodies Lab. Inc., Santee, CA) before repeating the test both for monkey and sheep ovarian tissue.

Antiovary antibodies in serum were detected by indirect immunofluorescence assay using commercial slides of monkey ovaries (Immco Diagnostics, Buffalo, NY) and frozen sections of fresh sheep ovary as antigen. The slides were read in a blind fashion. Although laparoscopic oophorectomy, cryopreservation, and transplantation were done by one individual (M.A.B.), the tissue and serum samples were coded before the slides were coated, stained, and read by another individual (N.E.). Serial dilutions of the serum samples (1:2 to 1:32) in phosphate-buffered saline were prepared and applied to unfixed 5- μ m-thick sections of sheep and monkey ovary.

The slides were washed in phosphate-buffered saline, and the presence of antiovary antibodies was detected by a second incubation with fluorescein isothiocyanate-conjugated rabbit anti-sheep immunoglobulin (Jackson ImmunoResearch Lab., Inc., West Grove, PA). The sections were examined under a fluorescent microscope (Leitz Dialux model; Leitz Wetzlar, GmbH, Wetzlar, Germany) at \times 400 magnification in a blinded fashion. Each sample was tested in duplicate in parallel with positive and negative controls according to the manufacturer's instructions. We determined the isotype of the antibody formation in the three animals, and in all positive sera, the antibody isotype was IgM. This again provided additional information that the autoimmune response was beginning to develop.

We detected antiovary antibodies in three serum samples obtained at transplant removal: two weakly positive samples (titer of 1:2) from sheep that received sliced tissue autograft and one positive sample (titer of 1:8) from a sheep that received an ovary autograft. All three sera were positive on the monkey and frozen sheep ovary slides (Fig. 1). There were no antibodies in the sera obtained preoperatively and at transplantation.

Anti-ovary antibodies have been reported in patients undergoing repeated IVF and in patients with systemic lupus erythematosus, premature ovarian failure, and endometriosis (5). These reports suggest that the ovary is either an immune target or an organ that contains autoantigens. During oocyte retrieval for IVF, autoantigens and altered immunogenic proteins may be released from internal ovarian layers, which may induce the immune response and result in ovarian antibodies.

It is not known which ovarian antigens are responsible for the induction of antiovary antibodies, but proteins derived from ovarian cells such as granulosa, interstitial cells, epithelial cells, and luteal cells, as well as zona pellucida and receptor sites for FSH and LH have been implicated (5, 6). Several factors may cause immune dysregulation and antibody formation, such as differences in autoantigen response and the duration of antigen exposure to the immune system.

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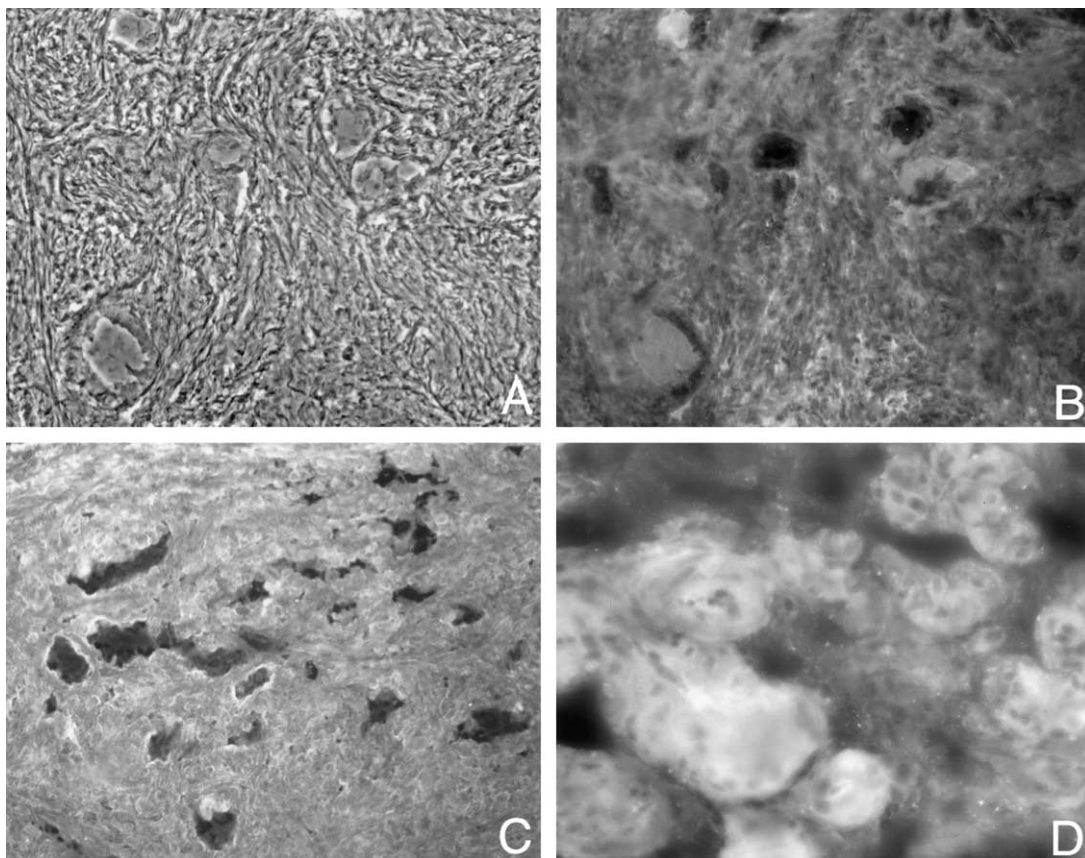
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FIGURE 1

Immunofluorescent analysis of antiovary antibodies using slide preparation of monkey ovaries. (A), Phase contrast. (B), Negative control. (C), Positive control. (D), Sheep ovary. (Magnification, $\times 20$ in A–C; $\times 40$ in D.)



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Although the ovary does not have an immunological barrier that is equivalent to the blood–testis barrier in males and cannot be considered an immunologically privileged site, there have been a number of observations that ovary may be compartmentalized with respect to its exposure to the immune system (7). Also it has been speculated that release of self-antigens and altered immunogenic proteins from internal ovarian layers during oocyte retrieval may induce the immune responses and result in formation of ovarian antibodies (8).

Several mechanisms may generate ovary autoantibodies: defects in the self-tolerance mechanism of the immune system; exposure of the immune system to large amounts of ovarian autoantigens after repeated follicular puncture or hormonal therapy; and dysfunction of regulatory T cells, which appears to inactivate self-reacting T-cell clones (5, 9). When antiovary antibodies are produced, they may negatively affect reproduction by [1] interfering with follicle function and oocyte maturation; [2] coating the surface of the oocyte and preventing sperm from penetrating the zona pellucida, [3] destroying the zona pellucida through complement-mediated cytotoxicity, and [4] preventing hatching from the zona pellucida, thereby impairing implantation.

Moreover, because the primary functions of the ovary are to produce the gametes and provide an endocrine function to support the developing gametes, the antibodies directed to FSH and/or LH receptors may block the receptors and affect normal follicular growth and function.

Several surgical manipulations during ovarian autotransplantation may expose large amounts of ovarian antigens and molecules to the immune system. More important, altered expression of self-antigens that result from physical damage to ovary cells during in vitro handling, cryopreservation, and heterotopic transplantation may encourage the development of autoantibodies. The fact that three ewes in our study developed antiovary antibodies after ovary autotransplantation supports the above hypothesis. However, this observation raises two further questions: [1] if ovary autografting provokes an immune response, why is there no evidence of their presence in other cases? and [2] what is the relationship between antiovary antibody production and transplant survival and its normal function?

Antibody production triggers soon after antigen exposure, and levels become detectable in serum after about a week. Although we

were able to detect the antibody titer in one animal, perhaps longer exposure (>7 days to 1 month) will induce the immune response more efficiently. Additional prospective studies are needed to evaluate the possible role of antiovary antibodies in fertilization and pregnancy outcome in patients undergoing ovary autotransplantation.

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