

## Effect of Blood Transfusion on the Survival Rate of Cryopreserved Mouse Ovaries Transplanted into Rat Uterine Cavity

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**Abstract:** Judgement of viability is essential for determining the effectiveness of cryopreservation method for ovaries. Freeze-thawed ovaries have been transplanted into the uteri of pseudopregnant rats, but the survival rate of after transplantation has been low. Since it has been reported that the survival rate of transplanted kidneys was improved when blood transfusion was performed before transplantation, the present study was designed to determine the effect of blood transfusion on the survival rate of transplanted mouse ovaries. Before transplantation of the ovaries in the uteri of rats, the recipient rats were given 1~5 intraperitoneal blood transfusions. The ovaries were frozen using the vitrification method. After dehydrated the ovaries, they were kept in liquid nitrogen. Immediately after thawed the ovaries, they were transplanted into uteri of pseudopregnant rats that had received blood transfusions, and the survival rate of the ovaries was investigated. The viability of the transplanted ovarian follicles was examined using a morphological technique. It was found that the number of healthy follicles increased as the number of blood transfusions was increased, indicating that pre-transplantation blood transfusion into the recipient is an effective method for increasing the survival rate of transplanted ovaries.

**Key words:** Ovary, Ovarian follicle, Cryopreservation, Transplantation, Blood fusion

The ovary is a repository of oocytes. Although each ovary contains an enormous number of egg cells, only a very small proportion of them are released at ovulation during the fertile years. Although superovulation can save ovarian follicles that have become atretic, it

only enables a small number of ova to be obtained [3]. The establishment of an effective method for cryopreservation of ovaries would have considerable economic benefits for the livestock industry [2]. It would also be of great value in the clinical field of medicine for transient preservation of ovaries during cancer therapy. In order to determine the effectiveness of cryopreservation, mammalian ovaries have been transplanted into the uteri of pregnant and pseudopregnant rats that had immunological tolerance, and the survival rate of the transplanted ovaries has been examined [2]. It was found that only a small proportion of the freeze-thawed ovaries survived. Since it has been shown that the survival rate of transplanted kidneys is improved when blood transfusion is performed before transplantation [7], the present study was designed to determine the effect of blood transfusion on the survival rate after transplantation of cryopreserved ovaries.

### Materials and Methods

#### Animals

Adult Wistar-Imamichi rats were obtained from the Imamichi Institute of Animal Reproduction and were laboratory-reared in an animal room that was airconditioned ( $23 \pm 2^\circ\text{C}$ ) and illuminated from 07:00 to 21:00 hr. Adult ICR mice were obtained from Clea Japan Inc. (Tokyo, Japan). For the removal of ovaries, the donor mice were deeply anesthetized by a combination treatment of sodium pentobarbital and diethyl-ether. All animals used were 10 weeks old at the time of the experiments.

#### Freezing

Ovaries were handled by the method of Rall and Fahy [1]. The ovaries were collected, and each ovary was divided into two pieces and placed in a freezing

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**Table 1.** Effect of blood transfusion on survival of transplanted ovaries

No. of blood transfusions	No. of recipients	No. of follicles that survived before freezing (A)	No. of follicles that survived after freezing	No. of follicles that survived after grafting (B)	Percentage of follicles that survived (B/A × 100)
0	10	45 ± 3*	40 ± 5*	6 ± 4*	13.3
1	10	51 ± 4	47 ± 3	11 ± 4	21.6
2	10	39 ± 7	36 ± 5	12 ± 3	30.8
3	10	42 ± 5	39 ± 4	11 ± 2	26.2
4	10	40 ± 5	38 ± 3	10 ± 3	25.0
5	10	47 ± 2	41 ± 3	11 ± 2	23.4

\*No. of healthy follicles larger than 250  $\mu\text{m}$  per 2 pieces of divided ovaries.

tube (Serum tube, Ms-4601, Sumitomo Bakelite Co. Ltd., Tokyo, Japan). The ovaries were washed in HB1 [1] solution, a modified Dulbecco's saline containing 1 mM phosphate buffer, 0.33 mM sodium pyruvate, 5.56 mM glucose, 3 ml<sup>-1</sup> bovine serum albumin and 100 IU per ml of penicillin G, and then placed in a 1:4 dilution of vitrification solution (25% VF1=5.125% W/V DMSO, 3.875% acetamide, 2.5% propylene glycol and 1.5% polyethylene glycol [relative molecular mass 8000]) in HB1 for 15 min at 20°C to allow the tissue to be completely infiltrated by the cryoprotectants. The ovary suspensions were then placed in a cold room (at about +4°C), where the ovaries were exposed to the vitrification solution in two steps: first to a 1:2 dilution of VS1 for 15 min and then to VS1. After exposure to VS1 for 15 min, the ovaries were immediately transferred to liquid nitrogen. The frozen ovaries were preserved in LN<sub>2</sub> for about 30 days.

#### Thawing

The frozen ovaries were warmed to 37°C, and the remaining cryoprotectants were washed away, first with 50% VS1 and then with 25% VS1. Finally, the ovaries were washed in HB1 solution and transplanted into a rat uterine cavity.

#### Transplantation of cryopreserved ovarian tissue

The procedure for homoplastic grafting of the rat ovary into the uterine cavity followed the method of Kagabu and Mamba [2]. At 10 weeks of age the recipient rats were allowed to copulate with vasectomized rats. Six days later, the pseudopregnant recipient rats were anaesthetized with 0.15 ml/rat Nembutal (Abbott, North Chicago, IL, USA), and a freeze-thawed ovary was placed in the empty uterine cavity of the recipient and the uterus was sutured.

#### Blood collection and transfusion

Blood was collected from the mice in heparinized tubes (2 ml). One-milliliter intra-peritoneal infusion were given to the recipients on days -17, -14, -11, -8 and -5 before transplantation (the day of transplantation being day 0).

#### Assessment of cell viability after transplantation

The ovaries and uterus complex were removed 7 days after transplantation. The complex was fixed in Lavdovski's solution, dehydrated, embedded in paraffin wax, serially sectioned at 10  $\mu\text{m}$  in thickness, and stained with hematoxylin and eosin. Follicles were classified as healthy or atretic according to the criteria of Braw and Tsafiri [3].

## Results and Discussion

The results are shown in Table 1 and Fig. 1. The conditions of the transplanted ovaries were judged according to the strict criteria of Braw and Tsafiri [3]: Follicles that had neither fragmentation or karyopyknosis of granulosa cells were judged to be healthy, and transplanted ovaries containing healthy follicles were judged to have survived. The survival rate after transplantation of cryopreserved ovaries was increased by blood transfusion. The types of transplanted ovary after freeze-thaw are shown in Fig. 1 as an ovary that did not survive (Fig. 1-2), an ovary that did survive, and its magnified view (Figs. 1-3 and 4, respectively).

Cryopreservation of ovaries has many possible applications and is a valuable technique in domestic animal reproduction. Investigation of the viability of tissue is essential for determining the most effective cryoprotective agent and freezing speed. In the case of frozen sperm, viability can be easily determined by observation of movement after thawing. However, viability of an ovary cannot be determined by this simple method

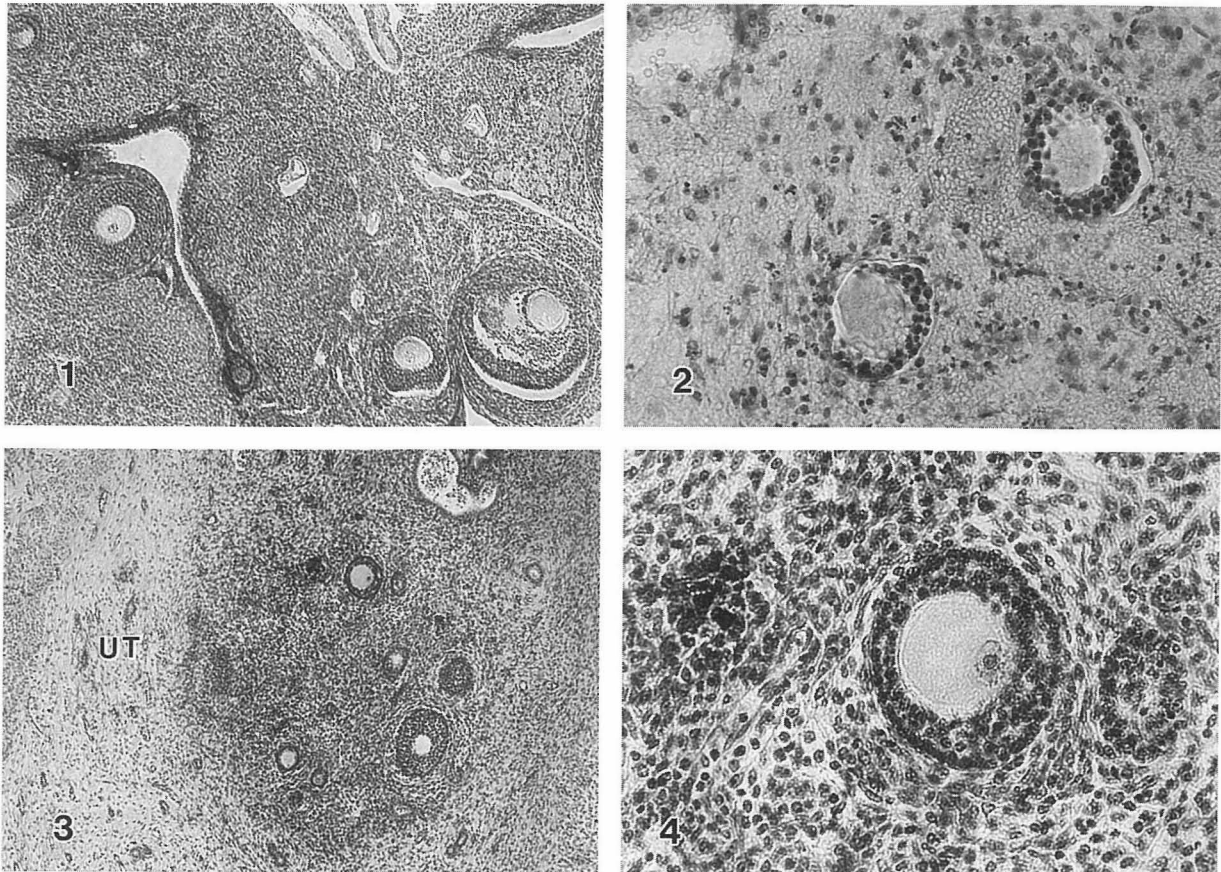


Fig. 1. Before and after transplantation of mouse ovaries. 1: after freeze-thaw, 2: an ovary that did not survive, 3, 4: surviving ovary. UT: uterus of recipient.

because there is almost no movement of ova. In the previous study [2], we therefore transplanted thawed ovaries in uteri and examined their viability using a morphological technique. The results showed a low survival rate of ovaries, indicating the need for improvement of the cryopreservation method.

Various attempts have been made to improve the survival rates of transplanted organs, and it has been shown that cyclosporin [4] and FK506 [5] as well as ultraviolet irradiation [6] are effective in increasing the survival rate of transplanted organs. In the case of cryopreservation of ovaries, the use of chemicals should be minimized to ensure survival of the ova.

Since it has been reported that the survival rate of transplanted kidneys was improved when blood transfusion was performed on the recipients before transplantation, we investigated the effect of blood transfusion on the survival rate of thawed ovaries transplanted in uteri. Blood that had been stored for more than two weeks was used in the present experiments because it

was reported that the use of blood that had been stored for more than two weeks for transfusion to the recipient before transplantation increased the viability of the graft and reduced the sensitivity of the recipient [8]. The results indeed showed that the survival rate of the transplanted ovaries increased when the recipients were given blood transfusions before the transplantation. Thus, blood transfusion to the recipient before transplantation is an effective method for improving the survival rate of thawed ovaries. However, since the highest survival rate found in the present study was only 30%, further study is needed to find other effective methods for increasing the survival rate of transplanted ovaries.

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