Regeneration of Oocytes After Chemotherapy: Connecting the Evidence From Mouse to Human

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In this issue, Dr Tilly’s laboratory presents another challenge to a half century—old dogma that ovarian reserve, made up of primordial follicles, cannot be replenished after birth. Their earlier work indicated that egg manufacturing continued into adult life in mice and that germ stem cells could have originated from the bone marrow; this work was met with much skepticism. It has been known for some time that bone marrow transplantation (BMT) and hematopoietic stem-cell transplantation (HSCT) are associated with extremely high rates of ovarian failure and infertility as a result of the preconditioning chemotherapy and radiotherapy. However, there are numerous reports of spontaneous pregnancies, even years after BMT or HSCT. Building on those observations and in response to various criticisms of their earlier work, Lee et al have tested the question of how BMTs can restore fertility in a rodent model. They found, to their surprise, that the donor-derived eggs from BMTs were not responsible for preservation of fertility in the recipients. All pups that were born after BMT were of recipient origin, even though a small fraction of immature oocytes of donor origin was found in recipient ovaries by cell tracking.

The current concept that the ovary has a static ovarian reserve is entirely at odds with the germ cell dynamics in its counterpart, the testis. The testis contains a sizeable number of germ stem cells and continuously generates new gametes, even into old age. Although sperm numbers may diminish slightly at the extreme end of aging, recent research in mice suggested that this is mainly a result of aging of the niche rather than a defect in germ cells. The message that is given by Lee et al is that the ovary is more like the testis, at least when exposed to chemotherapy. It is not a static organ and continues to produce new germ cells into adult life. Speculatively, perhaps chemotherapy does not simply take away a number of eggs from a fixed ovarian reserve but interferes with the ability of the ovary to regenerate oocytes.

If the mice born after BMT had the genetic background of the recipient, how did that procedure restore fertility? The fact that BMTs help maintain fertility despite the reduction in the size of follicle population after chemotherapy suggests that the transplantation may restore the lost germ stem cells, which then enables the ovary to maintain its reserve. Lee et al cleverly pointed out that the BMT treatment might have repaired chemotherapy-induced damage to the niche or the ovarian stroma. Indeed, our recent research showed that human ovarian samples taken from women who were previously exposed to various chemotherapy agents produce less estrogen than controls, indicating that chemotherapy may alter ovarian stromal function. In addition, there are clinical examples that suggest a role for ovarian niche in recovery from chemotherapy-induced ovarian failure. In a recent report, we described a Hodgkin’s lymphoma survivor who underwent subcutaneous ovarian transplantation with previously frozen autologous ovarian tissue. Before the transplantation, she was menopausal for 2.5 years as a consequence of an HSCT. Given the spontaneous recovery of ovarian function in the remaining menopausal ovary and recurrent spontaneous pregnancies concurrent with follicle development in the grafted ovary, we speculated that autologous ovarian transplantations may provide the necessary stromal oocyte recruitment/regeneration signals to the ovary that might have been damaged by previous chemotherapy.

There could also be additional explanations for the better maintenance of fertility after BMTs. Because the animals that were administered sublethal doses of chemotherapy followed by BMT had lower mortality than the controls, the improved fertility might simply have been a result of improvement of the overall quality of life by that procedure. It would have been interesting to see whether mice treated with BMT better maintained their ovarian cyclicity compared with controls. Unfortunately, vaginal smears were not obtained in the study by Lee et al. If a higher fraction of animals continued to cycle in the BMT group, this would explain the higher pregnancy rates.

The authors also observed that BMT had less benefit if mating was delayed for 2 months. Although they interpreted this as evidence for potential interaction between the hormonal milieu of pregnancy and BMT treatment in favor of future fertility, this could have also been a result of an accelerated loss of follicles during the months after chemotherapy. Previous work has suggested that primordial follicles that were exposed to chemotherapy and that seem to be morphologically normal under light microscopy may have ultrastructural damage and are eventually cleared from the ovary. In support of the interpretation of Lee et al, fertility was more severely impaired if BMT was also delayed for 2 months. Thus, it seems that, if BMT is to have a beneficial effect on fertility preservation, it should be performed as soon as the chemotherapy agents are cleared from the systemic circulation.

The study did not address the mechanism of fertility protection by BMT, but the authors ruled out the possibility of an...
immediate germ cell bolus or a salvaging effect on pre-existing oocytes by the transplantsations based on the fact that, even though the BMT-treated animals had severely diminished follicle reserve, the fertility rescuing effect was long lasting. They also found only a small number of donor-originated oocytes, which were not mature enough to be ovulated, and none of the newborns were of donor origin. Thus, the authors hypothesized that the BMT works through reinitiating new oocyte production in the host. It will be important to see whether the BMT-treated animals will have delayed reproductive senescence compared with controls because this would support the authors' hypothesis.

In support of possible new oocyte production in the adult ovary, we observed longer than expected ovarian function in patients undergoing ovarian transplantation with frozen-banked ovarian tissue. In one example, a breast cancer survivor underwent ovarian tissue cryopreservation at the age of 30 years, before undergoing HSCT. She then became menopausal. We transplanted her ovarian tissue 6 years later. Before ovarian transplantation, her follicle density was determined histologically, and given the low numbers, we only expected a short duration of graft function.15 Because of the initial ischemia after ovarian transplantation, further diminishment occurs in the oocyte reserve of grafts.16 Despite these circumstances, not only was this patient’s menopause reversed shortly after ovarian transplantation, but she also continues to have cyclical follicle development in her graft 4 years later. Furthermore, in a fetal ovarian xenograft model, we recently observed that there might be a recovery in primordial follicle numbers after chemotherapy. Because fetal ovary contains predominantly primordial follicles that cannot be distinguished from those in postnatal ovaries, we used it to study the impact of cyclophosphamide on human ovarian reserve in immunodeficient mice. Although 93% of follicles were lost 48 hours after chemotherapy, we observed an increase in the primordial follicle numbers 24 hours later (Fig 1). Although this is a preliminary observation and will need further confirmation, and although fetal ovarian tissue may have regenerative capabilities that are not retained in the postnatal life, this observation nevertheless shows that ovaries can at least partially recover from chemotherapy.

It should also be stressed that, even though BMT may be associated with return of fertility in a portion of patients, this is a rare event, and patients of reproductive age should still be referred for fertility preservation by established techniques, when feasible.18 It should also be remembered that BMTs are currently performed with purified hematopoietic stem cells and differ from the crude preparation used in this rodent study. As the authors showed in their previous work,2 the bone marrow cells that express germ cell preparation used in this rodent study. As the authors showed in their previous work,2 the bone marrow cells that express germ cell origin. Thus, the authors hypothesized that the BMT works through reinitiating new oocyte production in the host. It will be important to see whether the BMT-treated animals will have delayed reproductive senescence compared with controls because this would support the authors’ hypothesis.

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fertility preservation. In the meantime, it is extremely important to determine the mechanism by which the BMTs improve fertility in mice and to continue to counsel young patients regarding available cryopreservation technologies.18

**Fig 1.** Regeneration of primordial follicles after chemotherapy in human ovarian xenografts. Human fetal tissue was xenografted into severe combined immunodeficiency mice (n = 56), and the animals were administered either cyclophosphamide (CY) 200 mg/kg or the vehicle (control). Grafts were recovered 12 to 72 hours after chemotherapy, and follicle density (primordial follicles/mm²) was compared with controls. Although primordial follicle density remained steady from 12 to 72 hours after injection of CY in the controls, follicle numbers sharply declined until 48 hours after chemotherapy injection. However, there was a recovery in primordial follicle counts at 72 hours.

**REFERENCES**