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Ectopic autologous transplantation of ovarian tissue as a feasible technique to assess ovarian morphophysiology

Bárbara Rodrigues Nascimento, Guilherme Antonio de Gouvêa Lopes, Danielle Storino de Freitas and Paulo Henrique Almeida Campos-Junior

Laboratório de Pesquisa em Reprodução e Desenvolvimento, Universidade Federal de São João del Rei. Praça Dom Helvécio, 74 – Dom Bosco, São João del Rei, MG, 36301-160, Brazil

Short Communication

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Author for correspondence:
Paulo Henrique Almeida Campos-Junior. Universidade Federal de São João del Rei. Praça Dom Helvécio, 74 – Dom Bosco, São João del Rei, MG, 36301-160, Brazil.
E-mail: paulohenrique@ufsj.edu.br

Summary

The cryopreservation of murine ovarian tissue and its transplantation can be a promising technique for the preservation of fertility and an alternative for the future reconstitution of scientific valuable strains of mice. Accordingly, the aim of this study was to describe the entire surgical procedure for ovariectomy and dorsal subcutaneous autotransplantation in mice, and also some data about the efficiency of this procedure. Female C57Bl/6J mice ($n = 18$) were anaesthetised and bilaterally ovariectomized. After surgery, ovaries were autotransplanted in small subcutaneous pouches in the dorsal region of the forelimbs. The animals were inspected daily and, 23 days after transplantation, euthanasia and recovery of ovarian tissues were performed. Postoperative recovery, oestrous cyclicity, and folliculogenesis progression were evaluated. At 23 days after transplantation, the recovery of the ovaries was feasible, all classes (primordial to antral) of follicles were observed. Additionally, satisfactory efficiency rates were obtained, with 100% of anaesthesia survival rate, survival, graft recovery, folliculogenesis progression and oestrous cyclicity. In general, this short article describes ovarian ectopic autologous transplantation as an effective technique for maintaining rodent oogenesis and endocrine ovarian function. Even more broadly, we can still assume that the application of this technique may reduce the number of breeding matrices and experimental animals in the near future.

Introduction

The maintenance of reproductive colonies faces many logistical, financial, and ethical challenges (Hart-Johnson and Mankelow, 2021). Therefore, the cryopreservation of germplasm from valuable rodent models can be an alternative to overcome those bottlenecks. Embryo and gamete cryopreservation has been used as the main method (Hart-Johnson and Mankelow, 2021). However, such procedures are expensive due to the requirement for supplies and equipment (Campos-Junior *et al.*, 2016). Conversely, ovarian cryopreservation is a promising tool, allowing the storage of a large number of follicles (Lotz *et al.*, 2016; Donfack *et al.*, 2017). Ovaries can be obtained at any time of the oestrous cycle (Lotz *et al.*, 2016) and for further transplantation. Recipient mice can have their cyclicity and fertility recovered, with more than 60% of viable offspring (Terren *et al.*, 2019); however, this still requires the establishment of more efficient transplantation protocols. Most recently, our laboratory demonstrated that, despite cryopreserved and heterotopically transplanted ovaries having shown several transcriptomic modifications, they were able to sustain complete oogenesis. Therefore, this article aims to describe a surgical procedure for murine ovariectomy and subcutaneous autotransplantation while providing technical details and the efficiency of this technique.

Materials and methods

All procedures were approved by the Ethics Committee on the Use of Animals (025/2018) of the Federal University of São João del Rei. Female C57B1/6J mice ($n = 18$), 6 weeks of age, were used. The sample size was calculated by power analysis. All animals underwent the procedures during proestrus. There was no control group, and it is a descriptive study. The animals were kept under controlled light (12 h:12 h, light:dark cycle), temperature (21°C ± 2°C), and *ad libitum* diet.

Inhalatory anaesthesia with isoflurane was used (Zhao *et al.*, 2020). Meloxicam was administered subcutaneously before and 24, 48 and 72 h after surgery. The animals were trichotomized bilaterally above the hind limbs and on the dorsal midline at the height of the forelimbs, then, the skin was disinfected. A lateral incision (1 cm) was made in the flank region and above the posterior limb. After locating the ovary under the membrane of the peritoneum, an incision (0.5 cm) was made and then the ovary was exposed. After that, a single ligature was performed

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around the oviduct and the ovary was removed. The oviduct was re-inserted into the peritoneal cavity, which was sutured, and the skin incision was closed using metal clips.

After ovariectomy, the gonads were placed in a phosphate-buffered saline solution. The ectopic autotransplantation was performed in the dorsal subcutaneous region of the forelimbs. Two incisions (1 cm) were made forming pockets in which the ovaries were grafted. The incisions were closed with metal clips.

The stage of the oestrous cycle was determined by visual (Champlin *et al.*, 1973) and vaginal cytology methods (Byers *et al.*, 2012). Then, 23 days after transplantation, the animals were euthanized and the grafts were harvested. In some animals ($n = 9$), the transplanted ovaries were fixed and proceeded to follicle quantification (Pereira *et al.*, 2020). The grafts of other animals ($n = 9$) were collected and germinal vesicle (GV) oocytes were obtained according to Pereira *et al.* (2020), in which ovarian grafts were punctured with a 26G needle in a PBS solution supplemented with 20% fetal bovine serum, to assess their ability to support GV oocyte growth. There were no excluded animals, as no animals died during the procedures and there was no need for humane endpoints. The data are shown as mean ± standard error of the mean (SEM) and no comparison tests were used.

Results

All methodological steps were feasible and carried out successfully, as shown in Fig. 1. The results obtained by the autotransplantation surgery in mice are shown in Table 1. The ovaries were successfully recovered 23 days after the procedure (Fig. 2a) and follicles were observed at all stages of development (Fig. 2b). In addition, GV oocytes were recovered (Fig. 2c), which indicated the efficiency of the transplant and recovery of graft functionality.

Discussion

Ovariectomy can be performed through a single incision on the middle back, double dorsolateral incisions, or a single ventral transverse incision in the abdomen (Souza *et al.*, 2019). Due to the quicker postoperative recovery, we used and recommend access through dorsolateral double incisions. The anaesthesia survival rate was 100% (Table 1); currently, isoflurane is considered the anaesthetic agent of choice for laboratory animals (Cicero *et al.*, 2018). Postoperative monitoring is one of the most important steps in animal recovery and, as apparent, it was well done, as no animal was lost (Cicero *et al.*, 2018).

Complete gametogenesis and endocrine recovery can be achieved after ovarian transplantation and this technique would be useful to explore regulatory mechanisms of folliculogenesis (Cao and Lin, 2019). Different heterotopic ovarian autotransplantation sites have been explored to preserve ovarian function (Cao and Lin, 2019). Our study demonstrated that the dorsal subcutaneous region of the forelimbs is a promising site, once all grafts were successfully recovered (Table 1 and Fig. 2a).

The vaginal cytology reflects the endocrine changes of rodent females during an oestrous cycle. At 7–30 days after transplantation, the immature follicles develop until the preovulatory stage (Sugishita *et al.*, 2018), indicating that grafted ovaries resumed their endocrine function and maintained the cyclicity. These facts also corroborate our morphological analysis, in which all follicle classes were observed (Fig. 2b) indicating that ovarian autotransplantation can be used to preserve ovarian morphophysiology and investigate aspects of folliculogenesis (Cao and Lin, 2019).

Table 1. Surgical and follicular parameters for murine ovarian autotransplantation. Rates for surgery are in percentages (%) and follicular and GV oocyte quantities retrieved by graft are mean ± SEM

Success in anaesthesia rate (%)	100
Survival rate (%)	100
Graft recovery rate (%)	100
Oestrous cycle resumption rate (%)	100
Follicular progression to antral stage rate (%)	100
No. healthy follicles/graft (mean ± SEM)	2109.8 ± 15.9
No. atretic follicles/graft (mean ± SEM)	36.0 ± 4.9
No. de GV oocytes recovered/graft (mean ± SEM)	12.0 ± 5.0

GV, germinal vesicle; SEM, standard error of the mean.

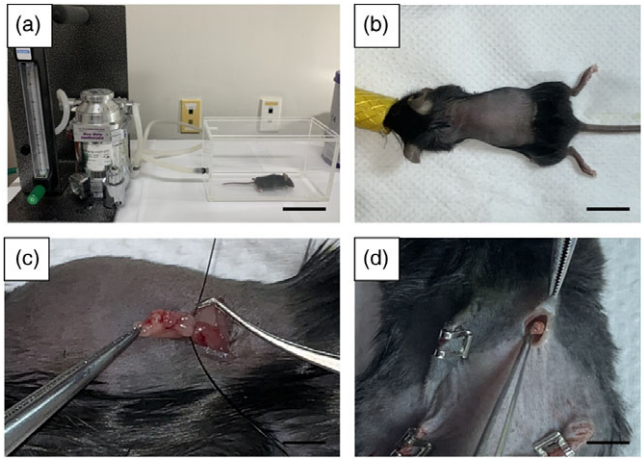

Figure 1. Ovariectomy and autotransplantation. (a) Anaesthetic induction chamber. (b) Animal sedated. (c) Exposed ovary. (d) Ectopic transplantation. Bars: (a) 8 cm; (b) 2.5 cm; (c) 1 cm; (d) 2 cm.

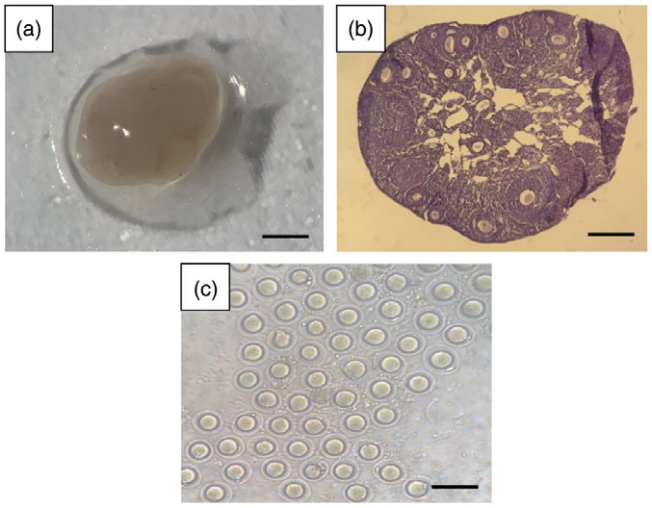

Figure 2. (a) Grafts recovered. (b) Histological picture of ovarian grafts. (c) Germinal vesicles. Bars: (a) 0.7 mm; (b) 200 µm; (c) 150 µm.

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Ovarian grafts are capable of producing oocytes giving birth to live pups (Waterhouse *et al.*, 2004). In our study, GV were recovered, indicating the efficiency of the transplant (Fig. 2c) as the collection of oocytes after the entire procedure is considered a 'gold standard' in the investigation of the graft functionality (Pereira *et al.*, 2020). Therefore, these results indicated that the method of ovary collection and autotransplantation here described has potential application to maintenance of endocrine function and progression of folliculogenesis, however this study has its limitations as the developmental ability of these oocytes was not evaluated.

In conclusion, this article describes briefly ovariectomy and ovarian autotransplantation to the dorsal subcutaneous region and demonstrated that the ovarian follicles developed until the ovulatory stage. Most importantly, this technique has potential use for experimental tests for the maintenance of oogenesis and ovarian endocrine function in rodents, it can be used in animal facilities to reduce the number of necessary laboratory animals.

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Author contributions. PHACJ designed and supervised the experiments, and revised the manuscript. BRN, GAGL and DSF performed the experiments, analyzed all data and prepared the manuscript.

Conflicts of interest. The authors declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Data availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author (paulohenrique@ufsj.edu.br) on reasonable request.

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