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Ovarian Tissue Transplantation in Mice and Rats: Comparison of Ovaries Age

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ABSTRACT

Transplantation of ovarian tissue has been shown to induce puberty and pregnancies successfully. The aims of the present study were to evaluate ovaries age effect on restoration function upon transplantation in mice and rats. Eighteen adult female rats were classified to three equal groups in addition to sex young females as donor of young ovaries. Similar classification was done using eighteen female mice and sex young females. The females of mice or rats were anesthetized for ovarian transplantation using (6.2 and 13.2 mg/kg BW) and (13.3 and 26.6 mg/kg BW) diazepam and xylazine, respectively. The female of control group was subjected to sham operation whereas the other two groups were subjected to ovarian transplantation from adult to adult and (adult-adult) and from young to adult (young-adult). After 8 weeks of ovarian transplantation, the females were mated with vasectomized males and were checked daily for vaginal plug. Females were killed for morphological and histological examination of transplanted ovaries. Blood samples were taken for analysis of complete blood picture. The results indicated presence of a neovascular capillary network around transplanted ovarian tissues. In addition, visible antral follicles and corpora lutea was demonstrated on the ovarian tissues upon transplantation. Histological examination indicated that transplanted young ovarian tissues restored function better than elder ones. It could be concluded that unilateral ovarian transplantation partially restored their developmental competence for producing ova. Such result might be helpful in assisted reproductive studies for treatment of infertility and conservation of species.

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Ovarian transplantation, Ovarian follicles, Mice, Rats, Ovaries age.

INTRODUCTION

In the last decade, interest of ovarian transplantation has grown as an option for infertility treatments or cryopreservation of genetic materials, which may applied for superior animals and/or endangered species and human as well. The results of different studies indicated that ovarian transplants restore function where folliculogenesis achieved upon ovarian transplantation in animals (Hernandez-Fonseca *et al*., 2004; Feng *et al*., 2010; Mohammed, 2012; Mohammed *et al*., 2012) and human. This trend has become an applied tool for studying *in vivo* follicular development in cancer patients. The murine animal has been used in several studies for investigating ovarian transplantation because of the limited availability of human and primates ovaries for such experiment (Eyck *et al*., 2010; Dath *et al*., 2010; Youm *et al*., 2015).

Ovarian restoration function might be affected by age and site of transplantation. Youm *et al*. (2015) compared different ovarian tissue transplantation sites as kidney, capsule back muscle, fat pad, and subcutaneous on the resulting outcomes of grafted ovarian tissue in mice. They found that the oocyte numbers aspirated from transplanted ovarian tissue were the highest in the kidney capsule group and the lowest were in the subcutaneous group. In addition, the percentages of matured oocytes were significantly higher in both kidney capsule and control groups.

In the recent years, the number of patients with cancer has been increased. Cancer treatments such as doses of chemotherapy and/or radiotherapy affect negatively on ovarian follicular structures resulting in infertility (Meirow *et al*., 2007). In addition, transplantation of ovarian tissues is necessitates upon sudden death of superior animals or animals threaten to extinct. Therefore, the aim of this study was to explore the effect of ovarian age on restoration of function upon transplantation in mice and rats.

MATERIALS AND METHODS

Animal management and experimental groups

The study was carried out at the lab animals of Human Medicine, Faculty of Assiut University. Eighteen adult female rats (320.6 ± 20.7 g body weight) were divided into three equal groups in addition to sex young females as donor of young ovaries for 3.0 months. Similar classification was done using eighteen adult female (6-8 weeks old) mice (22.67 ± 0.67 g body weight) and sex young females. The females of mice or rats were anesthetized for ovarian transplantation using (6.2 and 13.2 mg/kg BW) and (13.3 and 26.6 mg/kg BW) diazepam and xylazine,

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respectively (Mohammed *et al*., 2012; Mohammed, 2012). The female of control group was subjected to sham operation whereas the other two groups were subjected to ovarian transplantation from adult to adult (adult-adult) and from young to adult (young-adult). The diet and water were available *ad libtum* to animals during the study. Throughout the experimental period, an appropriate animal care protocol was applied for safety. Animals were left for two months after ovarian transplantation and were inseminated with vasectomized males.

Ovarian transplantation

Ovarian transplantation was done as previously indicated (Dorsch *et al*., 2004). Briefly, an incision at the right dorsal of the skin gave access to the right ovary on right side. The recipient female of ovarian transplantation was adult female. It was transplanted with adult ovary from adult female (adult-adult group) or young ovary from young female (young-adult group). Ovarian transplantation was performed through small incision in the fat surrounding the ovarian bursa to uncover the right ovary in two females at the same time. The recipient's right ovary was removed switched and transplanted into recipient adult female (adult-adult group) whereas young ovary was transplanted into adult recipient (young-adult group). Then, the slit in the fat surrounding the ovarian bursa was closed. The ovarian complex was returned to the body cavity, and the incision was closed.

Blood sample collection and analysis

Blood samples were aspirated from females of each group after four weeks of ovarian transplantation. Blood samples were collected from the orbital sinus as described by Hoff (2000). Whole blood samples were analyzed for complete blood picture using Sysmex XP-300 (Japan).

Tissue collection and processing

The females were killed by cervical dislocation after three days of mating with vasectomized male (Mohammed and Attaai, 2011). Ovarian tissues were collected and fixed in Bouin's fluid. Ovarian tissue processing were carried out according to the method of Myers *et al*. (2004). The samples were processed through graded alcohols and then embedded into paraffin wax. Paraffin-embedded ovaries were sectioned serially at 3 mm and stained with a modified Masson trichrome stain.

Reproductive evaluation

Upon ovarian transplantation, the females were mated with vasectomized males and were checked daily for vaginal plug. The animals were killed for morphological and histological examination of the ovaries. Ovaries with visible ovarian follicles and corpora lutea were considered active. In addition, the collected ovaries were fixed in in Bouin's fluid for histological examination. The numbers of secondary follicles, graffian follicles and corpora lutea per ovaries of each group were counted.

Statistical analysis

Data of histology and whole blood picture are presented as means ± SD. Differences between values of groups were determined by ANOVA, then followed by comparisons using the Duncan's multiple range test using SAS Program (2008). Differences with $P < 0.05$ between groups were considered significant.

RESULTS AND DISCUSSION

Reproductive evaluation

The numbers of secondary and graffian follicles in addition to copora lutea were significantly ($P < 0.05$) reduced upon ovarian transplantation. In addition, the aforementioned structures were none significantly higher upon young-adult ovarian transplantation compared with adult-adult ovarian transplantation (Tables I, II).

Table I.- Ovarian characteristics upon adult- adult and young-adult ovarian transplantation in mice.

Treatments	Control	Ovarian transplantation	
		Young-adult	Adult-adult
Females	6	6	6
Mated females	6	6	6
Secondary follicles	34.5a±2.08	31.0b±1.15	27.75c ± 1.70
Graffian follicles	3.5a±1.29	1.75b±0.50	1.5b ± 0.57
Corpora lutea	8.0a±0.81	6.25b±0.95	5.5b ± 1.29

a, b, c, values with the different superscripts in the same row differ significantly (P<0.05).

Table II.- Ovarian characteristics upon young and adult ovarian transplantation in rats.

Treatments	Control	Ovarian transplantation	
		Young-adult	Adult-adult
Females	6	6	6
Mated females	5	4	5
Secondary follicles	12.5a±1.29	8.25b±1.52	6.75b±1.0
Graffian follicles	2.0a±0.81	1.75ab±0.57	1.0b ±0.0
Corpora lutea	6.0a±1.15	4.75ab±0.57	4.25b±1.15

a, b, values with the different superscripts in the same row differ significantly (P<0.05).

Restoration of ovarian activity were occurred upon ovarian transplantation (Fig. 1). Visible antral follicles and copora lutea were observed on the ovarian surface upon transplantation. Number of growing follicles and corpora lutea were decreased upon ovarian transplantation in mice and rats compared with control (Tables I, II). The reduction was more pronounced in adult-adult compared to young-adult transplanted ovaries. This might be related to reduction of ovarian follicles in the adult animals compared to growing animals because of follicle degeneration occurred with age advancement. Development of ovarian follicles after transplantation has been reported in species as cattle (Semple *et al*., 2000), monkey (Candy *et al*., 1995), rat (Mohammed *et al*., 2012) and human (Gosden *et al*., 1994; Weissman *et al*., 1999). Surprisingly, restoration of ovarian activity upon ovarian transplantation occurred without blood vessels anastomoses in ovarian bursa. It has been indicated that the preferred site for grafting ovarian tissue into the body was under the kidney capsule (Weissman *et al*., 1999). The authors mentioned that the transplantation sites rich in vascularization have been chosen where the kidney receives about 20% of the cardiac output.

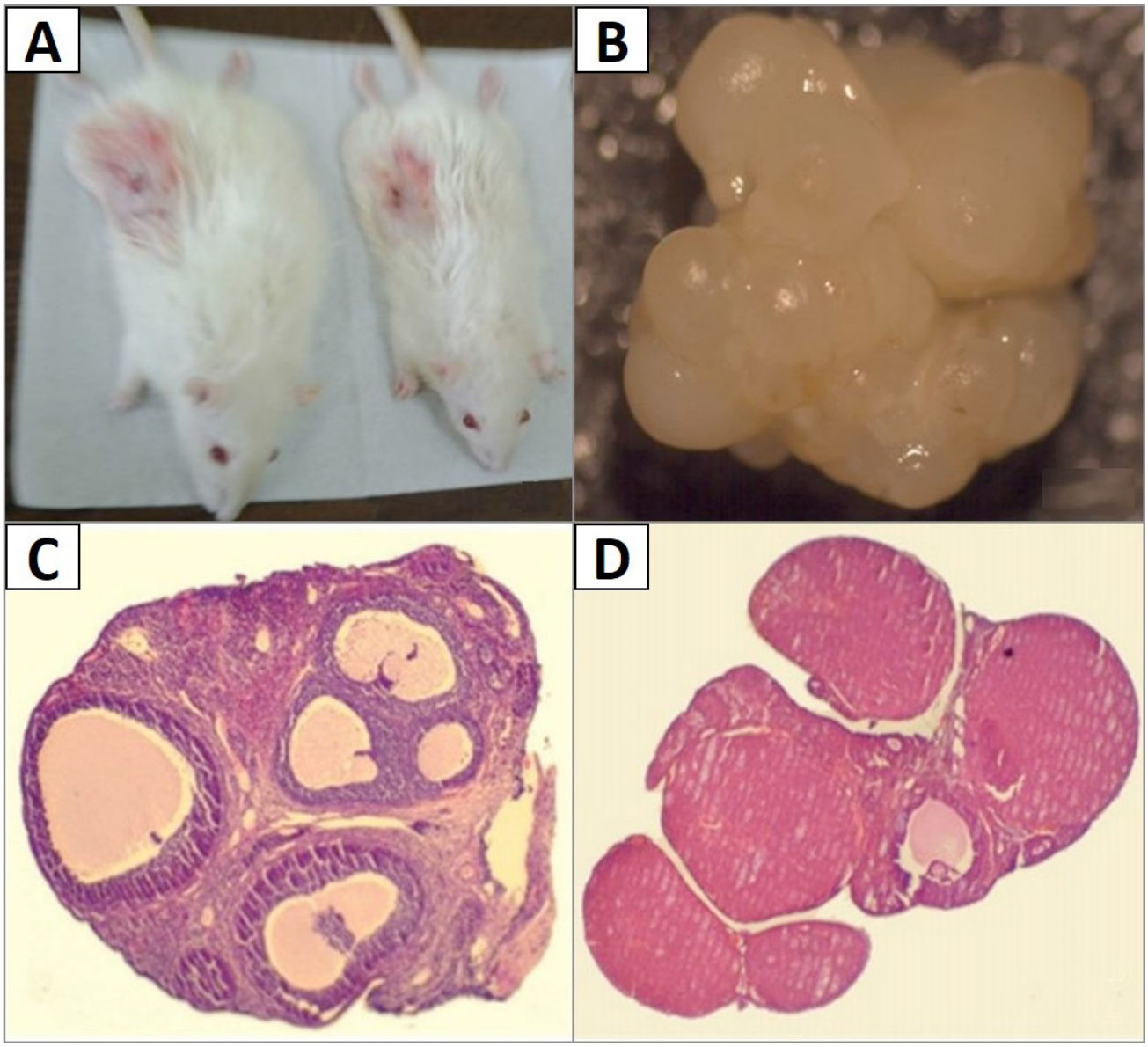

Fig. 1. Ovarian transplantation process and the resulting ovarian structure. **A,** ovarian transplantation process; **B,** morphology of active transplanted ovary; **C,** growing follicles of active transplanted ovary; **D,** copora lutea of active transplanted ovary.

In addition, kidney capsule was an appropriate securing place for ovarian grafts although the kidney capsule exerts pressure on the grafts, which might limit the expansion of growing follicles (Gosden *et al*., 1994). Thereafter, other studies examined the spaces that would provide adequate room for follicle development to occur (Gosden *et al*., 1994; Candy *et al*., 1995; Weissman *et al*., 1999) but the diameter of antral follicles did not exceed 6 mm, which may indicate that the site of transplantation is not critical. It has been suggested that follicles development in grafted ovarian tissues is enhanced in the absence of the host's own ovaries (cited in Nugent *et al*., 1997). In this study, restoration of transplanted ovarian tissue has been restored in the presence of host's own ovaries. Female and male mice as recipients of human ovarian tissue were compared (Weissman *et al*., 1999). The results proved that male mice had significantly more large follicles than female mice. The influence of the host's gonads on follicular development upon ovarian grafts needs to be addressed.

Table III.- Complete blood picture upon ovarian transplantation in mice.

Items	Control	Ovarian transplantation	
		Young - adult	Adult - adult
Body weight, g	333.6 ± 20.6	305.0 ± 20.7	323.3 ± 35.0
Red blood cells, 10^6/µl	9.27 ± 0.597	8.77 ± 0.31	8.80 ± 0.29
Hemoglobin, Hb g/dl	14.03 ± 0.99	13.36 ± 0.95	13.35 ± 0.96
Packed cell volume, PCV %	44.16 ± 3.00	43.86 ± 4.0	44.03 ± 3.86
Mean cell volume, MCV fl	49.73 ± 1.98	49.46 ± 2.11	49.43 ± 2.14
Mean cell hemoglobin, MCH pg	15.15 ± 0.53	15.43 ± 0.57	15.15 ± 0.62
Mean corpuscular hemoglobin concentration, MCHC g/dl	27.88 ± 1.26	26.8 ± 0.32	26.68 ± 0.27
White blood cells, 10^3/µl	8.56 ± 0.31	9.0± 0.38	9.13 ± 0.84
Lymphocyte, %	80.13 ± 3.72	81.5 ± 3.54	81.75 ± 3.4
Neutrophil, %	14.51 ± 2.9	13.34 ± 2.87	13.59 ± 2.73
Monocyte, %	3.70 ± 1.15	3.67 ± 1.03	3.33 ± 0.81
Eosinophil, %	1.25 ± 0.88	1.16 ± 1.12	1.0 ± 1.22
Basophil, %	0.41 ± 0.4	0.33 ± 0.25	0.33 ± 0.20
Platelets, µl	812 ± 121.65	727 ± 87.19	736 ± 79.75

Table IV.- Complete blood picture upon ovarian transplantation in rats.

Items	Control	Ovarian transplantation	
		Young - adult	Adult - adult
Body weight, g	22.98 ± 0.29	22.48 ± 0.83	22.57 ± 0.77
Red blood cells, 10^6/µl	7.62 ± 1.29	7.19 ± 1.23	7.60 ± 1.26
Hemoglobin, Hb g/dl	13.86 ± 2.40	13.03 ± 2.14	13.73 ± 2.26
Packed cell volume, PCV %	43.53 ± 2.52	42.33 ± 3.24	42.5 ± 3.09
Mean cell volume, MCV fl	55.85 ± 5.54	57.5 ± 4.44	54.73 ± 4.18
Mean cell hemoglobin, MCH pg	17.05 ± 1.118	16.33 ± 0.44	16.18 ± 0.35
Mean corpuscular hemoglobin concentration, MCHC g/dl	30.93 ± 5.24	27.5 ± 0.71	27.76 ± 0.65
White blood cells, 10^3/µl	9.45 ± 1.42	9.6 ± 0.464	8.716 ± 0.36
Lymphocyte, %	75.54 ± 2.45	75.86 ± 1.36	75.10 ± 1.50
Neutrophil, %	23.01 ± 2.77	22.7 ± 1.87	23.05 ± 2.06
Monocyte, %	0.37 ± 0.24	0.45 ± 0.23	0.37 ± 0.24
Eosinophil, %	0.83 ± 0.314	0.83 ± 0.314	0.83 ± 0.27
Basophil, %	0.25 ± 0.273	0.16 ± 0.25	0.20 ± 0.27
Platelets, µl	378.66 ± 84.77	381.33 ± 64.23	376.67 ± 60.36

Complete blood picture

The complete blood picture gives an important indication about red blood cell (RBC) and white blood cells (WBC). It helps to diagnose disorders as anemia and infection due to bleeding upon surgery. The results indicated that the ovarian transplantation did not change the characters of complete blood count (Tables III, IV).

Red blood cell (RBC) numbers and their characters (Hb, PCV, MCV, MCH and MCHC) are important in gas transport in the body (oxygen and carbon dioxide). In addition, white blood cells (WBC) protects the body against infection with virus, bacteria or other organisms. White blood cell types (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) play major roles in protecting the body against infection. This is an indication of the safety of ovarian transplantation on the body.

CONCLUSION

It could be concluded that unilateral ovarian transplantation partially restored their developmental competence for producing ova compared with control. The mechanism behind spontaneous restoration of ovarian activity needs to be explored. Further laboratory research will be needed to explore *in vitro* embryo production (maturation, fertilization and culture) of oocytes aspirated from antral follicles of transplanted ovarian tissues (Mohammed *et al*., 2008, 2010).

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Statement of conflict of interest

Author has declared no conflict of interest.

REFERENCES

Candy, C.J., Wood, M.J. and Whittingham, D.G., 1995. Follicular development in cryopreserved marmoset ovarian tissue after transplantation. *Hum. Reprod.,* **10**: 2334–2338. https://doi.org/10.1093/oxfordjournals.humrep.a136295

Dath, C., Eyck, A.S.V., Dolmans, M.M., Romeu L., Vigne, L.D., Donnez, J. and Van Langendonckt, A., 2010. Xenotransplantation of human ovarian tissue to nude mice: comparison between four grafting sites. *Hum. Reprod.,* **25**: 1734–1743. https://doi.org/10.1093/humrep/deq131

Dorsch, M., Wedekind, D., Kamino, K. and Hedrich, H.J., 2004. Orthotopic transplantation of rat ovaries as a tool for strain rescue. *Lab. Anim.,* **38**: 307–312. https://doi.org/10.1258/002367704323133691

Eyck, A.S.V., Bouzin, C., Feron, O., Romeu, L., Langendonckt, A.V., Donnez, J. and Dolmans, M.M., 2010. Both host and graft vessels contribute to revascularization of xenografted human ovarian tissue in a murine model. *Fertil. Steril.,* **93**: 1676–1685. https://doi.org/10.1016/j.fertnstert.2009.04.048

Feng, W., Cui, Y., Zhan, H., Shi, M., Cui, W., Guo, K., Li, Q., Song, C., Zhang, Y., Mori, T., Gershwin, M.E., Abraham, N.G. and Ikehara, S., 2010. Prevention of premature ovarian failure and osteoporosis induced by irradiation using allogeneic ovarian/bone marrow transplantation. *Transplantation,* **89**: 395-401. https://doi.org/10.1097/TP.0b013e3181ca86bb

Gosden, R.G., Boulton, M.I., Grant, K. and Webb, R., 1994. Follicular development from ovarian xenografts in SCID mice. *J. Reprod. Fertil.,* **101**: 619–623. https://doi.org/10.1530/jrf.0.1010619

Hernandez-Fonseca, H., Sirisathien, S. and Brackett, B.G., 2004. Effect of site of transplantation on follicular development of human ovarian tissue transplanted into intact or castrated immune deficient mice. *Fertil. Steril.,* **81**: 888-892. https://doi.org/10.1016/j.fertnstert.2003.10.017

Hoff, J., 2000. Methods of blood collection in the mouse. *Lab. Anim.,* **29**: 47-53.

Meirow, D., Baum, M., Yaron, R., Levron, J., Hardan, I., Schiff, E., Nagler, A., Yehuda, D.B., Raanani, H., Hourvitz, A. and Dor, J., 2007. Ovarian tissue cryopreservation in hematologic malignancy: Ten years' experience. *Leuk. Lymphoma,* **48**: 1569–1576. https://doi.org/10.1080/10428190701471957

Mohammed, A.A., Karasiewicz, J. and Modlinski, J., 2008. Developmental potential of selectively enucleated immature mouse oocytes upon nuclear transfer. *Mol. Reprod. Dev*., **75**: 1269-1280. https://doi.org/10.1002/mrd.20870

Mohammed, A.A., Karasiewicz, J., Kubacka, J., Greda, P. and Modlinski J., 2010. Enucleated GV oocytes as recipients of embryonic nuclei in the G1, S or G2 stages of the cell cycle. *Cell Reprogram.,* **12**: 427-435. https://doi.org/10.1089/cell.2009.0107

Mohammed, A.A. and Attaai, A.H., 2011. Effects of dietary urea on timing of embryo cleavages and blood components in mice. *Vet. World,* **4**: 360-363.

Mohammed, A.A., 2012. Anesthesia Induction and Reproductive Performance in relation to Diazepam and Xylazine injection in mice. *Egypt. J. Basic appl. Physiol*., **11**: 1-11.

Mohammed, A.A., Abdelnabi, M.A. and Modlinski, J.A.,

2012. Evaluation of anesthesia and reproductive performance upon diazepam and xylazine injection in rats. *Anim. Sci. Pap. Rep.,* **30**: 285-292.

Myers, M., Britt1, K. L., Wreford, N. G. M., Ebling, F.J.P. and Kerr, J.B., 2004. Methods for quantifying follicular numbers within the mouse ovary. *Reproduction,* **127**: 569–580. https://doi.org/10.1530/rep.1.00095

Nugent, D., Meirow, D., Brook, P.F., Aubard, Y. and Gosden, R.G., 1997. Transplantation in reproductive medicine: previous experience, present knowledge and future prospects. *Hum. Reprod. Update,* **3**: 267–280. https://doi.org/10.1093/humupd/3.3.267

Semple, E., Weissman, A., Gotlieb, L., Casper, R.F. and Leibo, S.P., 2000. Transplantation of fresh or cryopreserved bovine ovarian cortex to NOD-SCID mice. *Theriogenology,* **53**: 264.

Statistical Analysis System (SAS). 2008. *User's guide: Statistics*. Version 9.2. SAS Institute, Cary, NC, USA.

Weissman, A., Gotlieb, L., Colgan, T., Jurisicova, A., Greenblatt, E. M. and Casper, R.F., 1999. Preliminary experience with subcutaneous human ovarian cortex transplantation in the NOD-SCID mouse. *Biol. Reprod.,* **60**: 1462–1467. https://doi.org/10.1095/biolreprod60.6.1462

Youm, H. Y., Lee, J. R., Lee, J., Jee, B.C., Suh, C.S. and Kim, S.H., 2015. Transplantation of mouse ovarian tissue: Comparison of the transplantation sites. *Theriogenology,* **83**: 854–861. https://doi.org/10.1016/j.theriogenology.2014.11.026