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Subcutaneous ovarian transplantation for short term preservation in cat

Awban Aktham Al-Any , Baqer J. Hasan

[1-3] Department of Biology, College of Education, Al-Qurna, University of Basra

[2] Department of Chemistry, College of Education for Pure Sciences, University of Basra

[1]*E-mail: awban90aktham@gmail.com*

[2]*E-mail: Baqer.hikma.iq@gmail.com*

Abstract

An ovarian transplantation procedure in cat carried out without angioanastomosis on 10 donors in aimed of 10 recipient's cats. The ovaries of donors cats had been removed and sliced then placed in buffer saline followed by histopathological evaluation, transplanted into recipient cats subcutaneously. One month after surgery, the recipient cats treated with PMSG+hCG hormones to activate the transplanted ovaries. The response to hormonal activation were assessed using two methods, the first was ultrasonographic examination to monitoring follicular development and the second done by estimation of blood estrogen hormone after operation in comparison with before operation. The results show there are significant changes in serum estrogen concentration before and during different times (days) after hormonal treatment of recipient cats in comparison with before treatment which may indicate of ovarian activation containing primary and secondary follicles , this result confirmed by ultrasonography for follicular detection. In conclusion subcutaneous allo-ovarian transplantation could be in immunosuppressed cat with no any visible complications. The study also shows that there are remaining levels of estrogen hormone even after ovariectomy in cat. Finally administration of PMSG + hCG hormone could activate the subcutaneous transplanted ovaries by activation of follicular development.

Key words: ovarian, Transplantation , Cat , allo-ovarian transplantation

I. Introduction

The female cat reproductive system includes several important organs , such as the ovaries, uterine tubes, uterus, cervix, and vagina. The knowledge of these organs is important for both clinical veterinarians and feline reproduction (Hammond, 2009). Organ transplantation is one of the most advanced medical techniques to be developed so far, and how to manage its medical aspects appears in problems in which the body rejects an implanting organ and so becomes a cause of failure in organ transplantation, (Eilts,2013) (Kondapalli et al., 2012). Subcutaneous ovarian transplantation in the queen could be considered as a useful method for saving reproductive potential. It has been reported that this approach can help to sustain and preserve ovarian follicles (Vilela et al., (2019). There was an study reported 38.4% of the implanted ovaries presented a large population of primordial (59.75%) and primary follicles (40.25%) after 120 days (Crestana et al., 2006). Grafted ovarian tissue appeared revascularized within the first week, suggesting possible functional integration (Vilela et al., 2019). In study of histological analysis showed that the tissue architecture was maintained, and primary follicles were significantly larger in transplanted than in the control ovaries (Crestana et al., 2006). The study indicate that subcutaneous ovarian transplantation may be a useful alternative to improve reproductive longevity directly in female cats, however, more studies are necessary to further refine the implantation technique and to investigate long- term results (Crestana et al., 2006). In contrast subcutaneous transplantation has been found to be

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successful, as ovarian remnants within the peritoneal cavity allow for survivors, (DeNardo et al., 2001). The objectives of this research were to observe ovarian activity of transplanted ovaries and to investigate physiological changes in ovarian tissue following subcutaneous allo-ovarian transplantation.

II. Materials and methods

Animals od study: The study based on 20 sexually mature female cats (10 donor and 10 recipient cat) collected randomly from patient houses as adoption, their aged ranged from 1.5-3 years , their weight was ranged from 2.5-3 kg . The animals of study kept in separated cages (1-2 meter) in house animals belong to department of surgery and obstetrics, college of veterinary medicine – Baghdad University, Baghdad / Iraq, with a heavy diet consist of dry food , chicken meat with good management along the period of study .

Procedure

The recipient cats receive sliced ovary from histologically assessed donor cats subcutaneously after one month of operation . All females treated with prednisone 2 mg/kg for 6 days (Khelik et al., 2019) as short term immunosuppressive agent starting from transplantation day to prevent acute rejection (Matas et al., 2004)**.** Estimation of blood levels of Estrogen hormone in studied animals before PMSG+hCG hormones activation. Administration of PMSG 100 IU IM 1st day, then 50 IU IM on 2nd and 3rd day + hCG 500 IU on 7th day. The hormonal treatment starting from 14th day after implantation (Kutzler , 2007). Under aseptic condition, the animals cleaved and shaved, tincture iodine had been used for skin disinfectant, after that general anaesthesia using Xylazine 0.5 mg/kg and ketamine 5mg/kg had been done (Maryatmo et al.,2022) . A small incision (2-3cm) had been made in the donor cat's abdominal midline. The ovaries of both side which are found in medial and lateral sides of linea alba near the diaphragm had been remove carefully after that the collected ovaries kept in buffer isotonic saline ,then preserved in a cold solution tell transplantation complete . A small incision is made under the skin of the recipient cat under general anaesthesia , where the preserved ovary is implanted in subcutaneous tissue in dorsal part of animals near the neck (Leonel et al.,2018). The skin closed using non absorbable suture material (3.0 surgical Silk) then daily monitoring observation of cat for any complications which may occurs. Detection of animals response by Estimation of estrogen concentration in blood of all treated animals (15th , 30th days) after hormonal treatment in comparison with hormonal concentration before operation using CobasE411 device, (Roche company, Made in Germany) (Leonel *et al.*, 2018). Ultrasonographic monitoring of transplanted ovary performing two times every 2 weeks (after hormonal treatment) to evaluate the ovarian and follicular statement and development as similarly performed for age estimation of fetus, conception status, size of placentome, viability status of fetus and blood haematological traits related to reproduction in small ruminants (Al-Rawi, 2007; Hussein, 2017; Alwan, 2016; Khazaal et al., 2023).

III. Results

The results presented in (Table 1) demonstrate a high response rate to the combined hormonal treatment (PMSG + hCG) in subcutaneous ovarian transplantation cats (80% response and 20% no response). The chi-square test ($\chi^2 = 3.60$) and the P value (0.05) indicate a significant relationship between the treatment and successful ovarian response, and therefore, this hormonal protocol appears to promote graft function. These results are supported by other research groups that have shown that PMSG and hCG hormones are able to induce follicular growth and development in transplanted ovarian tissues, even if engraftment does not occur under physiological circumstances like the subcutaneous transplantation model. This high response rate is also supported by ultrasonographic observations (high multifollicular development was visible in 80% of treated cats). The presence of a number of follicles demonstrates that the transplanted ovary preserved its normal response to hormonal stimulation, and a sufficient potential capacity to produce more than single oocyte in the presence of the treatment used. According to Table 2, E2 concentration was evaluated at three different phases like, before transplantation of ovary and hormonal therapy and at 15 and 30 days after PMSG injection

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and transplantation. The low baseline estrogen level (7.19 ± 0.55 pg/mL, mean ± SE) was considered pre-transplant state in recipient cats. After transposition and hormonal protocol (PMSG + hCG) activation, it was found a similar significant increase of estrogenic hormones. The average estrogen concentration significantly increased to 23.45 ± 2.06 pg/mL at 15 days post-treatment, representing the early follicular stimulation and development of the transplanted ovarian tissue. This rise reflects the first phase of the graft response to exogenous gonadotropins. At day 30 after treatment, the serum estradiol levels of animals reached 42.32 ± 4.17 pg/mL, indicating a more develop and mature follicles (progressive development and maturation of the follicles after continuous stimulation of hormone). The significant differences that were observed among these stages (indicated by distinct superscripts, a, b, c) clearly indicate a progression with time of a functional endocrinological status of the ovary that is consistent with the ultrasonographic results of multiple growing follicles. This rising curve of estrogen shows a functional recovery and responsiveness of the transplanted ovarian tissue, and the treatment of hormone is effective for the maintenance of folliculogenesis and steroidogenesis after subcutaneous transplantation.

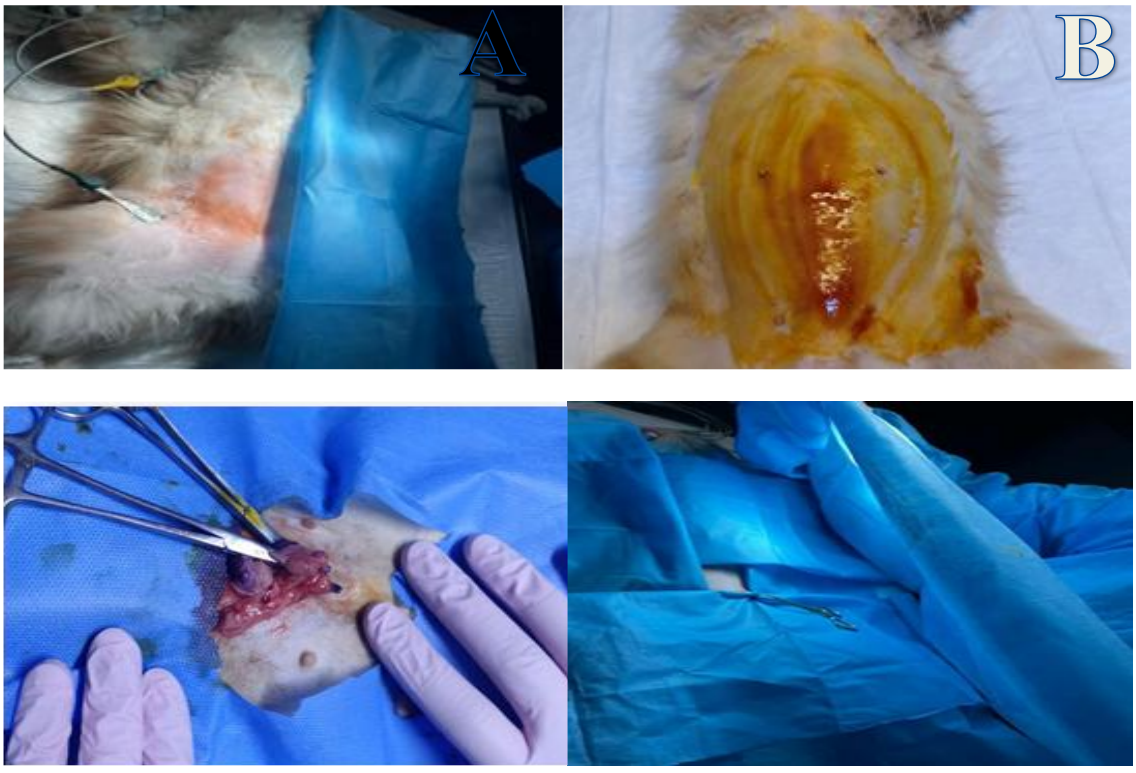

Figure (1) : Ovariectomy procedure in cat. **A**. clipping and shaving. **B**. general anesthesia (ketamine and Xylazine).**C.** Preparation of animal to operation. **D**. removing of ovaries.

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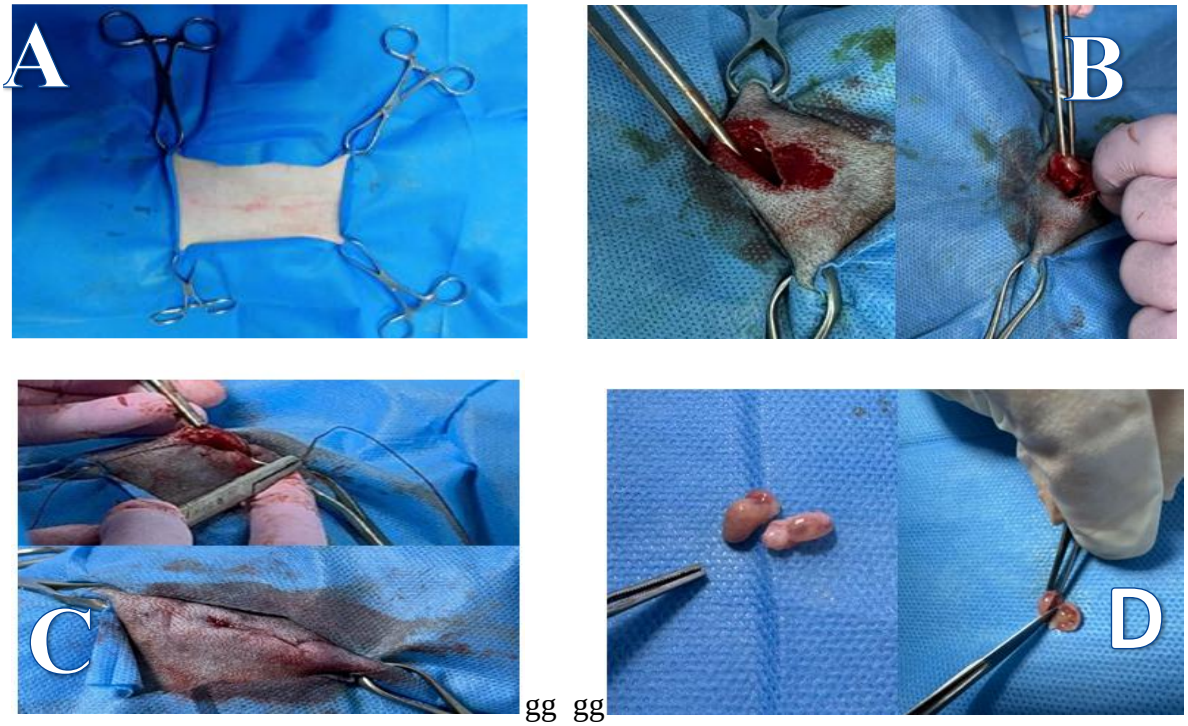

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Figure (2) : Procedure of **Allo-ovarian transplantation** in cat. **A** . **Preparation** of animal for surgery **B**. slicing of ovaries **C**. ovarian implantation **D**. skin closing

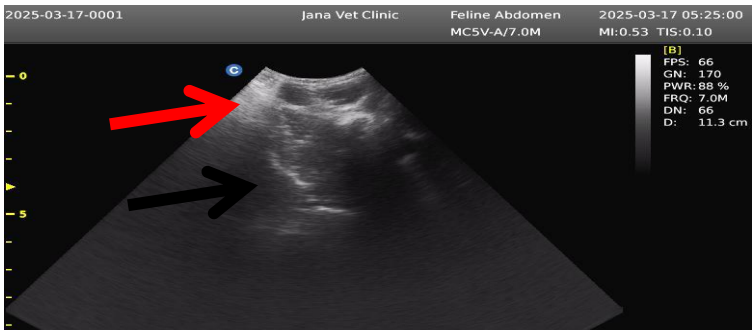

Figure (3): **Ultrasound Examination for Assessing Follicular Growth After Ovarian Transplant in Cats. (black arrow= ovary , red arrow = follicles)**

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Table (1): Animals response to hormonal treatment (PMSG + hCG hormones) in cats after subcutaneous ovarian transplantation

animals	number	percentage of response %	Chi-square value	P-value
responded	8/10.	80%	3.60	0.05
non responded	2/10.	20%		

.Table (2): Changes in blood estrogen concentration before and during different times (days) after hormonal treatment of recipient cats.

No	E2 before PMSG injection (pg/mL)	After 15 days of PMSG injection (pg/mL)	After 30 days of PMSG injection (pg/mL)
1	8.357	16.101	31.558
2	7.705	27.664	54.519
3	5.976	27.664	48.81
4	5.143	13.632	30.289
5	8.469	31.209	24.421
6	6.706	19.898	23.683
7	9.016	33.45	55.114
8	5.095	18.832	51.328
9	5.5	21.741	48.245
10	9.989	24.394	55.33
Mean± SE	7.19±0.55c	23.45±2.06b	42.32±4.17a
LSD		7.85	

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IV. Discussion

Most ovaries vesicles tissue is found survived post-transplantation or otherwise it maintained their morphology nevertheless have the other histopathological images were shown normal ovarian tissue with primary survival vesicles, these work agree with other authors (Salle,1998; Callejo ,1999; Salle, 2002),when they reported that different volume of capillaries were survived after transplantation examination of ovary that did not changes during normal saline maintenance due to the short period of time when transplantation done from donor to receptor and use channel antagonist drug and many other authors who used of lypholization technique, to transplant ovary tissue before encourage it to new vascularization due to rapprochement of ovary tissue with superficial blood vessels which surrounded (Liu J,2002). The transplanted ovary without blood vasculature anastomosis to survive and maintain these on ovary morphologic without injury is proved by ultrasonography (Liu ,2002) and disagree with other (Wang,2002).

Conclusions : the study concluded that subcutaneous allo-ovarian transplantation could be done in immunosuppressed cat with no any visible complications. There are remaining levels of estrogen hormone even after ovariectomy in cat. Administration of PMSG + hCG hormone could activate the subcutaneous transplanted ovaries by making follicular development and estrogen hormone levels elevation.

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