INTRAUTERINE TRANSPLANTATION OF EWE OVARIES TO PSEUDOPREGNANT RABBIT AS A MODEL OF THE GERM PLASM PRESERVATION FROM ENDANGARED SPECIES

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Introduction

The number of species and the genetic variability within species (biodiversity) is decreasing at alarming rate. The preservation of germ plasm from endengered species (genome resource bank) would augment captive breeding programs aimed at addressing this loss.

Xenografting provides an alternarive method for the in *vivo* maturation of oocytes and in this process; both nuclear and cytoplasmic maturation can be completed. (Cushman et *al*., 2002). Ovarian xenografting has provide a valuable experimental tool to study follicular growth in vivo for range of species including human and allows follicular development to the antral stage (Liu *et al*., 2002).

Mammalian ovaries have been transplanted into the uteri of pregnant or pseudopregnant rats that had immunological tolerance, and the survival rate of the transplanted ovaries has been examined. (Kagabu and Urnezu, 2001). This study aimed to determine the development follicles and oocytes viability of ewe ovaries post-transplantation intrauterine to pseudopregnant rabbit.

Materials and Methods

Ewe ovaries were collected from local slaughterhouse and transported (30 -35°C) to the laboratory in 0.9% NaCl solution immediately following collection. The ovaries were washed twice in 0.9% NaCl solution and sectioned into 4 pieces per ovary then ready to be transplant.

New Zealand White female rabbit (2.5 – 3.0 Kg) used as recipient was induced by artificial copulation. The artificial copulation conducted to the female rabbit was day 1 of pseudopregnancy. The procedure for grafting of the ewe ovaries intrauterine to pseudopregnant rabbit followed the method of Kagabu and Umezu (2001).

Posttransplantation in days 5 or 7, the transplanted ovaries were recollected and fixed in Bouin, prepared by paraffin method and stained with hematoxylin-eosin. Follicles were classified as Primordial, Primary, Preantral and Antral. To evaluate the viability of ewe oocytes after ewe ovarian transplantation, the oocytes were collected from the ovaries by slicing method and matured in Tissue Culture Medium (TCM)-199 and incubated in CO_2 incubator with 5% CO_2, 38°C for 24 h. After maturation, the oocytes were stained with 2% aceto-orcein to determine the nuclear status. The number of follicles and matured oocytes were analyzed by Anova and DNMRT.

Results and Discussion

The number of primary follicle in 5 and 7 days after transplantation were decreased significantly higher ($p<0.05$) from control (Table1).

Table 1. The avarage number of primordial, primary, preantral and antral follicle

Posttrans-plantation (davs)	Follicles				Total
	Primordial	Primary	Preantral	Antral	
0 (control)	(683.7±61.55)a	(452.2 ±41.46)a	(38.8±4.73)a	(5.7 ±1.83)a	11.808
5	(634.7±56.88)a	(345.1±43.57)b	(32.6±3.67)b	(5.4±0.84)b	10.178
7	(532.4±46.66)b	(240.1±35.30)c	(19.0±2.87)c	(3.2±1.03)c	7.947

Value within column with different superscript are significantly different ($p<0.05$)

The quality of ewe ovarian posttransplantation decreased as long as day of transplantation. Alternatively, differences in nutrient and oxygen supplysuplay to the ovarian transplant may inhibition of activation tissue metabolism of transplant that occur in rabbit uterine cavity. Normally, the ovaries were suplayed the energy to cells metabolism from blood vessel directly?. Without direct connection of blood vessel, the transplanted ovaries were reached the nutrient from solution (uterine rnitk) in the uterine cavity ?(Gunasena et *al.*, **1998;** Snow *et al.*, 2002).

Tabel 2. Nuclear status of ewe oocytes after maturation 24 hours

Day	Number of Oocytes	Number of oocytes with nuclear status... (%)			
		GVBD	M-I	M-II	NI
P5	119	32 (26,89)a	38 (31,92)a	**45** (37,82)b	**4** (3,36)
P7	103	**29** (28,16)a	**35** (33,98)a	33 (32,03)c	6 (5,83)
Kontrol	119	21 (17,65)b	27 (22,69)b	68 (57,68)a	3 (2,52)

GVBD: Germinal Vesicle Break Down, M-I: Metaphase I, M-II: Metaphase II, NI: no identified.
Value within column with different superscript are significantly different ($p<0.05$)

After maturation,the oocytes from transplanted ovaries could reach the M-II phase from five days post-transplantation 37.82% and seven days 32.03% decreased significantly ($p<0.05$) compared to the control 57.68% (Table 2). There was no directed connection betwen transplant and uterine endometrium due to the ewe ovaries only depent on the secretion of uterine gland. Kagabu and Umezu (2001) found that the transplanted ovaries in uteri resulted in low survival rate of ovaries, indicating the need improvement of the method.

Conclusion

It can be concluded that at all stages, the follicles and oocytes viability of ewe ovarium post-intrauterine transplantation to pseudopregnant rabbit were still preserved during intrauterine transplantation.

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