

HISTOPATHOLOGICAL OVERVIEW OF NEW ZEALAND WHITE PSEUDO-PREGNANT RABBIT UTERUS AFTER OVARY TRANSPLANTATION WITH VARYING DURATION OF OVARIES INSIDE THE UTERUS

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ABSTRACT

This study aims to understand the histopathological changes in the pseudo-pregnant rabbit uterus concerning the duration of ovaries inside the uterus during ovarian transplantation. The study used nine New Zealand White (NZW) pseudo-pregnant female rabbits aged 3-5 years, weighed 1.5-2.9 kg. After a 30-day adaptation period, all rabbits were divided into three treatment groups: K1 (n=3) underwent ovarian transplantation for 3 days, K2 (n=3) for 5 days, and K3 (n=3) for 7 days. Pseudo-pregnant was induced using PMSG and hCG. The rabbits were intramuscularly injected with 100 IU of PMSG, followed by intravenous injection of 75 IU of hCG three days later. Ovary transplantation was performed on the 8th day (day 0 being the day of hCG injection). Uterus was collected after the transplantation procedure according to the respective treatment groups. The results showed that in K1, K2 and K3, there was hyperemia in the endometrial epithelium and infiltration of inflammatory cells. Hyperemia and necrosis were more severe in K1 compared to K2 and K3. In K3, the histopathological appearance of the endometrium tended to be normal, with less damage. In conclusion, the histopathological findings of hyperemia and necrosis in the uterus undergoing ovarian transplantation lasted for 3 days compared to 5 and 7 days.

Key words: ovary, pseudo-pregnancy, transplantation

ABSTRAK

Penelitian ini bertujuan mengetahui peningkatan histopatologis uterus kelinci bunting semu seiring peningkatan lama ovarium di dalam uterus dalam kegiatan transplantasi ovarium. Dalam penelitian ini digunakan sembilan kelinci betina New Zealand White (NZW) bunting semu, berumur 3-5 tahun dan bobot badan 1,5-2,9 kg. Setelah adaptasi selama 30 hari, seluruh kelinci dibagi dalam tiga kelompok perlakuan yaitu, K1 (n=3) kelompok kelinci yang dilakukan transplantasi ovarium selama 3 hari, K2 (n=3) kelompok kelinci yang dilakukan transplantasi ovarium selama 5 hari, dan K3 (n=3) kelompok kelinci yang dilakukan transplantasi ovarium selama 7 hari. Induksi bunting semu dilakukan dengan menggunakan PMSG dan hCG. Kelinci diinjeksi dengan 100 IU PMSG secara intramuskulus dan diikuti tiga hari kemudian dengan injeksi 75 IU hCG secara intravena. Transplantasi ovarium dilakukan pada hari ke-8 (hari ke- 0 adalah hari ketika injeksi hCG). Uterus dikoleksi setelah proses transplantasi selesai dilakukan sesuai dengan kelompok perlakuan. Hasil penelitian menunjukkan bahwa pada K1, K2, dan K3 terlihat hiperemi pada epitel endometrium dan infiltrasi sel radang. Hiperemi dan nekrosis pada K1 lebih berat dibandingkan K2 dan K3. Pada K3, gambaran histopatologis endometrium cenderung normal dan tidak banyak terjadi kerusakan. Disimpulkan bahwa gambaran histopatologis berupa hiperemi dan nekrosis pada uterus yang mengalami transplantasi ovarium cenderung lebih berat pada transplantasi selama 3 hari dibandingkan selama 5 dan 7 hari.

Kata kunci: bunting semu, ovarium, transplantasi

INTRODUCTION

Rabbits have the potential to serve as both pets and sources of meat, addressing various human needs (Rinanto *et al.* 2018). Moreover, they are valuable in research due to their short reproductive cycles and ideal oocytes for a wide range of studies, thanks to their larger size compared to other model animals like mice and rats, and their ease of handling (Rahayu *et al.* 2019).

Pseudopregnancy in rabbits arises from ovulation and egg release. During this period, the corpus luteum develops, the uterus prepares for pregnancy, and mammary glands swell. Rabbits undergoing pseudopregnancy can be employed as experimental subjects in diverse research fields. These include exploring interspecies ovarian transpalation (Sumarmin *et al.* 2008), treating endometritis (Boiti *et al.* 1999),

studying reproductive endocrinology (Dugré *et al.* 1989), and investigating the morphology, histology, and immunohistochemistry of rabbit reproduction.

Ovarian transplantation entails moving part or all of the ovarian tissue, aiming to preserve ovaries and facilitate *in vivo* follicular development. In this process, each follicular development requires perfect maturation of primordial follicles, including the nucleus and cytoplasm. Interspecies, transplantation offers an alternative solution for ovarian preservation and transport, serving as a source of gametes (Sumarmin *et al.* 2008).

Transplantation's consequence is the occurrence of stress, resulting in immunosuppression and host immune system rejection of the transpalanted organ (Clipstone and Crabtree 1993). Corticotropin-Releasing Hormone (CRH, cortisol) produced in response to stress plays a pivotal role in regulating follicular

development. Elevated serum cortisol levels have been observed in stressed mouse models (Xu *et al.* 2018). Cortisol directly inhibits ovarian steroid hormone production, inducing apoptosis (Bambino and Hsueh, 1981 cited in Whirlledge and Cidlowski 2010). Yuan *et al.* (2016) reported that cortisol injections cause oocyte damage and granulosa cell apoptosis.

Stress can lead to uterine damage in pseudo-pregnant rabbits, stemming from surgery and anesthesia (Desborough 2000). Surgical incisions can also induce stress. Consequently, during the initial transplantation phase, it is anticipated that stress levels are higher, potentially resulting in more significant uterine damage. Additionally, prolonged pseudopregnancy increases endometrial gland secretion, which serves as micronutrients during early pregnancy (Burton *et al.* 2007). Enhanced secretion reduces the likelihood of adhesion between the ovary and uterus, thus minimizing uterine damage. Hence, research is essential to comprehend the histopathological characteristics of New Zealand White (NZW) rabbit uterus following ovarian transplantation with varying durations of ovaries residing within the uterus.

MATERIALS AND METHODS

In this study, 9 pseudo-pregnant female NZW rabbits, aged 3-5 years, with a body weight of 1.5-2.9 kg, were utilized. Before subjecting to treatment, all rabbits underwent a 30-day adaptation period. They were individually housed in cages measuring 60×43×48 cm to condition all animals to a clinically healthy state. Pellets were provided as food twice daily, and water was given *ad libitum*.

After the adaptation period, all rabbits were divided into three treatment groups, 3 rabbits in each group. Rabbits in K1, K2, and K3 underwent ovarian transpalation for 3 days, 5 days, and 7 days, respectively. Uterine specimens were collected after the transplantation process was completed.

Induction of Pseudopregnancy

Pseudopregnancy induction was performed using PMSG and hCG (Syafuddin *et al.* 2022). Ovarian transplantation was carried out on the 8th day (day 0 being the day of hCG injection).

Surgical Procedure

Recipient female rabbits induced for pseudopregnancy underwent general anesthesia with 0.1 mL of zoletil per kg of BW, intramuscularly. After anesthesia, the rabbits were placed on a surgical table in dorsal recumbency position, and the hair around the *linea alba* area was shaved. The surgical site was disinfected with 70% alcohol and 3% iodine tincture in a circular manner (Forcada and Lopez 2000 cited by Sumarmin *et al.* 2008).

Surgery was performed in the *linea alba* area with dorsal recumbency, approximately 3-4 cm in length (skin, transversus abdominis muscle, and *peritoneum*). Subsequently, a 1 cm incision was made in the uterine

corpus. Through this incision, sections of bovine ovarian tissue/ovarian *Intake* were inserted and pushed towards the uterine cornua. The bovine ovaries were divided into 4 parts, with one part transplanted into each left and right cornua. After transplanting the bovine ovaries, the incised uterus was sutured using continuous lambert sutures. This was followed by suturing the peritoneum with a continuous simple suture and the transversus abdominis muscle with the same suture pattern. Finally, the skin was sutured using a simple interrupted suture pattern to close the incision. Absorbable thread was used for suturing the uterus, peritoneum, and transversus abdominis muscle, while non-absorbable thread was used for suturing the skin. After completing the suturing process, betadine was applied over the stitches, and the rabbits were returned to their cages to recover. The recipient rabbits were then maintained and gentamicin ointment was applied every morning and evening to prevent infection.

Preparation of Histological Slides

Samples of uterine cornua were fixed in 10% *Neutral Buffered Formalin* (NBF) for 24 hours and then processed, starting with dehydration in graded alcohol concentrations. This initiated with two 2-hours immersions in 70% alcohol, followed by 80%, 96%, and absolute alcohol, each for two 2-hour immersions. The tissues were then *cleared* with xylene solution, soaked twice for 2 hours each. Subsequently, tissue infiltration in liquid paraffin was performed three times, each for 1 hour, in an incubator at 65° C. After that, the tissues were *embedded* in paraffin blocks. Paraffin blocks were sectioned using a rotary microtome with a thickness of 5 µm, and the sections were placed in a tissue bath at 45° C, attached to *glass slides*, and dried on a *slide warmer* at 37.5° C for 24 hours.

Hematoxylin-Eosin Staining

Staining commenced with deparaffinization, with two 5-minute immersions in xylene, followed by rehydration by immersing the tissue slides in alcohol, starting with absolute alcohol, then 96%, 90%, and 80%, each for two 2-minute immersions. The slides were rinsed in running water four times, followed by immersion in hematoxylin solution for 5 minutes. After rinsing in running water four times, the slides were placed in acid alcohol for three immersions and then into distilled water for four immersions. Subsequently, the slides were placed in eosin solution for 5 minutes. They were then dehydrated in alcohol (95%, absolute ethanol), each for two 3-minute immersions, followed by three immersions in xylene, each for 2 minutes. Finally, mounting was done using entellan adhesive. Observation was carried out using a microscope and photographed with a photomicroscope (magnifications of 100, 400, and 1000 times).

Research Parameters

The parameters observed in this study included the histopathological characteristics of the pseudo-pregnant

rabbit uterus experienced ovarian transplantation with varying durations of ovarian residency within the rabbit uterus. The histopathological observations included hyperemia and epithelial necrosis.

Data Analysis

All research data were presented in the form of images and analyzed descriptively.

RESULTS AND DISCUSSION

Histologically, the uterine wall consists of several layers, namely the perimetrium (serosal layer), myometrium (muscular layer), and endometrium (mucosal layer). The perimetrium is the outermost layer of the uterus, adhering to the outer part of the myometrium (Fabian 1981). The perimetrium functions as a protective barrier for the uterus. It is composed of blood vessels and connective tissues (Dellman and Carithers, 1996).

The myometrium is a muscular layer, with muscle tissue arranged longitudinally and circularly, as shown in research by Wray and England (2019). The myometrium contains inflammatory cells, such as leukocytes, which contribute to tissue protection and repair. The endometrium consists of the epithelial lamina and the lamina propria (Ozdemir and Atalar 2009). The epithelial lamina in the endometrium has ciliated columnar epithelium (Saruhan *et al.*, 2006). The lamina propria comprises loose connective tissue, collagen fibers, fibroblasts, and lymphocytes found between tissues (Gartner and Hiatt, 1997 cited by Harlita *et al.* 2015). The endometrial glands are round or oval and are located in the submucosa and basal layer of the endometrium (Liu *et al.* 2013). Unlike in humans and domestic animals, the uterine glands in rodentia do not have a circular and compact shape but are slightly branched (Brody and Cunha 1989).

After the transplantation of Aceh cow ovaries, the uterus of rabbits experienced histopathological changes, specifically hyperemia, as seen in Figure 1. Hyperemia is a condition characterized by excessive blood volume or congestion of blood vessels. Additionally, hyperemia can occur due to cell damage, leading to histamine release, resulting in capillary dilation and increased local blood flow (Budiman *et al.* 2008). In this study, hyperemia is suspected to be caused by injuries during implantation. Accumulation of inflammatory cells is believed to be a result of the rabbit uterus's rejection of the Aceh cow ovaries. The transplanted ovaries are foreign bodies that enter the rabbit's pseudo-pregnant uterus, leading to inflammation.

Inflammation is the body's response to tissue injury of infection caused by physical trauma, chemical agents, or microbiological substances (Mycek *et al.* 2001), such as viruses, bacteria, parasites, and hypersensitivity reactions (Ou *et al.* 2019). During inflammation, there is a vascular response (hyperemia), white blood cell (leukocyte) migration, and various chemical mediators present at the injured site. The

purpose is to neutralize harmful agents at the injury site, promote tissue repair, and prepare the environment for tissue regeneration (Anggraini 2009). Inflammation is categorized into acute and chronic forms. Acute inflammation occurs within minutes or days, while chronic inflammation can last for weeks, months, or even years (Wihastuti *et al.* 2016).

Based on the observations in Figure 1, it can be seen that hyperemia in the rabbit uterus in group K1 was more pronounced compared to K2 and K3. This is likely due to the presence of a foreign object, Aceh cattle ovary, which was implanted into the rabbit's uterus, thereby activating inflammatory cells to neutralize this foreign body. The inflammatory process led to an accumulation of blood cells in the incision area during the transplantation. Hyperemia observed in K2 and K3 did not severe as that observed in K1, possibly because of the nutritional support provided by the endometrial glands, which appear to be more abundant in K2 and K3.

In addition to hyperemia in the rabbit uterus, there is also evidence of cell necrosis, as presented in Figure 2. Necrosis refers to cell or tissue death resulting in damaged or abnormal tissue (Utami *et al.* 2016). Lestari and Mulyono (2011) stated that necrosis is cell and tissue death due to injury while the individual is still alive. Necrotic cells undergo irreversible changes, leading to cell death that damages the tissue. Necrosis can be characterized by the loss of tissue structure within cells (karyolysis), changes in the nucleus becoming dark and small (pyknosis), or fragmentation of the nucleus (karyorrhexis) (Dominika *et al.* 2023). Necrosis can be caused by various factors, including environmental factors, pathogens, food, and other substances entering the organism (Alif *et al.* 2021). In this study, necrosis is suspected to be caused by the incisions made during the transplantation.

Based on Figure 2, it can be concluded that the level of necrosis in the rabbit uterus is less severe in-group K2 compared to K1. In K3, which represents rabbits subjected to ovarian transplantation for 7 days, the uterus appears more normal and exhibits less necrosis. In this case, the severity of necrosis can be influenced by the extent of injury, the duration of organ damage, exposure to toxic substances, or infectious organisms (Dominika *et al.* 2023). Reduced necrosis in K3 is likely due to increased adaptation to the presence of Aceh cattle ovaries, allowing the uterus to repair damage caused by infection with the assistance of endometrial glands, which provide abundant nutrition. An increase in the number of endometrial gland secretions can reduce adhesion between the uterine wall and the ovaries. This is supported by the visible increase in the size and number of endometrial glands, as shown in Figure 3.

Based on Figure 3, in general, each treatment group exhibited different endometrial gland and lumen characteristics. The uterine lumen in the K3 group was smaller compared to K1 and K2. The endometrial glands in the K2 and K3 groups appeared more distinct than in the K1 group. This is consistent

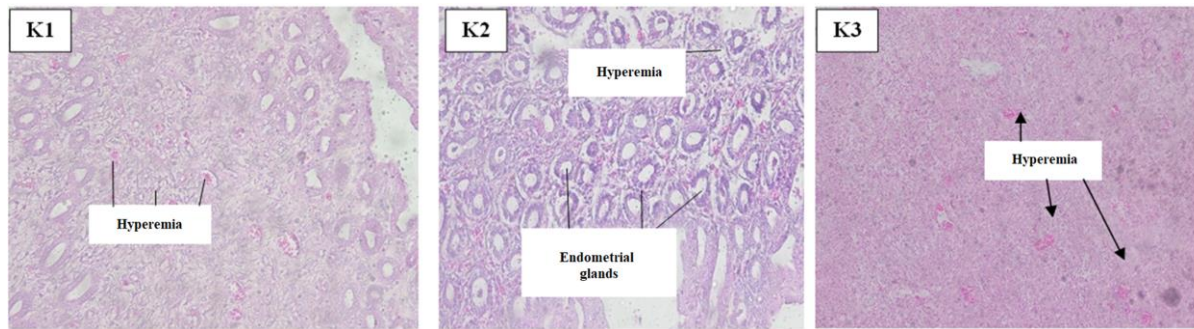


Figure 1. Hyperemia, endometrial glands in rabbit uterus following ovarian transplantation. K1: transplantation for 3 days; K2: transplantation for 5 days; K3: transplantation for 7 days, H&E, 200 $\times$.

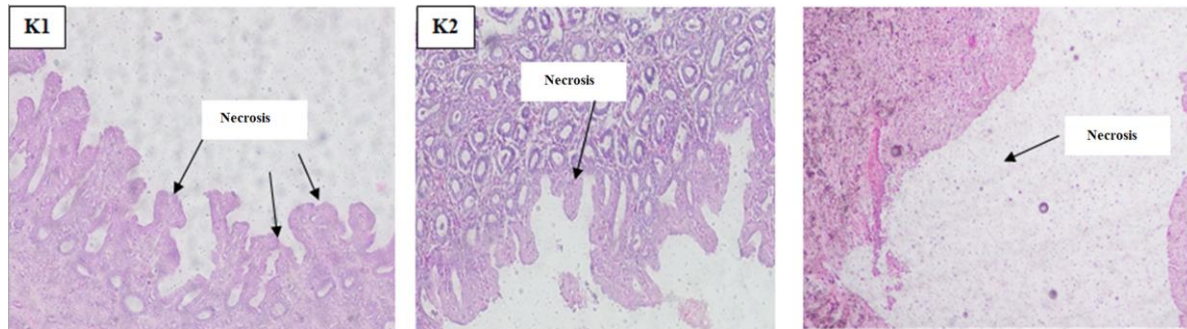


Figure 2. Necrosis in the rabbit uterus following ovarian transplantation. K1: transplantation for 3 days; K2: transplantation for 5 days; K3: transplantation for 7 days, H&E, 100 $\times$.

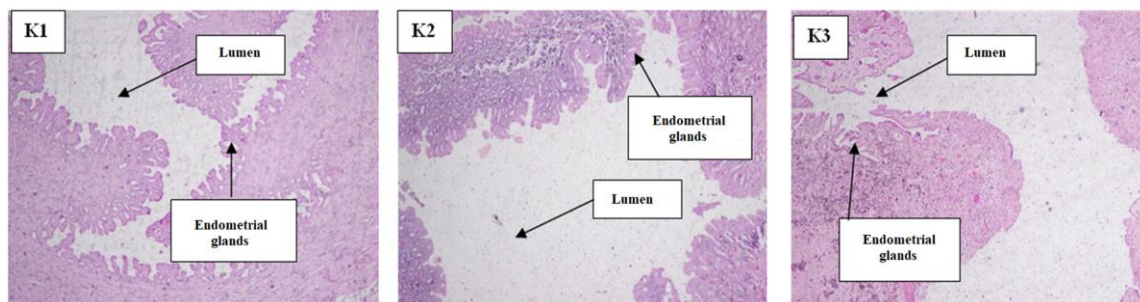


Figure 3. Endometrial glands and lumen of rabbit uterus undergoing ovarian transplantation. K1: transplantation for 3 days; K2: transplantation for 5 days; K3: transplantation for 7 days, H&E, 40 $\times$.

with the statement by Amira *et al.* (2011) that the size and number of endometrial glands increase during the pregnancy period and are regulated by progesterone hormones. Burton *et al.* (2007) also noted that endometrial glands function to transfer micronutrients during early pregnancy. According to Abd-Elkareem (2017), starting from the early stages of pregnancy, rabbit endometrial glands actively secrete secretions produced into the uterine lumen as a source of nutrition for the embryo before implantation. Endometrial glands are lined by columnar cells with oval basal nuclei and surrounded by myoepithelial cells. Endometrial glands contain secretions formed by apocrine activity.

In addition to the increased endometrial glands, the reduction in ovarian damage in K2 and K3 was suspected to be because the uterus has started to tolerate the presence of Aceh cow ovaries, resulting in a lower level of stress. Stress that occurs can be caused by surgical and anesthesia factors, resulting in higher cortisol levels in rabbits at the beginning of the study.

The results of the study by Syafruddin *et al.* (2023) showed that the presence of Aceh cow ovaries for five days transplanted into the local rabbit uterus with pseudo-pregnancy has a lower stress level compared to the treatment group for three days. Cortisol metabolite levels on the 3rd and 5th days post-transplantation were 433.94 ± 207.44 and 176.74 ± 83.0 ng/g, respectively. According to Lumanauw *et al.* (2016), anesthesia and surgery can cause stress responses as a reaction to tissue damage. The stress response causes the hypothalamus to produce CRH. CRH stimulated the pituitary gland to release *adreno-corticotrophic hormone* (ACTH), resulting in cortisol hormone production causing stress (Hernawati 2016).

CONCLUSION

In conclusion, the histopathological findings of hyperemia and necrosis in the uterus undergoing ovarian transplantation tend to be more severe during the 3-day transplantation compared to 5 and 7 days.

ACKNOWLEDGMENTS

The author would like to express gratitude to the Deputy for Strengthening Research and Development, the Deputy for Strengthening Research and Development, the Ministry of Research and Technology/BRIN, and the Rector of Syiah Kuala University, Indonesia, for facilitating this research through the Doctoral Dissertation Research Grant for the Fiscal Year 2022.

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