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Protective Effects of *Myrmecodia pendans* (Ant Nest) Extract on Cortisol Levels and Reproductive Tissue Integrity in Ovarian-Transplanted New Zealand White Rabbits

Syafruddin Syafruddin [1], Tongku Nizwan Siregar [2]*, Sri Wahyuni [3], Arman Sayuti [1], Roslizawaty Roslizawaty [1], Dede Rehaldi [1], Tazkia Aulia Nasution [4] and Sukma Prasasti Pertiwi [4]

[1]Laboratory of Clinic, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia
[2]Laboratory of Reproduction, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia
[3]Laboratory of Anatomy, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia
[4]Study of Program Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Aceh, Indonesia
***Corresponding author:** siregar@usk.ac.id

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ABSTRACT

Ovarian transplantation induces stress, which stimulates corticotropin-releasing hormone (CRH) secretion, pituitary release of adrenocorticotropic hormone (ACTH), and increased cortisol levels. Elevated cortisol may damage ovarian follicles and uterine tissues in pseudopregnant rabbits. Ant nest (*Myrmecodia pendans*) extract, a natural antioxidant, is considered to reduce these negative effects. This study aimed to evaluate the potential of *Myrmecodia pendans* extract to reduce cortisol concentration and improve ovarian and uterine integrity in New Zealand White (NZW) rabbits undergoing ovarian transplantation. Six pseudopregnant female NZW rabbits, aged 3–5 years and weighing 1.5–3.0kg, were used. After 30 days of adaptation, rabbits were divided into two groups (n=3). Group R1 received 200mg oral *Myrmecodia pendans* extract, while Group R2 received immunosuppressants (cyclosporine 2.5mg/kg intramuscularly and azathioprine 5mg/kg orally). Treatments were given twice daily from ovarian transplantation (day 8 after hCG injection = day 0) until day 5 post-transplantation. Fecal cortisol metabolites were measured on days 1, 3, and 5 using ELISA. Ovaries and uteri were collected on day 5 for histopathology. Cortisol concentrations in R2 were lower than R1 on day 1 (151.69±103.11 vs. 193.78±36.72ng/g) and day 3 (63.06±42.07 vs. 361.76±340.34ng/g) ($P<0.05$), but higher on day 5 (49.37±27.08 vs. 41.99±17.77ng/g) ($P<0.05$). Follicle counts varied between groups, with more intact tertiary follicles in R2. Uterine histopathology showed hyperemia, epithelial necrosis, and inflammatory infiltration. Necrosis was higher in R1, while epithelial proliferation was greater in R1 ($P<0.05$). *Myrmecodia pendans* extract has the potential to reduce cortisol concentration and ameliorate ovarian follicle and uterine tissue damage in NZW rabbits after ovarian transplantation.

Keywords: Ant nest, Cortisol, *Myrmecodia pendans* extract, Ovarian transplantation, Pseudopregnant rabbit.

INTRODUCTION

Aceh cattle are one of the indigenous local cattle breeds of Indonesia, distributed in Aceh Province and traditionally bred by local farmers. Aceh cattle have been designated as an Indonesian livestock genetic resource based on the Decree of the Minister of Agriculture No. 2907/Kpts/OT.140/6/2011 dated June 17, 2011, concerning the establishment of the Aceh cattle breed, and have been standardized according to the Indonesian National Standard (SNI 7651.3:2020) (National Standardization Agency 2021). Several advantages of Aceh cattle include strong resistance to extreme conditions, such as limited water and feed availability, parasitic diseases, and high environmental temperatures, as well as finer and denser meat structure compared to Brahman cross and Ongole crossbreeds (Hakim et al. 2022). Therefore, Aceh cattle require protection, conservation, and the development of their superior traits for breeding purposes through controlled mating programs (Rasyid et al. 2017).

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Several reproductive technologies have been applied to enhance the population quality and productivity of Aceh cattle, particularly through the assessment of estrous characteristics and steroid hormone profiles. Previous efforts include estrus synchronization using prostaglandin F2α (Hafizuddin et al. 2024), determination of the optimal timing of cervical dilation through endoscopic techniques (Ulfa et al. 2023), evaluation of estrus duration based on observations of cervical mucus characteristics (Mulfristia et al. 2022), and embryo transfer (Siregar et al. 2012). The embryo transfer (ET) process requires efforts to obtain high-quality embryos that meet transfer criteria for recipient animals. Embryos are collected from fertilized ova resulting from natural mating or artificial insemination. However, embryo collection is often constrained due to the relatively small cervical size of Aceh cattle (Siregar et al. 2012). One alternative to overcome this limitation is to produce embryos using *in vitro* fertilization (IVF) technology (Yilma 2022; Sharma et al. 2024).

Oocytes for IVF technology can be obtained from the slaughterhouse (abattoir) ovarian waste. The abundant supply of ovaries at slaughterhouses can serve as a source of fertilizable oocytes. However, ovaries cannot last long even when stored under cold conditions; thus, ovarian preservation technology is required to maintain follicle quality (Novita et al. 2025). Several researchers have reported that ovarian preservation through intra- or interspecies transplantation, with or without vitrification of the ovarian cortex or whole ovary, is a commonly applied reproductive technology (Sumarmin et al. 2008).

Rabbits are widely used as experimental hosts for ovarian transplantation. However, Jang et al. (2017) noted that rabbits are highly sensitive to stress, which may compromise graft survival. Transplanted ovarian tissue can also be damaged by immune-mediated mechanisms, particularly adhesion-mediated trogocytosis involving neutrophils and macrophages (Zhao et al. 2024). Furthermore, Song et al. (2025) reported that, despite the use of immunosuppressive therapies, transplantation rejection remains a major challenge because graft injury or stress frequently arises from immune activation. Consequently, early detection through reliable biomarkers or non-invasive imaging approaches is essential for improving graft monitoring and ensuring successful transplantation outcomes.

Elevated serum cortisol levels have been detected in stressed mouse models (Xu et al. 2018). The direct action of cortisol on the ovary inhibits steroid hormone production, leading to apoptosis (Whirledge and Cidlowski 2010). Yuan et al. (2010) reported that cortisol injection causes damage to oocytes and apoptosis of granulosa cells in ovarian follicles. Syafruddin et al. (2023) observed that ovarian follicle damage in Aceh cattle ovaries transplanted into pseudopregnant rabbits was associated with increased cortisol concentrations post-transplantation. Another impact of stress is uterine damage in pseudopregnant rabbits. Stress may result from surgery and anesthesia (Desborough 2000; Ilies et al. 2010). Additionally, surgical incisions can also induce stress. Syafruddin et al. (2022) reported that uterine damage in host rabbits was associated with increased cortisol concentration after transplantation. Rabbits in the five-day transplantation treatment group showed milder histopathological changes compared to those in the three- and seven-day groups, correlating with cortisol levels.

To mitigate transplantation-induced stress, immunosuppressive agents are commonly administered. However, the use of immunosuppressants such as cyclosporine often causes serious adverse drug reactions (ADR), including gingival hyperplasia, seizures, respiratory disorders, pruritus, hypertension, potassium retention, nephrotoxicity, hepatotoxicity, and a risk of opportunistic fungal and viral infections (Ma'at 2008). Therefore, plant-based (herbal) immunosuppressants are needed. Herbal therapies are considered to have relatively fewer side effects compared to synthetic drugs (Wahab et al. 2022).

Several herbal plants are thought to have the potential to reduce stress and act as antioxidants. One such herbal plant is the ant nest (*Myrmecodia pendans*), an epiphytic plant that grows attached to large trees. Phytochemical screening showed that the methanol extract of *Myrmecodia pendans* tubers from Aceh Jaya contains flavonoids, tannins, saponins, and terpenoids, while sarang semut tubers from Aceh Besar contain flavonoids, tannins, and terpenoids (Roslizawaty et al. 2023). According to Musman et al. (2015), an ant nest from Aceh belonging to the species *Hydnophytum formicarum* Jack (Rubiaceae) contains flavonoids and tannins with anticancer, antimicrobial, antiproliferative, cytotoxic, and antioxidant properties (Sayuti and Yenrina 2015), which may help reduce stress. However, studies on the effect of sarang semut extract on the uterus of pseudo-pregnant rabbits as hosts for ovarian transplantation in relation to cortisol reduction have not yet been reported. Therefore, this study aimed to evaluate the protective effects of *Myrmecodia pendans* (ant nest) extract on cortisol levels and the structural integrity of ovarian and uterine tissues in New Zealand White rabbits undergoing ovarian transplantation. Specifically, the study assessed whether oral administration of the extract could reduce transplantation-induced stress, mitigate graft-related histopathological damage, and improve reproductive tissue preservation compared to conventional immunosuppressant therapy.

MATERIALS AND METHODS

Ethical approval

This research was conducted at the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh. This study was carried out based on the ethical clearance certificate from the Research Animal Ethics Commission, Faculty of Veterinary Medicine, Universitas Syiah Kuala, number 101/KEPH/V/2021.

Experimental animals

A total of six adult pseudopregnant female New Zealand White (NZW) rabbits aged 3–5 years and weighing 1.5–3.0kg were used. All animals were acclimatized for 30 days in individual cages (60×43×48cm³) under standard husbandry conditions. Rabbits were fed pellet diets twice daily, and drinking water was provided ad libitum.

Ant plant extract

Ant plant extract was prepared using methanol 95% according to Roslizawaty et al. (2023). Fresh ant-nest

tubers were thoroughly cleaned, washed, and drained before being thinly sliced. The slices were air-dried in a shaded, well-ventilated area without direct exposure to sunlight for approximately 10 days, depending on environmental conditions. Once completely dried, the tubers were cut into smaller fragments and milled into a fine powder using a blender. The powder was then sieved to obtain a uniform particle size and stored in clean glass containers. A total of 2,000g of simplicia powder was placed into maceration vessels and immersed in methanol (75ppm). The mixtures were kept in sealed containers, stirred intermittently, and stored in the dark for 5 days. After maceration, the samples were filtered, and the residues were subjected to a second maceration using 25 parts of fresh methanol for an additional 2 days. The combined filtrates were concentrated using a rotary evaporator at 45°C until a viscous extract was obtained.

Induction of pseudopregnancy

Pseudopregnancy was induced following Syafruddin et al. (2022). Rabbits were intramuscularly injected with 100IU PMSG (Folligon™, Intervet, Holland), followed three days later by 75IU hCG (Chorulon™, Intervet, Holland). Ovarian transplantation was performed on day 8 (hCG injection = day 0).

Experimental design

Six healthy female New Zealand White rabbits were used in this study. Prior to the experiment, all animals underwent a 7-day acclimatization period under controlled environmental conditions (22–24°C, 50–60% humidity, and a 12h light–dark cycle) with ad libitum access to commercial feed and water. After acclimatization, the rabbits were randomly assigned to two treatment groups (n = 3 per group) using a simple randomization method. Group R1, ant-plant extract treatment: Rabbits in this group received ovarian transplantation followed by oral administration of *Myrmecodia pendans* extract at a dose of 200mg/kg BW, delivered twice daily using an oral gavage. Group R2, immunosuppressant treatment (positive control): Rabbits underwent ovarian transplantation and were treated with a standard immunosuppressive regimen consisting of cyclosporine (2.5mg/kg, intramuscular) and azathioprine (5mg/kg, orally once daily). All treatments commenced on the day of transplantation and continued until the scheduled endpoint. Ovarian and uterine tissues were collected for subsequent hormonal and histopathological assessments. Animal health and welfare were monitored daily, and all procedures adhered to institutional animal ethics guidelines.

Surgical procedure

Premedication was administered using atropine sulfate (0.04mg/kg BW), followed by anesthesia with Zoletil (0.1mg/kg BW, intramuscular). A midline laparotomy was performed, and a 1cm incision was made in the uterine horn. Bovine ovarian tissue fragments (one ovary divided into four pieces) were transplanted into each uterine horn. Incisions were sutured with 3-0 Vicryl and silk. Postoperative care included topical betadine twice daily.

Fecal sample collection and preparation

Fecal samples were collected on days 1, 3, and 5 post-transplantation. Approximately 0.55g of each fecal sample was processed to extract fecal hormone metabolites using 4.5mL of 80% methanol following a field-friendly extraction protocol (Nugraha et al. 2017; Gholib et al. 2018). The resulting supernatants containing the hormone metabolites were stored at –20°C until further analysis.

Cortisol metabolite analysis

Fecal cortisol metabolites were measured using an ELISA kit (cortisol commercial kit, 3α,11β-dihydroxy-CM ELISA kit, microplate washer, microplate ELISA reader, vortex mixer). Diluted extracts (50µL) were added to pre-coated plates coated with goat anti-rabbit IgG, followed by antibody and enzyme addition, overnight incubation, streptavidin-peroxidase treatment, substrate reaction with TMB, and colorimetric reading at 450nm using an ELISA reader (Gholib et al. 2018).

Histological analysis

Ovarian grafts and uterus were collected on day 5 post-transplantation, fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin-eosin (HE) for histological evaluation.

Data Analysis

Cortisol metabolite levels (day 1, 3 and 5) were analyzed statistically using Student's t-test. Differences were considered to be significant for values of $P<0.05$.

RESULTS

Cortisol concentrations in pseudopregnant rabbits following ovarian transplantation are presented in Table 1. On days 1 and 3, cortisol concentrations in group R2 were significantly lower than those in group R1, with mean values of 151.69±103.11ng/g and 193.78±36.72ng/g on day 1 and 63.06±42.07ng/g and 361.76±340.34ng/g on day 3, respectively ($P<0.05$). Conversely, on day 5, cortisol concentration in R2 was significantly higher than in R1 (49.37±27.08ng/g vs. 41.99±17.77ng/g; $P<0.05$). These findings indicate that administration of immunosuppressive agents in R2 initially suppressed cortisol secretion more effectively than sarang semut (*Myrmecodia pendans*) extract, although the pattern reversed by day 5 post-transplantation (Table 1).

Table 1: Fecal cortisol concentration (ng/g) in New Zealand White (NZW) rabbits following ovarian transplantation treated with *Myrmecodia pendans* (sarang semut) extract (R1) and a combination of cyclosporine and azathioprine (R2)

Group	The day after transplantation		
	1 (d1)	3 (d3)	5 (d5)
R1 (*Myrmecodia pendans* extract)	193.78±36.72[a]	361.76±340.34[a]	41.99±17.77[b]
K2 (cyclosporine and azathioprine)	151.69±103.1[b]	63.06±42.07[b]	49.37±27.08[a]

Values are expressed as mean ± standard deviation (SD). Different superscripts (a, b) within the same column indicate significant differences ($P<0.05$).

Histopathological observations of Aceh cattle ovarian tissue transplanted into rabbit uteri revealed varying degrees of follicular degeneration in both treatment groups (Fig. 1–3). Despite structural alterations, follicles at different developmental stages, including primordial, primary, secondary and tertiary follicles, were still observed. The number of intact tertiary follicles appeared higher in R2 compared to R1, suggesting better follicular preservation following immunosuppressant administration. The mean counts of intact ovarian follicles in each group are summarized in Table 2.

Table 2: Mean (± SD) number of intact Aceh cattle ovarian follicles transplanted into the uterus of pseudopregnant New Zealand White (NZW) rabbits across treatment groups

Groups	Total follicles			
	Primordial follicles	Primary follicles	Secondary follicles	Tertiary follicles
R1 (*Myrmecodia pendans* extract)	22.33±3.05	16.33±5.51	6.00±4.36	1.00±1.00
R2 (Cyclosporine and azathioprine)	22.00±23.43	18.00±16.64	9.67±10.78	4.33±3.21

Not significantly different (P>0.05).

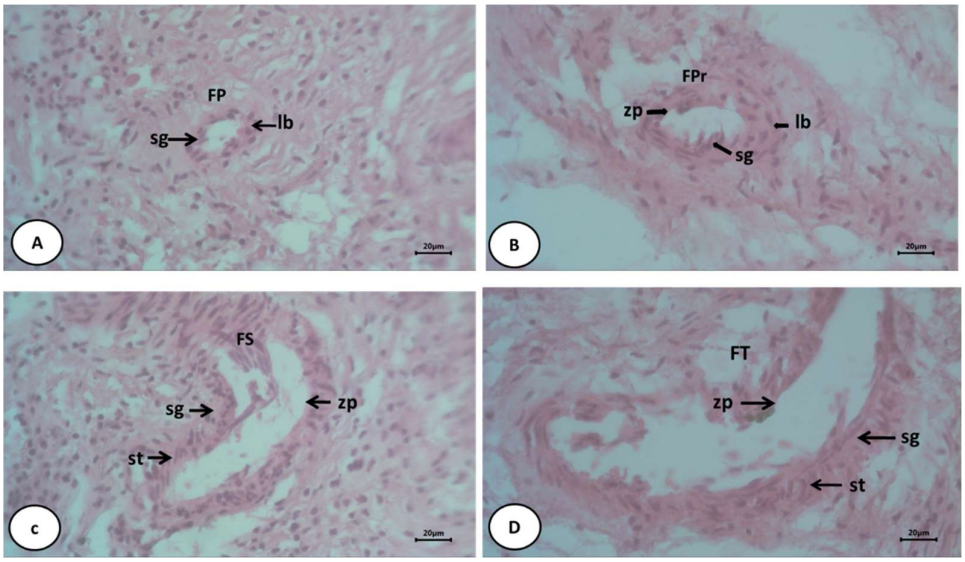

Fig. 1: Histopathological features of Aceh cattle ovarian follicles transplanted into the uterus of control rabbits (K0). (1A) Primordial follicle (FP), (1B) Primary follicle (FPr), (1C) Secondary follicle (FS), and (1D) Tertiary follicle (FT) showing structural alterations. Oocytes (O) were still observed in FPr; the theca cell layer (St) and granulosa cells (Sg) were disorganized and detached from the basal lamina (Lb). Granulosa cells were separated from the layer and entered the follicular antrum (A). The zona pellucida (Zp) in FS and FT appeared discontinuous. Hematoxylin–eosin staining, 1000×magnification.

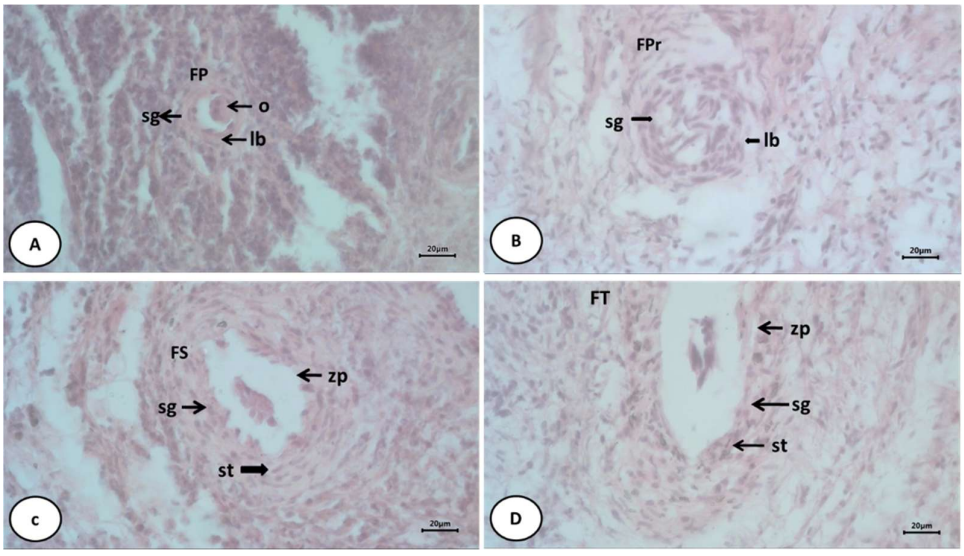

Fig. 2: Histopathological features of Aceh cattle ovarian follicles transplanted into the uterus of rabbits treated with *Myrmecodia pendans* (sarang semut) extract (K1). (2A) Primordial follicle (FP), (2B) Primary follicle (FPr), (2C) Secondary follicle (FS), and (2D) Tertiary follicle (FT) showing histopathological alterations. Theca (St) and granulosa (Sg) cell layers were disrupted and detached from the basal lamina (Lb). Granulosa cells were separated and entered the follicular antrum (A). The zona pellucida (Zp) in FS and FT was discontinuous. Hematoxylin–eosin staining, 1000× magnification.

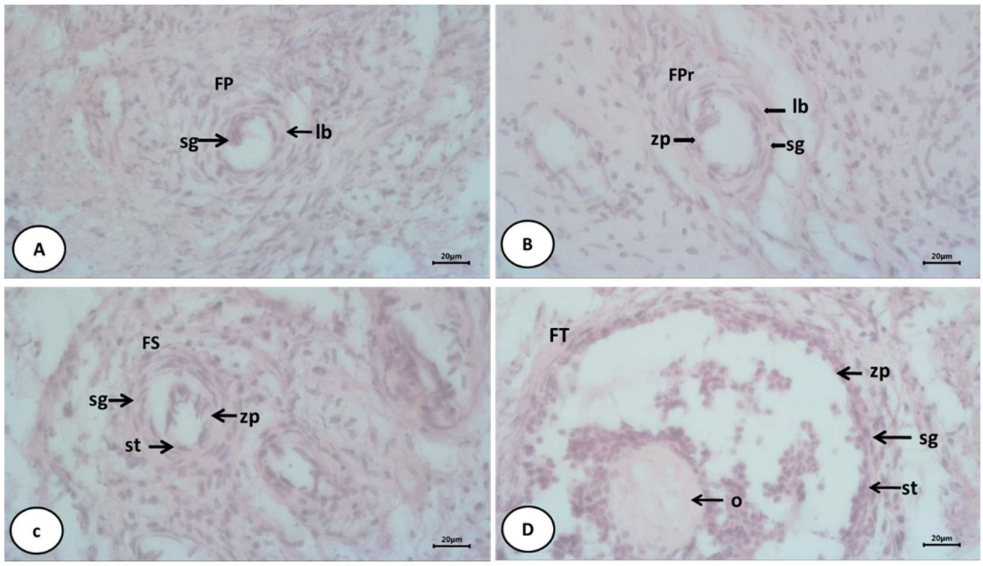

Fig. 3: Histopathological features of Aceh cattle ovarian follicles transplanted into the uterus of rabbits treated with potent immunosuppressive drugs (K2). (3A) Primordial follicle (FP), (3B) Primary follicle (FPr), (3C) Secondary follicle (FS), and (3D) Tertiary follicle (FT) showing histopathological alterations. Oocytes (O) were still observed in FPr and FT. Theca (St) and granulosa (Sg) cell layers in FP were disrupted, with Sg detached and entering the follicular antrum (A) in FS and FT. The zona pellucida (Zp) in FS and FT was discontinuous. Hematoxylin–eosin staining, 1000× magnification.

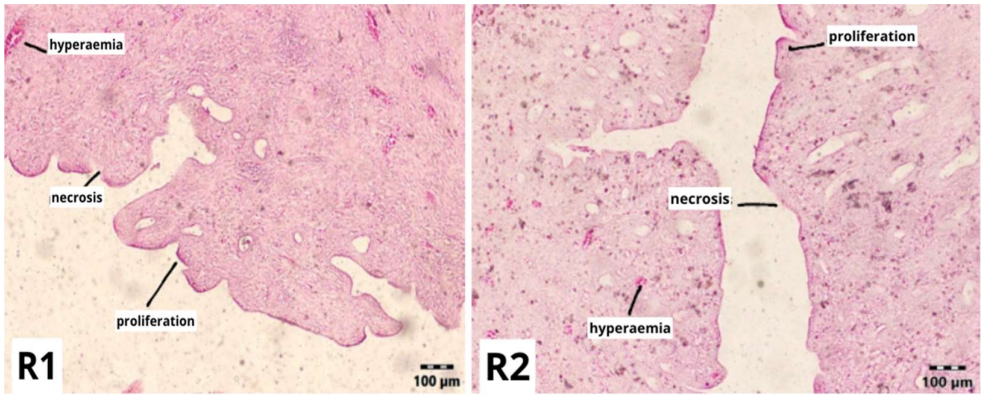

Fig. 4: Histopathological features of the uterus in rabbits after ovarian transplantation, showing necrosis, hyperemia, and epithelial proliferation in R1 and R2 groups (R1 = Treatment with *Myrmecodia* extract; R2 = treatment with cyclosporine and azathioprine.

In the R1 group (treated with *Myrmecodia pendans* extract), ovarian follicles showed partial disruption of the theca (St) and granulosa (Sg) cell layers, with detachment from the basal lamina (Lb) and granulosa cells dispersed into the follicular antrum (A). The zona pellucida (Zp) of secondary (FS) and tertiary (FT) follicles appeared discontinuous (Fig. 2). In contrast, the R2 group (treated with cyclosporine and azathioprine) displayed milder disruption in follicular architecture, with oocytes (O) remaining visible in both primary and tertiary follicles (Fig. 3). However, signs of degeneration and follicular atresia were still evident.

Uterine histopathology demonstrated hyperemia, epithelial necrosis, and inflammatory cell infiltration in both groups (Fig. 4). The degree of necrosis was more pronounced in R1, whereas epithelial proliferation was greater in this group compared to R2 ($P<0.05$). These results suggest that *Myrmecodia pendans* extract mitigates tissue necrosis and promotes epithelial regeneration in the uterus of ovarian-transplanted rabbits (Table 3).

Table 3: Mean (±SD) values of necrosis and uterine epithelial cell proliferation in rabbits following transplantation of Aceh cattle ovaries

Groups	Necrosis	Uterine cell proliferation
R1 (*Myrmecodia pendans* extract)	91.36±10.95[a]	12.28±18.31[b]
R2 (Cyclosporine and azathioprine)	71.81±7.82[b]	11.58±26.48[a]

Values are expressed as mean ± standard deviation (SD). Different superscripts (a, b) within the same column indicate significant differences ($P<0.05$).

DISCUSSION

Cortisol concentration

Cortisol hormone concentration is one of the important indicators in assessing the physiological stress response after transplantation. In this study, administration of *Myrmecodia pendans* extract significantly reduced cortisol levels on day 5 compared to day 1 and day 3, particularly in group R1. This reduction indicates that the effectiveness of *Myrmecodia pendans* extract in suppressing cortisol

secretion is highly influenced by the duration and possibly also by the dosage of administration. On day 5 after transplantation, cortisol concentration was lower than on days 1 and 3, with a significant difference ($P<0.05$) between R1 and R2 groups. These findings suggest that the potential of *Myrmecodia pendans* extract in reducing cortisol levels is affected by the duration of treatment. The decreased cortisol concentration on day 5 (H5) may be associated with the dose of *Myrmecodia pendans* extract administered. A higher extract dose might reduce cortisol levels earlier, on day 1 and day 3. The effect of increasing dosage on the rate of antioxidant activity enhancement has been reported by Palupi et al. (2019), who found that ethanolic *Centella asiatica* extract normalized blood glucose levels at 1200mg/kg BW after 28–35 days and at 600mg/kg BW after 35 days of administration.

The cortisol concentrations on day 5 post-transplantation after *Myrmecodia pendans* extract (R1) and cyclosporine–azathioprine (R2) administration were much lower than those reported by Syafruddin et al. (2023), which reached 174.79±101.70ng/g. The ability of *Myrmecodia pendans* extract to reduce cortisol hormone concentration is attributed to its bioactive compounds, flavonoids, tannins, phenolics, glycosides, and terpenoids, which act as antioxidants (Roslizawaty et al. 2023). Flavonoids, a type of polyphenolic compound, play an essential role in protecting the body from oxidative stress. They act as anti-carcinogenic agents by suppressing reactive oxygen species (ROS) and inducing apoptosis in malignant cells (Immaniar et al. 2022). The flavonoid and tocopherol content in *Myrmecodia pendans* works by releasing hydrogen atoms from hydroxyl groups, which subsequently bind to free radicals (Ariani et al. 2014).

A similar trend was observed in the R2 group, where prolonged administration tended to decrease cortisol levels. Routine application of immunosuppressive therapy may help prevent graft damage or rejection. Wennberg et al. (2001) stated that the success of animal transplantation experiments depends on an appropriate immunosuppressive protocol. Essentially, long-term graft survival can be achieved through two different approaches: pharmacological immunosuppression management or induction of immunological tolerance to donor tissues (Diehl et al. 2017).

Azathioprine acts by suppressing the immune system, thereby supporting the acceptance of newly transplanted organs. In autoimmune diseases, azathioprine prevents the immune system from attacking healthy cells or tissues, while cyclosporine inhibits the production and release of interleukin-2 (IL-2), thus blocking T-lymphocyte activation. Cyclosporine is a key drug used to prevent transplant rejection and can also be applied in treating autoimmune or dermatological disorders such as psoriasis, pemphigus, and lichen planus (Aranti and Kemal 2013).

Number of intact follicles

The development of ovarian follicles after transplantation is an important parameter to assess the success rate of ovarian tissue transplantation. Evaluation of follicular morphology and numbers at various developmental stages provides an overview of the extent to which ovarian tissue can maintain folliculogenic activity after the transplantation process. In this study, histological observations were performed on Aceh cattle ovaries transplanted into the uterus of rabbits under different treatments to assess follicular development and tissue damage levels.

The ovary contains several types of follicles distinguished by their morphology during folliculogenesis, namely primordial, primary, secondary, and tertiary follicles. Each type has distinct morphological features characterized by the presence of theca and granulosa cell layers. Primordial follicles contain a small oocyte surrounded by a single layer of flattened granulosa cells. These follicles gradually enlarge as they develop into primary, secondary, and tertiary follicles. Primary follicles are characterized by a slightly larger oocyte surrounded by a single layer of cuboidal or columnar granulosa cells. Secondary follicles are larger and located deeper in the ovarian cortex, containing oocytes surrounded by multiple granulosa cell layers. Tertiary follicles are identified by the presence of a large oocyte surrounded by several layers of granulosa cells, a distinct zona pellucida, and the formation of an antrum that coalesces into a single cavity (Figueiredo et al. 2018).

The transplantation procedure in this study was considered successful, as follicles at multiple developmental stages were still present in the grafted ovarian tissue. This finding is consistent with Sumarmin et al. (2008), who emphasized that the persistence of follicles serves as a reliable indicator of successful ovarian transplantation. Comprehensive evaluation of transplantation outcomes typically involves histological assessment, quantification of neovascularization density, analysis of granulosa cell proliferation activity and monitoring of hormonal profiles and estrous cyclicity (Liu et al. 2025).

Moreover, evidence from heterotopic transplantation of cryopreserved human ovarian tissue has demonstrated that long-term endocrine function can be sustained for extended periods, reaching up to seven years. Such durable restoration of hormonal activity indicates that the transplanted ovarian tissue retains sufficient follicular viability and functional integrity. These findings highlight the clinical relevance of high-quality ovarian grafts, particularly for young cancer survivors who experience premature ovarian failure and require long-term endocrine recovery.

The presence of follicles suggests that the epithelial structure protecting the ovarian cortex remains intact, ensuring normal metabolic activity. The number of intact follicles in R1 and R2 groups was as follows: primordial follicles (22.33±3.05 vs. 22.00±23.43), primary follicles (16.33±5.51 vs. 18.00±16.64), secondary follicles (6.00±4.36 vs. 9.67±10.79), and tertiary follicles (1.00±1.00 vs. 4.33±3.21). Although the differences among follicular stages were not statistically significant ($P>0.05$), the numbers of primordial and primary follicles tended to be higher than those of secondary and tertiary follicles.

Ovarian follicle damage observed after transplantation included disruption of the theca and granulosa cell layers, detachment of granulosa cells into the antrum, disintegration of the zona pellucida, and the formation of vacuoles between the granulosa and theca layers. Other histopathological changes included follicles lacking oocytes or containing oocytes with abnormal morphology.

According to Rosadi et al. (2011), morphologically normal follicles are characterized by intact oocytes with a round nucleus and visible nucleolus, surrounded by well-organized, non-pyknotic granulosa cells. Based on Fig. 1–3, differences in the degree of ovarian tissue damage were observed between R1 and R2 ovaries, although the general histopathological features of ovarian and follicular tissue damage were relatively similar between groups.

The observed follicular damage in Aceh cattle ovaries in this study was likely caused by several factors, including stress experienced by the rabbit host during interspecies transplantation. Syafruddin et al. (2023) reported a relationship between increased cortisol metabolites and follicular damage in Aceh cattle ovaries transplanted into pseudo-pregnant local rabbits. Stress arises from surgical procedures involving anesthesia, incision wounds, and the presence of foreign tissue (Aceh cattle ovary) in the rabbit uterus. Stress triggers activation of the hypothalamic–pituitary–adrenal (HPA) axis, leading to cortisol secretion by the adrenal gland. Prasad et al. (2016) stated that elevated cortisol levels stimulate oxidative stress and induce granulosa cell apoptosis. Granulosa cell apoptosis disrupts folliculogenesis and impairs communication between granulosa cells and oocytes, resulting in compromised nutrient supply and oocyte maturation.

Uterine tissue damage

Physiological stress induced by the transplantation procedure can affect the condition of the host uterus and consequently influence the success of ovarian transplantation. Uncontrolled stress responses may disrupt endometrial function, which serves as a microenvironment supporting the survival of donor tissue. Pathological changes in the uterus, such as decreased numbers of endometrial glands, cellular degeneration, and inflammatory reactions, indicate physiological disturbances caused by oxidative stress. Therefore, histological evaluation of uterine tissue is essential to determine the extent to which stress and oxidative activity affect tissue integrity and the effectiveness of the treatments applied.

Increased stress levels in rabbits may lead to uterine damage through a reduction in the number and activity of endometrial glands, which provide micronutrients for the transplanted ovary. Chukwuebuka et al. (2020) reported similar tissue damage in the ovaries of rats subjected to stress with different types of stressors. The stress response in ovarian tissue is characterized by cellular degeneration, inflammatory cell infiltration, and follicular atresia.

ROS play an important role in the physiological coordination of the ovary and are crucial for follicular survival and development (Sugino 2005). Oxidative stress occurs when excessive oxidants in the body are not adequately counteracted by antioxidant defense systems or cellular repair mechanisms. Elevated ROS levels may exceed cellular antioxidant capacity and trigger oxidative stress, resulting in direct oxidative damage to biomolecules, including proteins, lipids, and DNA (Dizdaroglu and Jaruga 2012; Kamat et al. 2016).

Histopathological evaluation of R1 and R2 uterine tissues revealed the presence of necrosis, haemorrhage, and proliferative responses. Dhyantari et al. (2014) stated that inflammation is a physiological response to tissue injury or infection. This process involves vascular reactions that allow leukocytes and blood components to migrate to the affected site as part of the body's protective mechanism to neutralize harmful agents and restore tissue function. The hyperemia observed in this study was presumably caused by the presence of a foreign object, the transplanted ovary, within the pseudopregnant rabbit uterus.

Inflammation can be classified into three types based on duration: acute inflammation occurs immediately after injury and lasts a few days; chronic inflammation may persist for months or even years; and subacute inflammation represents a transitional phase lasting two to six weeks (Hannoodee and Nasruddin 2021). Necrosis of the lamina mucosa epithelium may result from temperature extremes, radiation, inadequate blood supply, toxins, or mechanical trauma (Duwiri et al. 2019). Swarayana et al. (2012) defined necrosis as cell death caused by tissue injury, microscopically characterized by loss of chromatin, nuclear shrinkage, hyperchromatic and pyknotic nuclei, nuclear fragmentation (karyorrhexis), and eventual nuclear dissolution (karyolysis).

Endometrial damage observed in this study was likely associated with stress-induced ROS accumulation in the uterus. Oxidative stress resulting from excessive ROS can stimulate apoptosis in granulosa cells and oocytes, thereby reducing oocyte quality. Chukwuebuka et al. (2020) similarly reported stress-induced uterine damage in rats exposed to various stressors. In this study, uterine damage was likely caused by stress related to transplantation procedures, anesthesia, surgical incisions, and the introduction of foreign tissue into the uterus.

Higher necrosis levels were observed in R1 compared to R2. Administration of *Myrmecodia pendans* (ant-plant) extract did not significantly reduce uterine necrosis, although this extract contains flavonoids, tannins, phenolics, glycosides, and terpenoids, compounds known for their antioxidant activity (Engida et al. 2013). Flavonoids, which are polyphenolic compounds essential in protecting cells against oxidative stress, act as anticancer agents by suppressing ROS formation and inducing apoptosis in malignant cells (Immaniar et al. 2022). The lack of reduction in necrosis might be due to suboptimal dosage or treatment duration, as this study represents an early stage of investigating the use of *Myrmecodia pendans* extract in ovarian transplantation.

Reduced necrosis in R2 is attributed to the use of cyclosporine and azathioprine, which are well-documented for their ability to mitigate post-transplant stress. Routine immunosuppressive therapy is applied to prevent graft rejection or tissue damage. Wennberg et al. (2001) emphasized that an appropriate immunosuppressive protocol is crucial for successful experimental transplantation in animals. Long-term graft survival after transplantation can be achieved either through optimized immunosuppressive management or by inducing immunological tolerance to the donor tissue (Diehl et al. 2017).

Cellular proliferation represents a mitotic process stimulated by mitogenic factors such as growth factors and stress. Stress activates the hypothalamic–pituitary–adrenal (HPA) axis, leading to cortisol secretion from the adrenal gland. According to Prasad et al. (2016), elevated cortisol induces oxidative stress and granulosa cell apoptosis,

which disrupts folliculogenesis and interferes with oocyte–granulosa cell communication, thereby impairing nutrient supply and oocyte maturation.

Stress observed in this study was likely induced by surgical and anesthetic procedures, resulting in elevated cortisol levels in rabbits at the beginning of the experiment. Lumanauw et al. (2016) reported that anesthesia and surgery can trigger stress responses due to tissue injury, activating the hypothalamus to release corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to secrete adrenocorticotropic hormone (ACTH), subsequently increasing cortisol production. The higher proliferation observed in R1 compared to R2 ($P<0.05$) suggests that *Myrmecodia pendans* extract can alleviate stress in rabbits, allowing the uterus to develop tolerance toward the presence of transplanted ovarian tissue.

Conclusion

Myrmecodia pendans extract demonstrated promising biological activity in reducing stress responses and supporting reproductive tissue integrity following ovarian transplantation in pseudopregnant rabbits. The extract effectively decreased fecal cortisol concentrations, most notably on day 5, and helped preserve ovarian follicular structure and uterine epithelial organization. Although conventional immunosuppressive therapy produced stronger early protective effects, *Myrmecodia pendans* showed potential as a complementary or alternative agent for enhancing graft tolerance. Further research using larger sample sizes, optimized dosing, longer observation periods, and molecular biomarkers is required to elucidate its mechanisms and therapeutic value in ovarian graft survival.

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