JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2018.e297
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

December 2018
VOL 5, NO. 4, PAGES 445–453

ORIGINAL ARTICLE

Anatomical and histological changes of uterine horn of Aceh cattle with repeat breeding

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ABSTRACT

Objective: This study aimed to observe the changes in anatomical pathology, histopathology, and histomorphometry of uterine horn in Aceh cattle with repeat breeding (RB).

Materials and methods: In this study, five uterine horns were collected from fertile Aceh cattle (N1, N2, N3, N4, and N5) and Aceh cattle with RB (RB1, RB2, RB3, RB4, and RB5). Changes in the anatomical pathology of uterine horn were observed prior to histopathological preparations. The results were analyzed descriptively.

Results: The observation of anatomical pathology revealed that the uterus of fertile cattle was yellowish white with horn-like shape consisting of hollow muscles, while RB cattle were reddish-pale. Furthermore, uterine horn swelling with purulent exudate was observed in the uterine horn of RB1 and RB4, while swelling with serous exudates was found in RB2, RB3, and RB5. Thin-walled uterine horn with caruncular atrophy was observed only in RB3. Histopathological observations showed erosion and hyperplasia of endometrial columnar epithelial and uterine glands in RB1, RB2, RB4, and RB5. Atrophy of uterine gland was found in RB3 and edema of caruncular was observed in RB1 and RB4. In addition, hemorrhage and inflammatory cell infiltration (neutrophils, lymphocytes, and macrophages) were found in all RB cattle. Histologically, cow uterus is divided into three layers, endometrium, myometrium, and perimetrium with the uterine gland found in the endometrium. Histomorphometric measurements found that the uterine horn wall (endometrial, myometrial, and perimetrium) of RB cattle were thicker than of fertile Aceh cattle, 208.06 ± 39.90 *vs.* 187.39 ± 29.09 µm, 400.138 ± 51.96 *vs.* 277.91 ± 42.88 µm, and 23.59 ± 9.67 *vs.* 18.53 ± 4.40 µm. However, the endometrial gland diameter of RB cattle is smaller than that of fertile Aceh cattle, 4.04 ± 0.88 *vs.* 4.99 ± 1.37 µm.

Conclusion: The changes in anatomical pathology and histopathology of the uterine horn of Aceh cattle with RB indicate endometritis which caused by subacute and chronic bacterial infections. Endometritis causes disrupted blood circulation, which is characterized by hemorrhage, edema, and thickening of the endometrium, myometrium, and perimetrium, resulting in an increased diameter of the endometrial gland in RB Aceh cattle.

ARTICLE HISTORY

Received September 03, 2018
Revised October 30, 2018
Accepted October 20, 2018
Published November 30, 2018

KEYWORDS

Aceh cattle; repeat breeding; histopathology; histomorphometric; uterine horn

Introduction

Low reproductive efficiency is a common problem among cattle in Indonesia. One of these problems is a repeat breeding (RB). RB is a condition in which cow failed to get pregnant even after breeding three times or more with fertile males without any observed abnormalities [1]. Cows that undergo RB are generally characterized by long calving interval (18–24 months), low conception rate (<40%), and high service per conception (>3) [2]. RB disorders can also lead to infertility [3].

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How to cite: Thasmi CN, Siregar TN, Wahyuni S, Aliza D, Panjaitan B, Nazaruddin N, et al. Anatomical and histological changes of uterine horn of Aceh cattle with repeat breeding. J Adv Vet Anim Res 2018; 5(4):445–53.

There are two main factors causing RB, namely failure of fertilization and early embryonic death. Factors affecting early embryonic death are genetic, infection, environment, hormonal imbalance, nutritional defect [4], maternal age, and number of embryos or fetuses in the uterus [5]. Uterine infection and abnormalities are one of the causes of reproductive failures in cattle [6]. According to Ferreira [7], almost all cattle uterine with RB disorders showed inflammation based on clinical symptoms and histopathological examination. The transformation of the uterine lining in RB Aceh cattle is important because the uterus is one of the reproduction organs which closely related to RB incidence.

One of the inflammations in the uterus that can cause RB is endometritis. Endometritis is inflammation of the endometrium and is the most common inflammation on the uterus. Endometritis can cause infertility in cattle [8]. Endometrial inflammation can be caused by the transmission of various microorganisms or by secondary inflammation from other parts of the body. Endometritis can lead to infertility and sterility, including embryonic death or failure of implantation [5]. Anatomical pathology and histopathological description of endometritis in cattle have been reported by Rhyaf [9]; the uterine lining in endometritis is characterized by thick and swollen walls containing serous exudates. In addition, Chethan et al. [10] added that the uterine wall lining in buffalo with chronic endometritis characterized by purulent exudate. Histopathological features show uterine epithelial hyperplasia and inflammatory cell infiltration in the subepithelial layer, especially neutrophils and macrophages [9].

Several causes of reproductive disorders in Aceh cattle have been reported by Siregar et al. [11] and [12]. Low progesterone level in the early luteal phase [13] apparently cannot be proven to be the cause of RB incidence among Aceh cattle [12] Nevertheless, the main cause of high RB incidence among Aceh cattle is still unknown. An initial study, conducted by Rafika [14], claimed that the main cause of RB in Aceh cattle is a bacterial infection in the reproductive tract, especially in the uterine horn. Therefore, it is necessary to conduct a study on the anatomical pathology, histopathology, and histomorphometry of uterine horn of Aceh cattle with RB to investigate the bacterial infection.

Materials and Methods

Animals and sampling

Our study used five uteri of fertile Aceh cattle (N1, N2, N3, N4, and N5) and five Aceh cattle with RB (RB1, RB2, RB3, RB4, and RB5). Intact uteri were collected from the cows slaughtered at Banda Aceh City Slaughterhouse. Selection of specimens for fertile cattle was >2 months postpartum cattle with a history of successful pregnancy with one insemination and have two regular cycles. The criteria of specimens for Aceh cattle with RB were cattle that failed to be pregnant even after three or more inseminations but have a normal estrus cycle. Determination of fertile and RB status was based on inseminator records in Blang Bintang Area, Aceh Besar District. All cattle aged were ranging from 3 to 6 years old.

Anatomical pathology examination

The uterus of both fertile and RB Aceh cattle was inspected for its color, consistency, shape, and pathological lesions. Prior to anatomical pathology examination, a small part of the organ (uterine horn) of Aceh RB and fertile cattle which showed pathological changes will be cut into 2 × 2 × 2 cm preparate. Uterine horn tissues were immersed in 10% neutral buffer formalin (NBF 10%) fixative for 24 h.

Tissue preparation and histological techniques

Samples of uterine horn that had been fixed in 10% NBF solution were then washed and dehydrated in an ethanol series with increasing concentrations (80%, 96%, and absolute alcohol), cleared in xylene, and embedded in paraffin wax. The wax block was then sectioned at a thickness of 5 µm, followed by hydration in an ethanol series of descending concentrations, then stained with hematoxylin-eosin to observe histopathological changes and Masson's trichrome for histomorphometric examination.

Hematoxylin-eosin staining

Hematoxylin-eosin staining was carried out according to [15] method with several modifications. Staining started with the deparaffinization using xylene, followed by rehydration with decreasing alcohol concentration (absolute, 96%, and 90%). Subsequently, the slides were immersed in hematoxylin and eosin solution, rehydrated with 96% and absolute alcohol, and cleared with xylene, then mounted with adhesive material Entellan® slides. Histopathological observation of uterine horn tissues was carried out under a light microscope and documented by photomicrograph.

Masson's trichrome staining

Tissue deparaffination was carried out prior to Masson's trichrome staining. The slides were then soaked in Bouin solution followed by stained with hematoxylin. Tissue staining started with soaking slides in ponceau 2R dye solution, stained with orange G dye for 5 min and followed by immersion in the light green dye. Washing with 1% acetic acid was done at the end of every staining phase. The staining result of each stage was observed under the light microscope. After the staining phase is complete, the entire slide is dehydrated with absolute alcohol, cleared in xylene and ended by covering the slide with glass cover (mounting) using Entellan® adhesive.

Uterus histomorphometry

Histomorphometry of cattle uterus was examined by measuring the uterine layers using microscope aided by ToupView software. We measured the diameter of uterine glands in endometrium and thickness of the endometrium, myometrium, and perimetrium layers.

Data analysis

The data were analyzed descriptively. Changes in anatomical, histopathological, and histomorphometry pathologies of the uterus in RB and fertile cattle are presented in the form of images. Thickness of each uterine layers and diameter of the endometrial gland will be presented in mean ± standard deviation (SD).

Results and Discussion

Anatomical pathology of uterine horn in Aceh cattle with repeat breeding

Uterine horn of fertile cattle appeared yellowish-white or pale with a horn-like shape consisting of hollow muscles (Fig. 1A). This is consistent with Ball and Peters [16] who stated that the fertile uterine horn shape resembles a horn consisting of hollow muscles. When the uterine horn is sliced, we found prominences or commonly called caruncle on its endometrial surface and blood vessels on each caruncle surface. This finding is similar to Senger [17] who found prominent non-glandular areas rich in blood vessels on the ruminant animal endometrial surface.

Anatomical pathology of the uterine horn of Aceh cattle with RB is shown in Table 1. There was a change in color to pale reddish. Changes in shape were also observed due to uterine swelling with containing thick yellow purulent exudates and thickened uterine horn wall (Fig. 1B). In RB2, RB3, and RB5 cattle, the swelling was accompanied by serous exudate (Fig. 1C). Thinning of uterine horn wall with caruncular atrophy was observed only in RB3 cattle (Fig. 1D). It can be assumed that the changes in the anatomical pathology of the uterine horn of RB cattle were caused by endometritis. This condition supported by Sayyari et al. [18], who stated that cows with endometritis showed endometrial swelling and exudate in the endometrial lumen with various types of secretions, namely serous, mucoid, and purulent.

Swollen uterine horn with purulent exudate is the characteristic of chronic purulent endometritis or pyometra [19]. Hardjopranjoto [5] added that the thickened uterine wall was caused by scar tissue without tonus in the uterus. Pyometra in cattle could be caused by sexually transmitted diseases such as brucellosis and non-specific bacteria namely coccus, coli, and pyogenes.

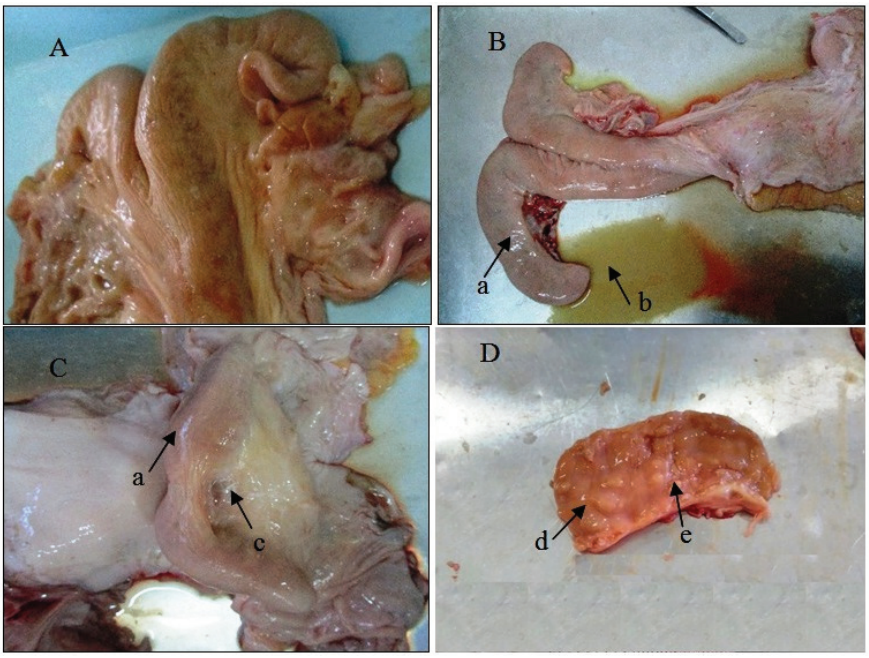

Figure 1. Photographs of the anatomical pathology of uterine horns of Aceh cattle with RB. (A) Fertile Aceh cattle uterine horns appeared yellowish or pale white, (B) uterine horn swelling with yellow purulent exudate, (C) uterine horn swelling with serous exudate, (D) thin uterine horn wall with caruncular atrophy. (a) Uterine horn swelling with, (b) purulent exudate, (c) serous exudate, (d) thin uterine wall, and (e) caruncular atrophy.

Table 1. Changes in the anatomical pathology of the uterine horn of Aceh cattle with repeat breeding (n = 5).

Uterine horn	Pathologic lessions		Pathologic category
Uterine horn RB1, RB4	Color	Pale reddish	Chronic purulent endometritis or pyometra
	Shape	Swelling with thickening	
	Exudate	Purulent	
Uterine horn RB2, RB5	Color	Pale reddish	Subacute endometritis
	Shape	Swelling with thickening	
	Exudate	Serous	
Uterine horn RB3	Color	Pale reddish	Chronic endometritis or hydrometra
	Shape	Swelling with thickening	
	Exudate	Serous	

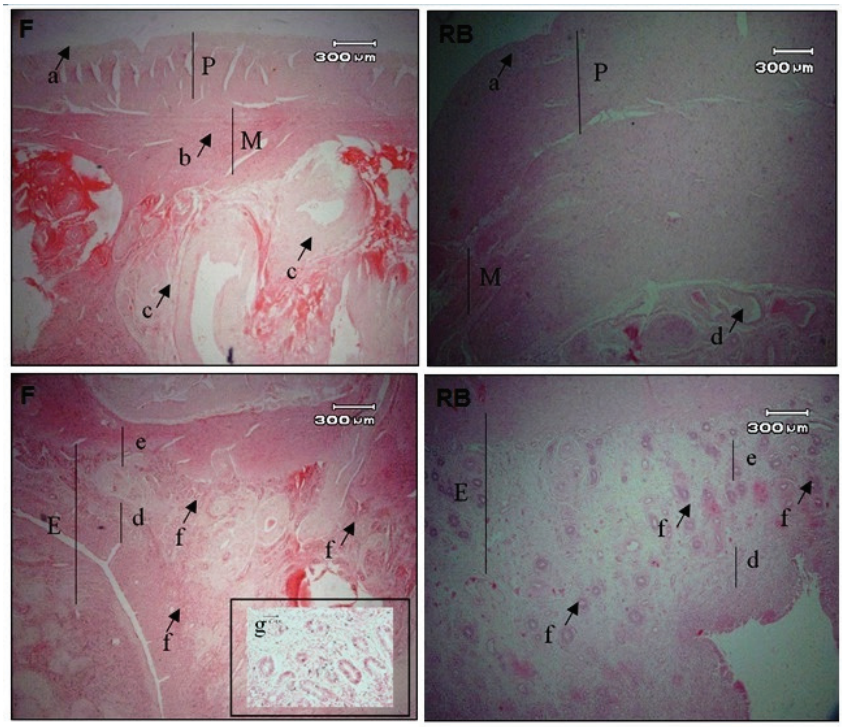

Figure 2. Histopathological features of uterine horns from a fertile cow (F) and RB cow (RB). Both uterine horn tissues are divided into three layers: perimetrium (P), myometrium (M), and endometrium (E). (a) Mesothelium, (b) muscle bundle, (c) caruncle, (d) functional layer, (e) basal layer, and (f) uterine gland. (H&E, 4×), insert: uterine gland (g) (H&E, 40×).

Changes in the anatomical pathology of uterine horn accompanied by serous exudates and thin uterine horn wall with caruncular atrophy are the characteristic of hydrometra [10].

Histopathology of uterine horns of Aceh cattle with repeat breeding

Histopathological examination of fertile Aceh cattle uterine horns showed that the uterus has three layers: perimetrium, myometrium, and endometrium. Perimetrium consists of connective tissue and mesothelium, myometrium consists of smooth muscle bundles, and endometrium consists of the basal layer and the functional layer (simple columnar epithelium), caruncles without uterine glands (Figs. 2 and 5).

The result of this study is in accordance with Eurell and Frappier [20] who stated that histologically, cow uterus consists of three layers: endometrium, myometrium, and perimetrium. Endometrium consists of two zones with different structures and functions, functional layer with pseudostratified columnar and/or simple columnar epithelial cells, and basal layer. (Fig. 6). In ruminant animals, there are wrinkles or thickening limited to the endometrium (caruncle) without uterine glands. The myometrium consists of the thick layer of circular/longitudinal smooth muscle. Perimetrium consists of loose connective tissue which is covered by peritoneal mesothelium and smooth muscle.

Histopathological examination of uterine horns of Aceh cattle: RB1, RB2, RB4, and RB5 showed erosion

of endometrial columnar epithelium and hyperplasia of endometrial columnar epithelial and uterine gland. Those histopathological changes were not found in RB3, where we found uterine gland atrophy. In addition, RB1 and RB4 also showed edema that was not observed in the other two samples (Fig. 3). We also found hemorrhage (Fig. 3) and inflammatory cells infiltrate such as neutrophils, lymphocytes, and macrophages from histopathological examination (Fig. 4).

Endometritis causes erosion and hyperplasia of columnar epithelial layers in the functional layer and uterine gland epithelial. A similar result was reported by Bajaj et al. [21] who found the columnar epithelial erosion in the functional layer in buffaloes with endometritis. The epithelial erosion occurred due to the contact between epithelial cells and pathogens that entered the uterus. Hyperplasia is an increase in the number of cells in a tissue or organ, resulting in enlargement of the tissue or organ. Hyperplasia occurs due to cell adaptation processes [22]. The same result was reported by Chethan et al. [10], who found columnar epithelial hyperplasia in the functional layer in buffalo with endometritis. This occurs due to the response to a specific stimulus, resulting in cell proliferation.

Inflammation of the endometrial lining also caused edema in RB1 and RB4 and uterine gland atrophy which was only observed in RB3. Edema is the excessive fluid collection in the cell body or various body cavities [23]. This result is in agreement with [24] study that reported uterine tissue edema as a histopathological feature of chronic endometritis in Buffalo. Rhyaf [9] added that the edema in endometritis is a response to inflammation which caused changes in hydrostatic pressure in the microcirculation and blood vessel permeability, resulting in protein and fluid leakage into the tissues [23].

Atrophy is shrinkage of cellular size and reduced cellular function [22]. The same results were reported by Ali and Ameen [25] who revealed that histopathological features of chronic endometritis in dairy cows included uterine gland atrophy. Furtermore, Chethan et al. [10] added that the uterine gland atrophy was caused by decreased uterine gland number and its secretory activity due to fluid in the uterine lumen.

Endometrial inflammation also causes hemorrhage in uterine tissue. Hemorrhage is blood that escapes from cardiovascular circulation due in the heart and blood vessels such as arteries, veins, and capillaries [22]. Hemorrhage in endometrial tissue is caused by the inflammatory response which results in blood vessels dilatation, resulting in red blood cells escaping from the vessel into the tissue [9]. Moreover, Price and Wilson [23] stated that blood vessels dilatation occurs due to blood vessel wall widening, thus, blood flowing out of the local microcirculation. Red blood cells that escape from the vessel into the tissue are immediately phagocyted by macrophages as part of the inflammatory response.

Inflammatory cell infiltration such as neutrophils, lymphocytes, and macrophages in endometrial tissue was an indicator of endometrial inflammation in Aceh cattle with RB caused by subacute and chronic bacterial infection. [26] stated that inflammatory cell infiltration in goats with endometritis shows the categories of inflammation, namely acute, subacute, and chronic phases. In the acute phase, neutrophils could be found in the caruncle, intercaruncular, and uterine glands. In the subacute phase, the lymphocytes, neutrophils, and macrophages are found in lamina propria and caruncle, whereas in the chronic phase lymphocytes and macrophages are found in the lamina propria and endometrial lumen.

The uterine horns of Aceh cattle with RB due to subacute and chronic endometritis could cause anatomical and histological changes in the uterine horn layer. Rhyaf [9], Chethan et al. [10], Sayyari et al. [18], and Ali and Ameen [25] also reported that acute, subacute, and chronic endometritis can cause anatomical and histological changes in the uterus.

Histomorphometry of uterus

The result of uterine histomorphometry measurements in Aceh cattle with RB and fertile Aceh cattle is presented in Table 2.

Table 2 showed the difference in mean ± SD thickness of each uterine layer (endometrium, myometrium, and perimetrium) between RB and fertile Aceh cattle. The thickness of all uterine layers of RB cattle was higher than the fertile one, however, the endometrial uterine gland diameter of Aceh cattle with TB was smaller than the fertile one.

The thicker endometrial layer of RB Aceh cattle can be caused by pathological conditions such as endometritis. Endometritis is the inflammation of the endometrium caused by a bacterial infection. This infection occurs because the bacteria ascended to the uterus via the vagina to uterine cervix and contaminates uterus during parturition. In addition, endometritis can also be sequelae of dystocia, uterine prolapse, placental retention, and unhygienic environment. An unhygienic environment will facilitate the entry of microbes which contaminate uterine lumen and interfere with embryonic development. This condition can cause premature embryonic death [27]. Based on the rectal examination, it is known that uterus with endometritis is larger and its wall is thicker. These abnormalities are generally detected in one of the uterine horns, but can also occur in both [5].

Factor that caused perimetrium layer thickening of RB Aceh cattle is pathological conditions such as perimetritis. According to Hardjopranjoto [5], perimetritis is an inflammation that occurs in the outermost layer of the uterine

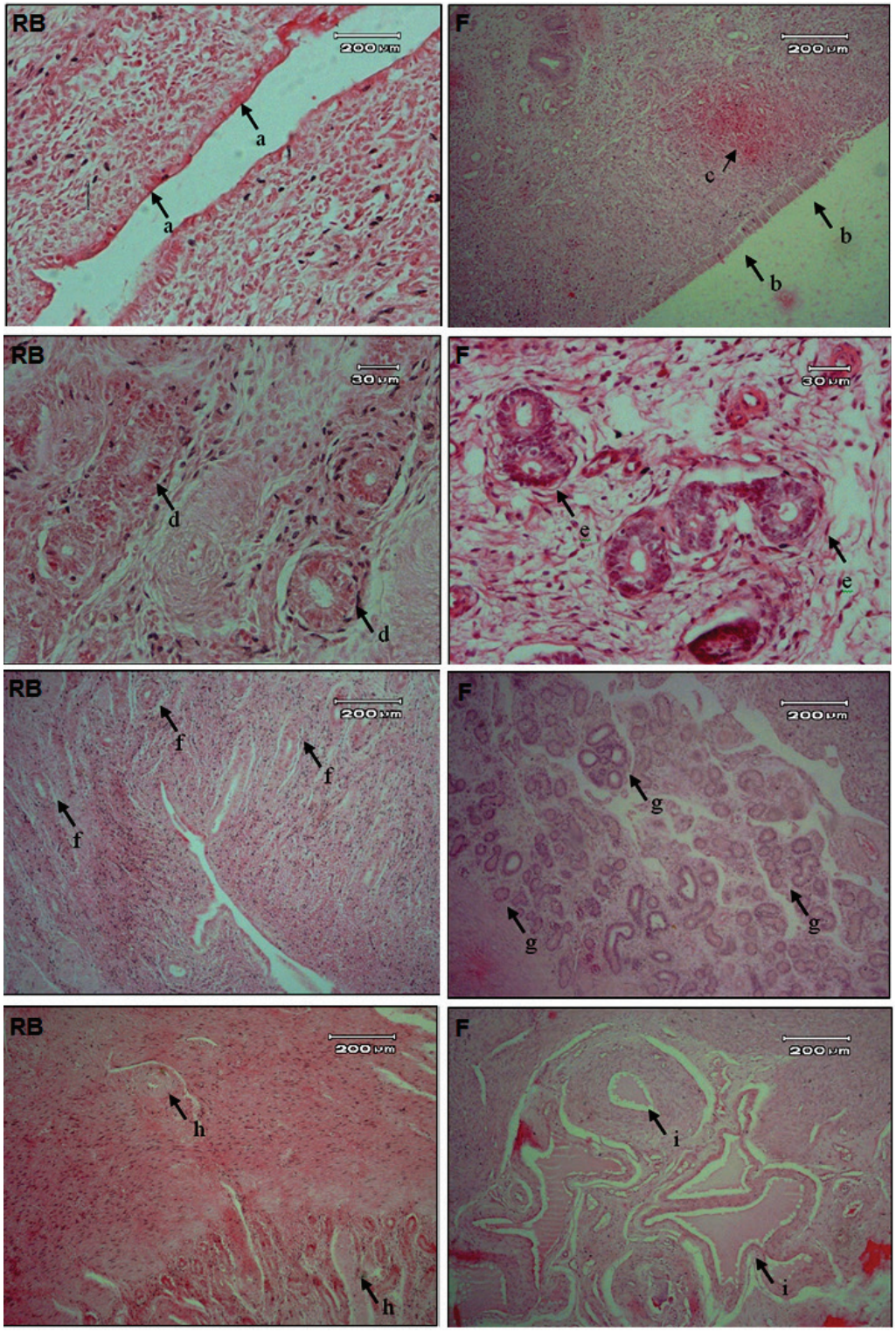

Figure 3. Histopathological changes showed in RB cow uterine horns (RB) compared with a fertile cow (F). (a) Endometrial columnar epithelium, (b) erosion of endometrial columnar epithelium, (c) Hemorrhage (H&E, 10X), (d) uterine gland epithelium, (e) hyperplasia of uterine gland epithelium (H&E, 40×), (f) uterine gland, (g) atrophy of uterine gland, (h) caruncle, and (i) edema in caruncle (H&E, 10×).

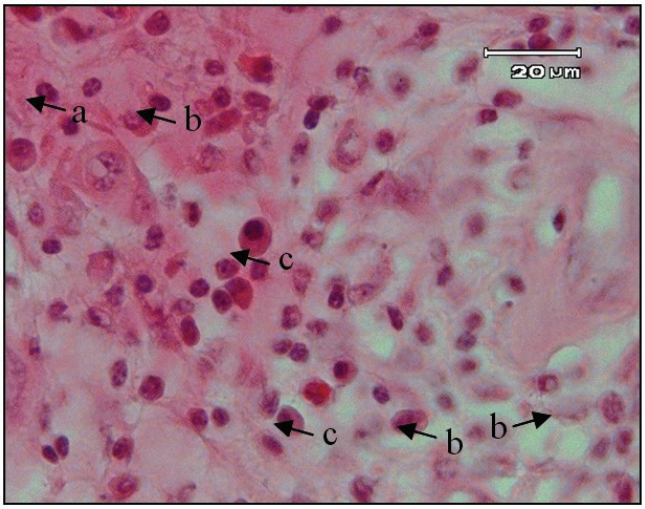

Figure 4. Infiltration of inflammatory cell in uterine horns of Aceh cows with RB. Infiltrating inflammatory cells include (a) neutrophil cells, (b) macrophages, and (c) lymphocytes. Hematoxylin and Eosin staining.

Table 2. Histomorphometry of Aceh cattle with repeat breeding (RB) and fertile cattle.

Uterine Hystomorphometry (µm)	Female Cattle	
	Repeat breeding (RB)	Fertile
Thickness		
Endometrium	208.06 ± 39.90	187.39 ± 29.09
Myometrium	400.138 ± 51.96	277.91 ± 42.88
Perimetrium	23.59 ± 9.67	18.53 ± 4.40
Endometrial uterine gland diameter	4.04 ± 0.88	4.99 ± 1.37

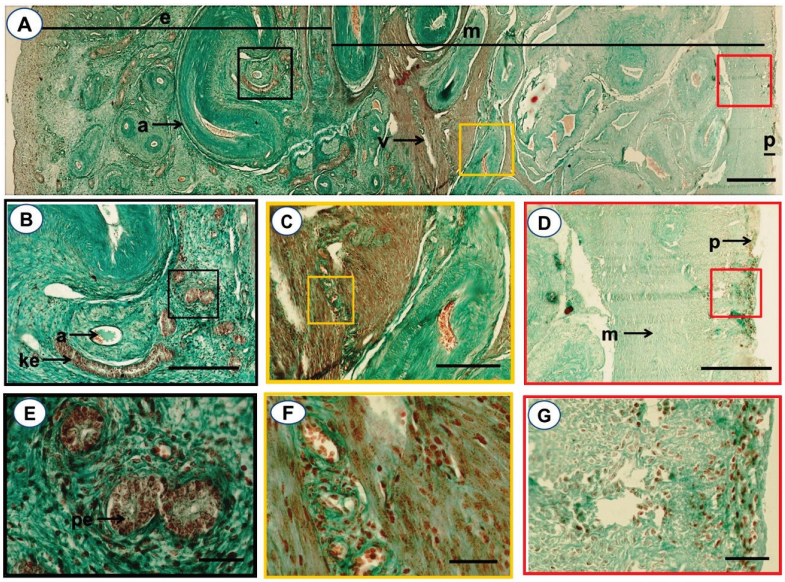

Figure 5. Histological structure of RB cows. (A) The uterus is divided into endometrium (e), myometrium (m) and perimetrium (p). (B) and (E) the endometrial layer; (C) and (F) the myometrial layer; (D) and (G) the perimetrium layer. Endometrial gland (ke), artery (a), pseudostratified epithelium (pe). Masson's trichrome staining. Magnification of 40× (A), 100× (B–D), and 400× (E–G).

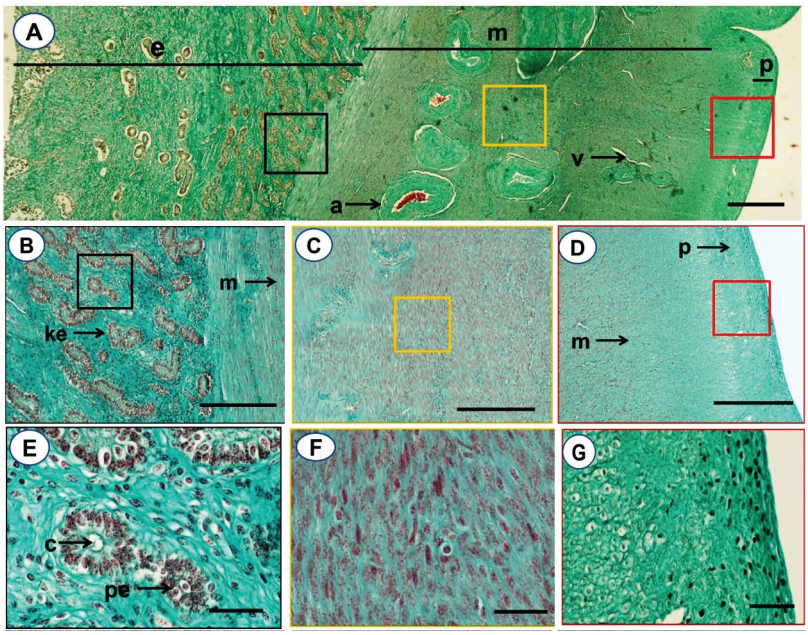

Figure 6. The histological structure of the fertile cow's uterus. (A) The uterus is divided into endometrium (e), myometrium (m), and perimetrium (p). (B and E) the endometrial layer; (C and F) the myometrial layer; (D and G) the perimetrium layer. Endometrial glands (ke), arteries (a), veins, pseudostratified epithelium (pe), cilia (c) Masson staining's trichrome. Magnification of 40× (A), 100× (B–D), and 400× (E–G).

wall (serous). Signs of this pathological condition are the presence of adhesion between the uterine wall and the wide ligament and organs in the pelvic cavity and peritoneal cavity. Perimetritis is sometimes accompanied by perimetrial abscess, resulting in a thicker layer.

Based on the measurements of uterine glands diameter in the endometrium, there were differences in both groups. The diameter of RB endometrial gland is smaller (4.04 ± 0.88 μm) than that of fertile cows (4.99 ± 1.37 μm). This difference is thought to be influenced by the differences in progesterone concentrations between RB cattle and fertile one in the early luteal phase. In the luteal phase, morphological changes occur in endometrial cells. At the start of the luteal phase, progesterone level is increased, as well as the number and the size of glands, and endometrial layer becomes thicker [28]. This is in accordance to Thasmi et al. [12] who reported that gland diameter in Aceh cattle with RB is smaller than that of the fertile one due to the recurrent mating which leads to low progesterone level and high estrogen level.

Conclusion

The changes in anatomical pathology and histopathology of the uterine horn of Aceh cattle with RB indicate endometritis which caused by subacute and chronic bacterial infections. Endometritis causes disrupted blood circulation, which is characterized by hemorrhage, edema, and thickening of the endometrium, myometrium, and perimetrium, resulting in an increased diameter of the endometrial gland in RB Aceh cattle.

Acknowledgments

This work was supported by the Ministry of Research and Technology, and Higher Education, Government of Indonesia, under the project Hibah Penelitian Disertasi Doktor 2018.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' contribution

The manuscript was written by Cut Nila Thasmi, Sri Wahyuni, and Dwinna Aliza and edited by Budianto Panjaitan and Nazaruddin Nazaruddin. Planning and execution of this work were under the supervision of Tongku Nizwan Siregar. Firschilia Nurul Sabila and Miranda Fallatanza collected the samples and conducted an experiment under the supervision of Cut Nila Thasmi, Sri Wahyuni, and Dwinna Aliza. All authors read and approved the final manuscript.

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