การสะสมของไกลโคเจนและลักษณะทางจุลกายวิภาคของมดลูกสุกรระบบโรงเรือนเปิดและสุกรระบบโรงเรือนปิด

Glycogen Accumulation and Histological Characteristics of Swine Uterus in Outdoor and Indoor Housing Systems

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ABSTRACT

Stress is a significant factor affecting irregular estrus cycles. This study aimed to investigate uterus histological characteristics of outdoor and indoor swine methods. The sample of outdoor and indoor swine uterus was carried out through histological technique and dyed with H&E and PAS. The results show that indoor swine had significantly thicker endometrial layers ($p < 0.05$). Indoor swine exhibited significantly higher glycogen accumulation than outdoor swine ($p < 0.05$). In conclusion, the study has presented the histological characteristics of the uterus in two distinct types of swine, offering valuable insights into the uterine health status of these animals. This information holds potential significance for consumers.

Keyword: Swine, Uterus, Stress, Endometrium, Glycogen accumulation

บทคัดย่อ

ความเครียดเป็นปัจจัยที่ทำให้เกิดความไม่สมดุลของฮอร์โมนส่งผลให้วงรอบการเป็นสัดมาไม่สม่ำเสมอ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาลักษณะทางจุลพยาธิวิทยาของมดลูกของสุกรระบบโรงเรือนเปิดและสุกรระบบโรงเรือนปิด ตัวอย่างเนื้อเยื่อมดลูกสุกรทั้งสองชนิดถูกย้อมด้วยสี Hematoxylin and Eosin (H&E) และ Periodic Acid-Schiff (PAS) ผลการวิจัยพบว่าสุกรระบบโรงเรือนปิดมีชั้นเยื่อบุโพรงมดลูกหนากว่าอย่างมีนัยสำคัญ ($p < 0.05$) สุกรระบบโรงเรือนปิดมีการสะสมไกลโคเจนสูงกว่าสุกรระบบโรงเรือนเปิดอย่างมีนัยสำคัญ ($p < 0.05$) โดยสรุปการศึกษานี้ได้นำเสนอลักษณะทางเนื้อเยื่อวิทยาของมดลูกในสุกรสองประเภทที่แตกต่างกัน ซึ่งให้ข้อมูลเชิงลึกที่เกี่ยวกับสุขภาพของมดลูกของสุกร ข้อมูลนี้อาจมีความสำคัญต่อผู้บริโภค

คำสำคัญ: สุกร มดลูก ความเครียด เยื่อบุโพรงมดลูก การสะสมของไกลโคเจน

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Introduction

Chronic stress can interfere with hormonal control in the brain, affecting both male and female fertility. In males, it can disrupt sperm formation and quality. In females, the normal function of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), responsible for oocyte growth and release, as well as estrogens and progesterone, which regulate the estrus cycle and prepare the uterus for embryo implantation, can be compromised by chronic stress. This disruption often manifests as decreased gonadotropin-releasing hormone (GnRH) secretion, leading to amenorrhea or increased GnRH exposure, resulting in anovulation. Moreover, chronic stress can influence estrogen and progesterone levels, leading to reduced libido and sexual frequency, thus decreasing the chances of conception. It can also impact embryo implantation due to altered hormone levels. (Andrea Rodrigo, 2022).

Stress can be a major contributor to infertility, as it may induce abnormal hormone production (Ramya, Poornima *et al.* 2023) Cold stress can reduce the thickness of the endometrial layer in the uterus (Wang, Cheng *et al.* 2020). Oxidative stress can cause changes in the uterus, like increased height of luminal epithelium cells and significant apoptosis of glandular and luminal epithelium cells. It also leads to inflammation in the endometrium, with increased infiltration of eosinophils, polymorphonucleocyte lymphocyte, macrophages in the myometrium and endometrium layers, and vascular congestion (Alchalabi, Rahim *et al*. 2016). Oxidative stress can also result in histopathological changes such as degeneration/necrosis of the circular muscle layer and a high rate of apoptotic cells (El-Din, Ghareeb *et al.* 2023). Moreover, heat stress can bring about histopathological changes in reproductive organs like the ovaries and uterus, affecting their normal functions. This, in turn, can lead to reduced embryo implantation and pregnancy rates, negatively impacting female reproduction through histopathological changes in reproductive organs (Rebez, Sejian *et al*. 2023). Another study found that the repeated administration of adrenocorticotropin hormone (ACTH) in swine after ovulation resulted in elevated cortisol concentrations and had no significant effect on the distribution of inflammatory cells, particularly lymphocytes. However, there was a tendency for an effect on the distribution of neutrophils in the connective tissue of the subepithelial layer in the swine endometrium. (Kaeoket, Mwanza *et al*. 2002)

The estrous cycle of swine, consisting of proestrus, estrus, metestrus, and diestrus stages, is a complex series of events that involve both hormonal and histological changes in the ovaries and the uterine lining. This cycle plays a pivotal role in the reproductive process of swine. Proestrus: Proestrus marks the onset of the follicular phase, characterized by the secretion of estrogen and follicle-stimulating hormone (FSH). These hormones increase in preparation for estrus, the next stage. Additionally, small pulses of luteinizing hormone (LH) commence, contributing to the overall hormonal milieu. The uterine histology during proestrus reveals a relatively thin uterine lining. In some cases, there may be observable signs of endometrial gland development. Estrus: Estrus is the pinnacle of the follicular phase, where the oocyte reaches maturity. Estrogen, FSH, and LH secretion reach

their zenith, prompting the rupture of the follicle and ovulation. Ovulation typically takes place within 24 to 48 hours of peak estrogen release. The uterine histology during estrus exhibits a thickening of the uterine lining, or endometrium, which becomes vascularized. This is in preparation for potential implantation. Metestrus: Metestrus is characterized by a decline in estrogen, LH, and FSH levels, leading to the cessation of the female's sexual receptivity to the male swine. Ovarian follicles that release oocytes during ovulation undergo luteinization, transforming into the corpus hemorrhagicum and, subsequently, the corpus luteum. With the formation of the corpus luteum, progesterone secretion begins. The uterine histology during metestrus reflects changes in the endometrial glands as they become more tortuous and filled with secretions, in response to the presence of the corpus luteum. Diestrus: Diestrus, the longest stage of the estrous cycle, is pivotal for recognizing pregnancy. During this phase, progesterone levels continue to increase, typically around day 12 of gestation. The uterine histology during diestrus displays a thickened, glandular endometrium with a well-developed vascular network, serving to support a potential pregnancy. In the absence of fertilization and implantation, the corpus luteum will regress, initiating the start of a new estrous cycle. (Kaeoket, Persson *et al*. 2001) (Hines 2023)

Stress is not exclusive to humans; it is also experienced by swine. Several well-established stressors in the swine farming environment are known to contribute to chronic physiological stress. These stressors encompass issues such as overcrowding, high temperatures, limited access to food, the inability to engage in natural foraging behaviors, uncomfortable flooring, and suboptimal handling practices (Lagoda, Marchewka *et al.* 2022).

In Thailand, two primary categories of swine are raised for consumption: outdoor swine and indoor swine. Outdoor swine are defined as swine raised within production systems that grant them access to outdoor areas for activities such as foraging, rooting, and engagement in natural behaviors. This form of production allows swine to manifest their innate behaviors and provides them access to a more natural environment, thus significantly contributing to their overall welfare and well-being. Notably, outdoor swine production aligns itself with niche markets that prioritize natural and organic pork products. These markets often necessitate outdoor or bedded settings while prohibiting the use of subtherapeutic antibiotics or growth promoters. Indoor swine, conversely, are reared in confinement systems where they remain housed indoors throughout their entire production cycle. Indoor swine production presents a controlled environment conducive to superior management of various factors, including temperature, nutrition, and health. Additionally, this system offers a more efficient utilization of available space and resources, proving particularly advantageous in regions characterized by limited land availability (Honeyman 2005).

These different farming methods can cause potential stress, which can be a factor in physical health, especially the reproductive system, causing abnormalities that may be reflected by the morphology and histology of swine uterus specimens. In this context, this study aimed to investigate uterus histological

characteristics of indoor and outdoor swine housing. Knowledge from this study will illustrate the uterus health status of two kinds of swine for the consumers' reference.

Materials and Methods

Ethical Considerations

This study was consistent with the Laboratory Animal Use Convention published by the National Institutes of Health. All animal experimental procedures were approved by the animal ethics committee of the Center for Animal Research, Naresuan University (Approval no. NU-AEE620505).

Sample collection and histological analysis

Twelve samples of swine uterus obtained from four-month-old swine were bought from indoor and outdoor farm enterprises in Phitsanulok and Nakhon Pathom, Thailand. The housing temperature 28-33 °C, changes based on the surrounding weather. Three pieces of tissues from the uterus were cut into 0.5 × 1 cm size. Then, the cut tissues were fixed in 10% neutral buffered formalin for 7 days and each piece was individually labeled for histological preparation. The fixed tissues were processed for embedding in paraffin and sectioned at 5 μm thickness in a rotary microtome. From each paraffin block, sections were derived from the superficial, middle, and deep layers of the tissue. All 108 tissue slides were subjected to staining, employing both routine Hematoxylin and Eosin (H&E) and Periodic Acid-Schiff (PAS) staining techniques. Finally, each of the prepared slides was mounted. Photos were taken using a slide scanner and recorded through the Aperio Image Scope version 12.3.3.5048 software to examine the histological characteristics of the uterine walls in both types of swine detecting variances in the endometrium and myometrium layers of the uterine wall. Specifically, the study focused on assessing changes in the thickness of the endometrial layer, lymphocytic diffusion, and apoptotic cells. Simultaneously, the myometrium layer was examined for alterations in thickness and the accumulation of glycogen in both types of swine.

Statistical analysis

The data acquired from the IBM SPSS Statistics 29.0.1.0 program were analyzed using the T-Test and subjected to comparison via the Independent Samples Test to assess statistical differences. In each experiment, data were computed and presented as mean ± standard error of the mean (SEM). A p value of < 0.05 was considered a statistically significant difference.

Results and Discussion

Histological characteristics of indoor and outdoor swine uterus

The histology of indoor and outdoor swine uteri was examined under 4X magnification, revealing three distinct layers 1) Perimetrium: This is the outermost protective layer. 2) Myometrium: Positioned in the middle, this highly muscular layer expands during pregnancy and contracts to facilitate parturition. 3) Endometrium: Located as the innermost lining of the uterus, the endometrium plays a pivotal role in uterine function. Within this layer, one can find lymphocytic diffusion and apoptotic cells.

The endometrium comprises two layers: the basal layer, which houses endometrial stem cells responsible for generating the functional

layer. The functional layer is the part that undergoes growth at the commencement of each estrus cycle and subsequently sheds during menstruation. We observed variations in the structure of certain layers of the swine uterine wall.

Thickness of the Myometrium

When studying the thickness of the myometrium, it was observed that indoor swine housing had a thickness that was not significantly different from outdoor swine housing (2.78 ± 0.27 and 2.16 ± 0.23, respectively) (Figure 1)

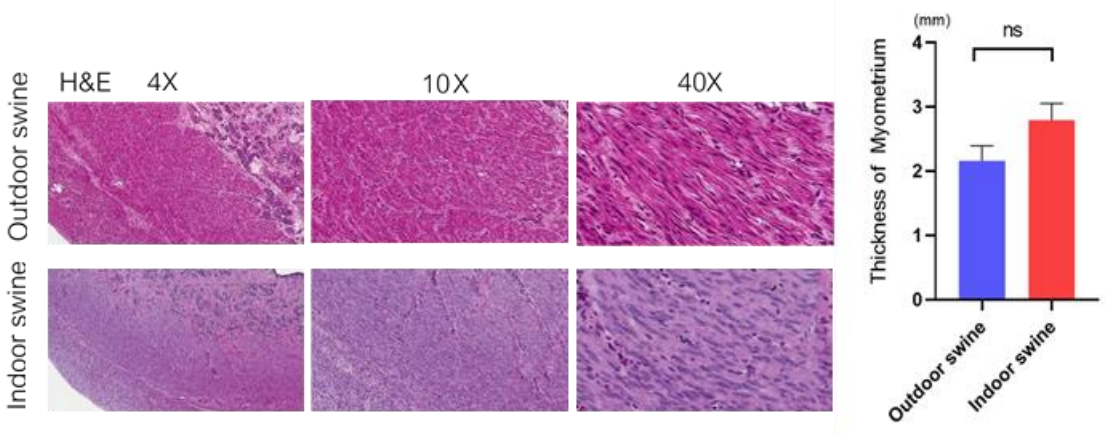

Figure 1 The histology presenting thickness of myometrium of outdoor and indoor swine using H&E staining

Thickness of the Endometrium

However, in the case of the endometrium, it was found that indoor swine displayed a significantly greater thickness (2.78 ± 0.178 compared to outdoor swine (1.90 ± 0.26) with statistical significance (p < 0.05), (Figure 2)

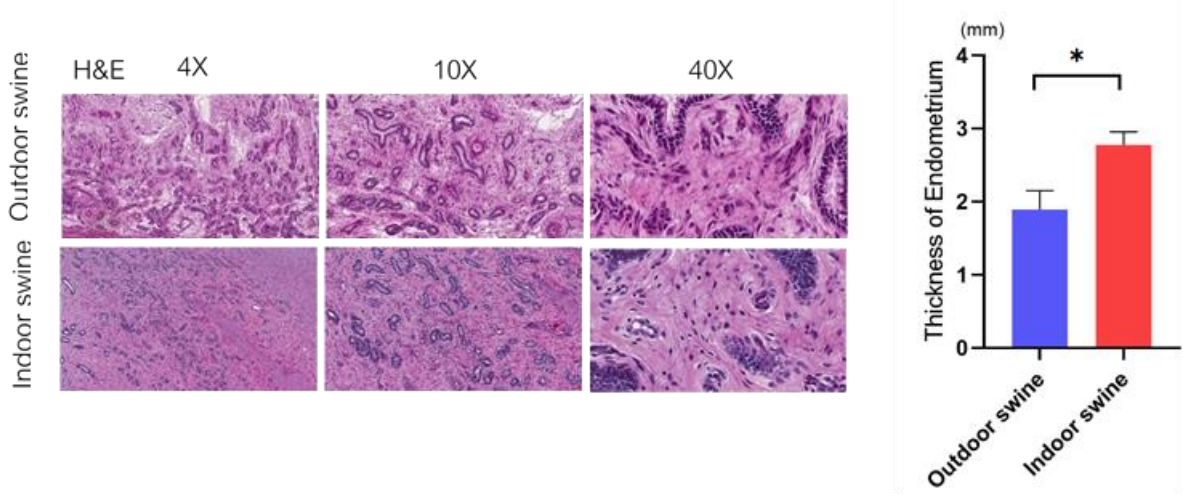

Figure 2 The histology presenting thickness of endometrium of outdoor and indoor swine using H&E staining, * indicates p < 0.05.

The effect of stress on the uterine wall can vary among individuals and may not follow a consistent pattern of thickening or thinning. Stress can influence the reproductive system in many aspects. In some cases, chronic stress may lead to hormonal imbalances, potentially affecting the estrus cycle and resulting in changes in the thickness of the endometrial lining. However, stress responses can differ from animal to animal. Some individuals may experience irregular menstruation or even amenorrhea due to stress, which could result in a thinning of the uterine wall. Others may experience different effects, and stress may not always have a direct and predictable impact on uterine thickness. Stress can lead to an increase in the production of reactive oxygen species (ROS). The production of free radicals and reactive oxygen species affects this reduction in myometrial thickness *(Mir, Butt et al.*2020) The impact of stress on the myometrium, the muscular layer of the uterine wall, is complex and may not be as direct as its effects on the endometrium.

Stress can harm reproductive health and hormonal balance, potentially affecting ovulation (Rooney and Domar, 2018) If ovulation does not occur, progesterone is not released, and the lining is not shed. The endometrium may continue to grow in response to estrogen. The cells that make up the lining may crowd together and may become abnormal. This condition, called hyperplasia, can lead to cancer (Gynecologists, 2022)

Number of Lymphocytes

When studying the number of lymphocytes in the endometrium, it was observed that indoor swine tended to have higher numbers (15.00 ± 2.61) compared to outdoor swine (9.83 ± 1.76). Stress is known to have various impacts on the immune system, including changes in immune cell populations and activity. The infiltration of lymphocyte in uterine tissue may be indicative of an immune response or the presence of free radicals, which could contribute to endometrial oxidative damage and the pathogenesis of endometritis *(Alchalabi, Rahim et al.* 2016) In the results of this experiment, no statistically significant differences were observed between the two types of swine. Nonetheless, it is worth noting that there was a tendency for indoor swine to have a higher lymphocyte count compared to outdoor swine, (Figure 3)

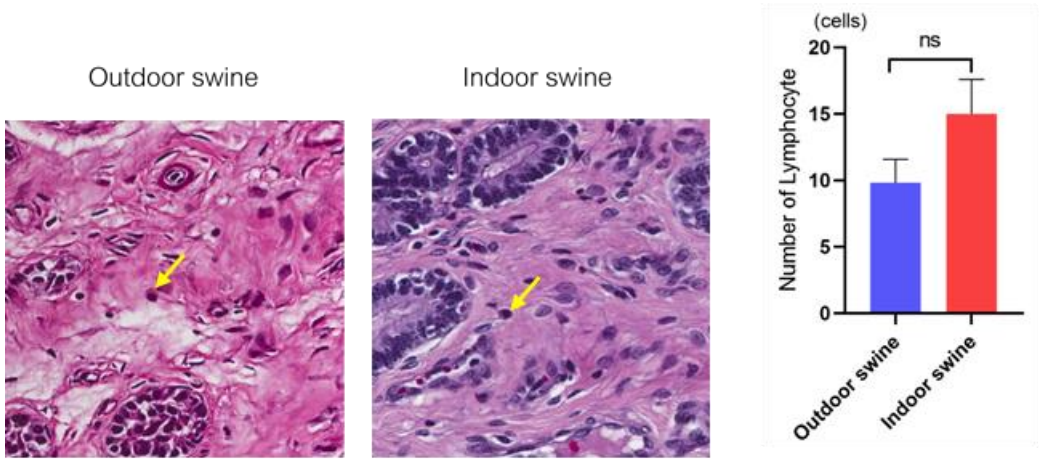

Figure 3 The histology presenting number of lymphocytes of outdoor and indoor swine using H&E staining, lymphocyte indicated by yellow arrow 40x

Number of Apoptotic cells

When studying the number of apoptotic cells in the endometrium, it was found that the number of apoptotic cells in indoor swine tended to be higher (12.94 ± 2.53) compared to door swine (9.11 ± 1.51). Stress can induce cell death in the uterus, and oxidative stress, associated with an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, can lead to apoptotic cell in uterine tissues (Alchalabi, Rahim *et al.* 2016). In the results of this experiment, no statistically significant differences were observed between the two types of swine. Nonetheless, it is worth noting that indoor swine tended higher apoptotic cell counts compared to outdoor swine, (Figure 4)

Figure 4 The histology presenting number of apoptotic cells of outdoor and indoor swine using H&E staining, apoptotic cells indicated by yellow arrows 40x

Accumulation of glycogen

We found significant glycogen accumulation in the outdoor swine compared to indoor swine. In outdoor swine, the accumulation of glycogen was 26.04% (26.04 ± 2.74), and in indoor swine, the accumulation of glycogen was 41.71% (41.71 ± 4.67) ($p < 0.05$). Stress has been reported to result in higher cortisol levels during the luteal phase (Montero-López, Santos-Ruiz *et al.* 2018). And there is a peak in glycogen accumulation during the luteal phase (Dean 2019). It could be interpreted that stress stimulation also affects glycogen accumulation. Typically, glycogen accumulation in the uterus is associated with hormonal fluctuations, the estrus cycle, pregnancy, and certain physiological processes, (Figure 5)

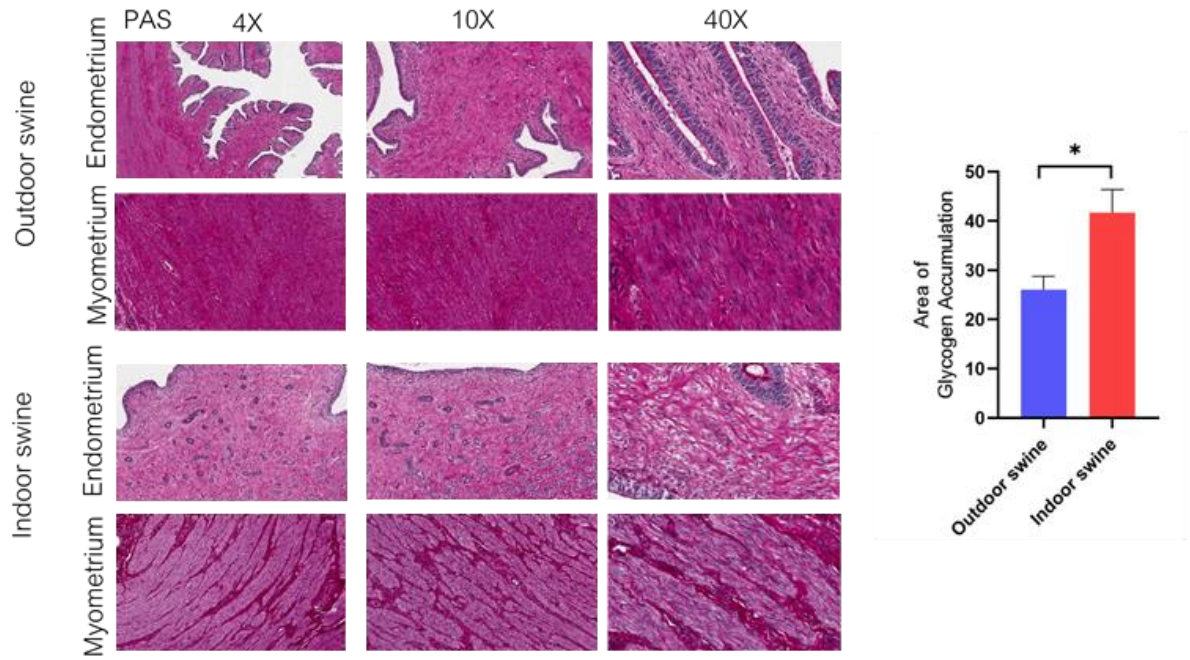

Figure 5 The histology presenting an accumulation of glycogen of outdoor and indoor swine using PAS staining, * indicates $p < 0.05$.

Conclusion and Suggestion

Swine production has a vital economic role in Thailand, primarily serving domestic consumption needs. The practice of raising swine encompasses a wide spectrum, from personal consumption in households to large-scale industrial operations. However, to date, there have been no reported studies investigating whether different rearing methods result in distinct histological characteristics in swine uteri.

From this study, it was observed that the histological characteristics of the myometrium in both outdoor and indoor swine were similar. However, a significant difference was noted in the thickness of the endometrial layer, with outdoor swine exhibiting greater thickness compared to indoor swine.

Nevertheless, it's important to note that comprehensive histological features of the normal uterine wall in swine have yet to be reported.

Consequently, further studies are required to comprehensively compare and analyze the differences identified in this study.When assessing the quantity of lymphocytes within the endometrial layer of outdoor and indoor swine, there was no significant difference observed. Similarly, when examining the count of apoptotic cells within the endometrial tissue of both outdoor and indoor swine, no notable differences were detected between the two swine types. We found significant glycogen accumulation in the outdoor swine compared to indoor swine. The significant difference suggests that environmental factors or management practices between outdoor and indoor settings may influence glycogen levels in swine. Additionally, it's important to consider other factors that might contribute to this difference, such as diet, exercise, or any environmental conditions that may affect glycogen metabolism in swine. Further research may be needed to explore the underlying mechanisms and implications of this glycogen accumulation difference between outdoor and indoor swine. In summary,farming methods and living environment impact swine's welfare, which might lead to differences in their organ histology.

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