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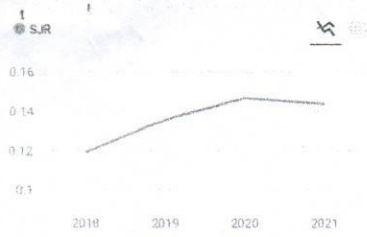

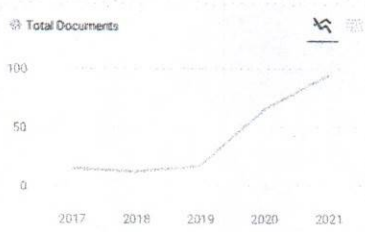

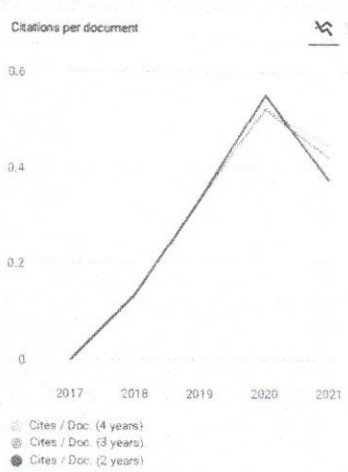

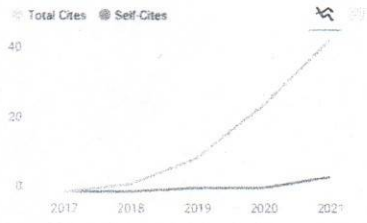

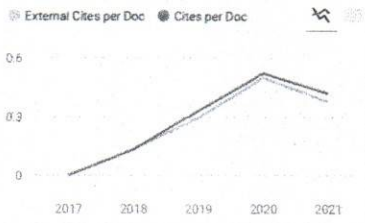

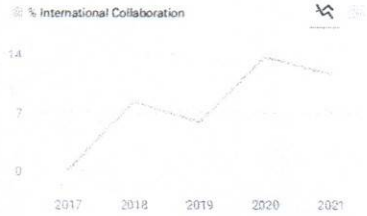

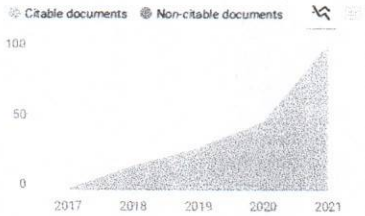

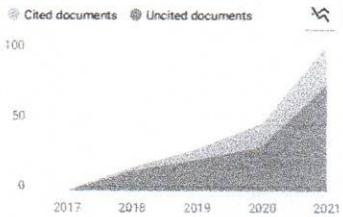

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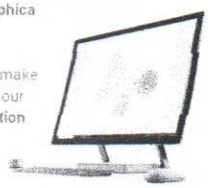

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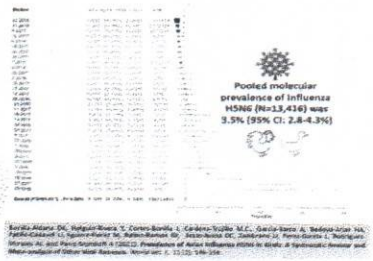
Systematic Review

Prevalence of Avian Influenza H5N6 in Birds: A Systematic Review and Meta-analysis of Other Viral Zoonosis

Bonilla-Aldana DK, Holguin-Rivera Y, Cortes-Bonilla I, Cardona-Trujillo M.C., García-Barco A, Bedoya-Arias HA, Patiño-Cadavid LJ, Aguirre-Florez M, Balbin-Ramon GJ, Erazo-Arana DC, Zambrano LI, Perez-Garcia L, Rodriguez-Morales AJ, and Paniz-Mondolfi A.



World Vet. J. 11(2): 146-156, 2021; *pii:S232245682100020-11*

DOI: https://dx.doi.org/10.54203/scil.2021.wvj20

ABSTRACT: Avian influenza viruses (AIV) are zoonotic pathogens that can potentially affect humans and potentially be epidemic in a region. Birds (such as poultry and wild birds) serve as potential reservoirs for these viruses, highlighting the importance of determining AIV prevalence in the avian population. No systematic reviews have been published on this issue in the world so far. The present systematic literature review following the PRISMA standard, with meta-analysis, used three databases to globally assess the Influenza H5N6 infection in birds (including poultry and wild birds). A model of random-effects meta-analysis was performed to calculate the pooled prevalence and 95% Confidence Interval (95% CI) for the prevalence of Influenza **H5N6** infection in birds. A total number of 14,605 articles published from 2015 to 2020 were retrieved. After screening the abstract/title, 37 articles were selected for full-text assessment, and 15 were included for qualitative and quantitative analyses. Of the total number of birds (n = 13,416 birds), the pool prevalence by RT-PCR was 3.5% (95% CI: 2.8-4.3%). From the total, 39.67% of the birds assessed were ducks (*family Anatidae*), in which *pool prevalence was 7.7% (95% CI: 4.4-11.0). In chickens (Gallus gallus domesticus), the pool prevalence was 3.3% (95% CI* 1.9-4.8). Vietnam was the country with the highest pool prevalence; 7.9% (95% CI 4.0-11.7%). Bangladesh was the country with the lowest pool prevalence of 0.4% (95% CI 0.2-0.7%). A considerable proportion of infected birds tested positive highlighted the relevance of individual animals as reservoirs of H5N6. Ducks and chickens were found to be positive by RT-PCR in over 3% of the cases. These data suggest their relevance in maintaining zoonotic transmission and their potential implications for epidemics and even pandemics in the near future.
Keywords: H5N6, Influenza, Meta-Analysis, Molecular diagnosis, RT-PCR, Systematic Review

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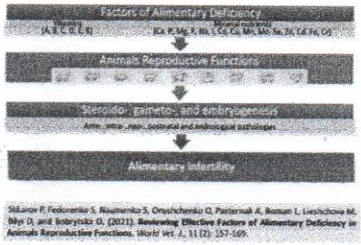
Review

Reviewing Effective Factors of Alimentary Deficiency in Animals Reproductive Functions

Skliarov P, Fedorenko S, Naumenko S, Onyshchenko O, Pasternak A, Roman L, Lieshchova M, Bilyi D, and Bobrytska O.



World Vet. J. 11(2): 157-169, 2021; pii:S232245682100021-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj21

ABSTRACT: Animal reproduction is one of the main factors limiting the efficiency of livestock production. Its optimal level is possibly achieved when certain conditions are created for animals. As reproduction is a complex reflex process depending on neuroendocrine regulatory

mechanisms, the character and strength of stimuli, which, in turn, is due to a number of factors. Under normal conditions, the body of animals is affected by many different factors, which are appropriately transformed and specified by positive or negative reactions. Inhibitory factors include air pool, saturated with harmful substances and gases, ionizing radiation, poor water quality along with altered redox properties, hypokinesia combined with poor unbalanced feeding, systematic chronic stress, presence of toxic substances in feed, and the deficiency of vitamins and other bioantioxidants in feed or their excessive spending. Of the wide range of genetic and paratypic factors of negative impacts on reproductive capacity, the most common one is alimentary, which causes impaired reproductive function due to deficiencies in the rules, regulations, and feeding regime of animals. In particular, the alimentary can be associated with both general malnutrition (starvation) and overfeeding (obesity). However, the alimentary form of infertility mostly occurs due to low-quality diets when the diet lacks vital components (mainly vitamins, macro-, and micronutrients) or the quantitative ratios of the ingredients are violated. This is possible even if the total nutritional value of the diet meets the established requirements for the physiological needs of the body. Vitamins, micro-, and macronutrients are ecologically deficient factors of disturbance of animal reproductive function, the influence of which is observed on all processes of reproduction, from fertilization to the postpartum period and the preservation of young animals. The pathogenesis of their insufficiency is associated with the violation of steroido-, gameto-, and embryogenesis and the emergence of ante-, intra-, neo- and postnatal pathologies, respectively. Therefore, treatments and prevention measures should be aimed at providing animals with biologically complete balanced feeding and replenishment of the body with vitamins and minerals. However, all these issues remain incompletely studied and need further research.

Keywords: Alimentary deficiency, Animals, Reproductive function.

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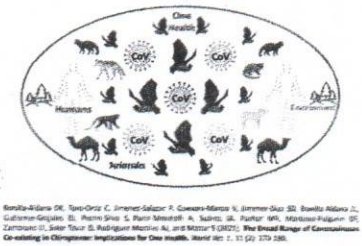
Review

The Broad Range of Coronaviruses Co-existing in Chiropteran: Implications for One Health



Bonilla-Aldana DK, Toro-Ortiz C, Jimenez-Salazar P, Guevara-Manso V, Jimenez-Diaz SD, Bonilla-Aldana JL, Gutierrez-Grajales EJ, Pecho-Silva S, Paniz-Mondolfi A, Suárez JA, Pachar MR, Martinez-Pulgarin DF, Zambrano LI, Soler-Tovar D, Rodriguez-Morales AJ, and Mattar S.

World Vet. J. 11(2): 170-180, 2021; pii:S232245682100022-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj22

ABSTRACT: Bats are a group of mammals that harbor the most significant number of coronaviruses. The aim of present review article was to analyze the broad spectrum of the coronavirus coexisting in Chiropterans hosts. Bats have certain types of cell receptors that allow them to be the potential hosts of a large number of viruses without the presence of any clinical manifestations, and to be a source of contagion infections for other animals and human species. Emphasis can be placed on five coronaviruses, such as Porcine Epidemic Diarrhea Disease, Severe Acute Diarrhea Syndrome, Middle East Respiratory Syndrome, Severe Acute Respiratory Syndrome, and Severe Acute Respiratory Syndrome 2, which have had significant impacts causing epidemic outbreaks in different parts of the world, and generating implications for both human and animal health. In conclusion, recent research indicated the importance of bats as potential hosts of multiple coronaviruses leading to some zoonotic diseases.

Keywords: Bats, Coronaviruses, Cross-species, Evolution, Spillover, Transmission

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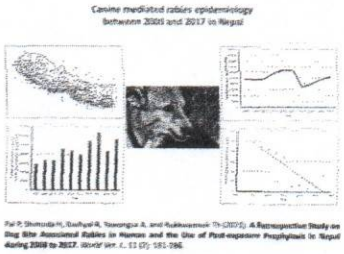
Research Paper

A Retrospective Study on Dog Bite Associated Rabies in Human and the Use of Post-exposure Prophylaxis in Nepal during 2008 to 2017



Pal P, Shimoda H, Bashyal R, Yawongsa A, and Rukkwamsuk Th.

World Vet. J. 11(2): 181-186, 2021; pii:S232245682100023-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj23

ABSTRACT: A 10-year (2008-2017) retrospective canine-mediated human rabies epidemiology was studied to assess the burden of rabies in Nepal. To this end, the number of dog bites, the use of post-exposure prophylaxis (PEP), and human death records from 2008 to 2017 were retrieved from Sukraraj Tropical Hospital, Kathmandu, Nepal. The findings revealed that the number of human rabies occurrences was consistent with minor fluctuations throughout the study period. There were 252,297 dog bite cases in humans recorded between 2008 and 2017. Every month, 2,102 people were bitten by mostly stray dogs. There was a gradual increase in PEP use throughout 10 years. On average, 36,995 PEP dosages were used per year for stray dog bites. The PEP consumption and the number of human deaths were negatively correlated. A total of 482 human rabies deaths were recorded in Nepal during the study period. On average, 49 people died of canine-mediated rabies each year. Although there was an increase in the use of PEP, the number of human deaths and street dog bites recorded were still high. The high mortality due to rabies could then be attributed to the flawed surveillance system and stray dog population management, and not merely the lack of PEP services. Hence, it is recommended that the government agencies and other concerned stakeholders should organize mass vaccination and population management program for stray dogs in order to reduce the country's rabies burden.

Keywords: Dog bite, Epidemiology, Prophylaxis, Rabies

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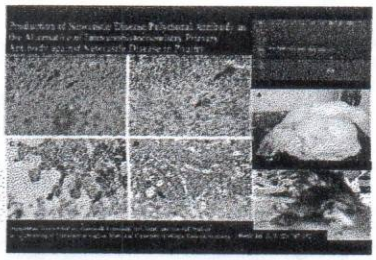
Research Paper

Production of Newcastle Disease Polyclonal Antibody as the Alternative of Immunohistochemistry Primary Antibody against Newcastle Disease in Poultry



Naf'an MKh, Kurniasih K, Untari T, and Prakoso YA.

World Vet. J. 11(2): 187-192, 2021; pii:S232245682100024-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj24

ABSTRACT: Newcastle disease (ND) is the most pathogenic viral infection in poultry. Furthermore, the availability of laboratories that support the molecular diagnosis of ND is still limited in Indonesia. The present study aimed to produce ND polyclonal antibody as the alternative of immunohistochemistry primary antibody against ND in poultry. Two adult male New Zealand White rabbits weighed 2.5 kg were vaccinated seven days after the adaptation using intraperitoneal injection of the ND live vaccine at multilevel doses weekly. The serum was collected inactivated, and purified in the sixth week. A total number of 31 chicken samples were collected and their samples of brain, lung, spleen, and intestine were tested using immunohistochemistry and Reverse Transcription Polymerase Chain reaction (RT-PCR). The result showed that 19/31 (61%) were positive against immunohistochemistry and RT-PCR and a total of 12/31 (39%) were negative. Based on the obtained results, immunohistochemistry using ND polyclonal antibody had a similar accuracy with RT-PCR. It can be concluded that ND polyclonal antibody produced by vaccination in the rabbit could be used as the alternative immunohistochemistry primary antibody for diagnosing ND in poultry.

Keywords: Immunohistochemistry, Newcastle disease, Polyclonal antibody, Poultry, RT-PCR

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Research Paper

651 notifications concerning the different exported food products were analyzed. Among the 663 notifications for the presence of parasites, 161 (24.3%) were border rejections. A total number of 20 countries have been detected with the presence of parasites in their exported fish and fish products. The main fish species concerned with this hazard were Hake (26%), Silver Scabbardfish (10.5%), and Angler (9.3%). In Morocco, among the 651 notifications, 373 concerned with seafood (57.2%). The number of border rejections of fishery products was 220 that is 33.8% of overall notifications. Fish and fish products category are the most concerned with 170 rejections (26.1%), with 64 notifications due to the presence of parasites (37.6%). The Silver Scabbardfish was the species most affected by parasite infestations (23.5%), followed by European Anchovy (12.5%) and Swordfish (10.9%). In conclusion, the nematode *Anisakis* is the most common parasite in fish infestation while the plerocercoïd larvae of the Cestoda *Gymnorhynchus gigas* seems to have a predilection to infest the Atlantic Pomfret (*Brama brama*).
Keywords: Fish, Morocco, Notification, Parasite, Rapid alert system for food and feed

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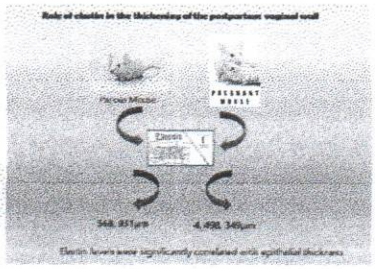
Role of Elastin Expression in Thickening the Postpartum Vaginal Wall in Virgin and Postpartum Rat Models



Setyaningrum T, Listiawan MY, Tjokroprawiro BA, Santoso B, Prakoeswa CRS, and Widjiati W.

World Vet. J. 11(2): 228-234, 2021; pii:S232245682100029-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj29

ABSTRACT: Childbirth induces a number of alterations, including ligament weakening and increased vaginal distensibility. The occurrence of vaginal laxity or distensibility is associated with the vaginal wall and introitus overstretching during vaginal parturition while the pathophysiology is due to increased levator dimension and trauma to the levator ani muscle through avulsion (macrotrauma) or overdistension (microtrauma). Elastin is an extracellular matrix protein that confers elastic properties to organs and tissues, particularly those requiring elasticity. Elastin plays a vital role in the functioning of numerous tissues, such as the lungs, blood vessels, heart valves, ligaments, tendons, and skin. It is also a component of the vaginal mucosa. The aim of the present was to evaluate the role of elastin in the thickening of the postpartum vaginal wall composed of epithelial mucosa, and to understand the mechanism underlying vaginal laxity or distensibility within parous and nulliparous animal models. A total of 32 female white rats (*Rattus norvegicus*) were used in the present study. They were divided into two groups, each group consisting of 16 rats. The control group (C0) consisted of virgin nulliparous rats, which were sacrificed on the second day after vaginal parturition. Pregnant rats (group C1) were sacrificed on the second day after vaginal parturition. The median elastin expression in group C1 was higher (3 ± 0.56) than group C0 (2.85 ± 0.75). The mean thickness of the vaginal mucosal epithelium in group C0 (56,8 931µm) was greater than group C1 (44,98 349µm). The comparison of vaginal mucosal epithelium thickness between the two groups indicated a significant difference between groups C0 and C1. Elastin levels were significantly correlated with epithelial thickness. The expression of elastin significantly affects the vaginal wall thickness, which further affects vaginal laxity or vaginal distensibility.
Keywords: Distensibility, Elastin, Vaginal wall, Animals

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Research Paper

Molecular Characterization of Chicken Anaemia Virus Circulating in Commercial Poultry Flocks in Egypt during 2020

Abdelhalim A, Samir A and Yehia N.

World Vet. J. 11(2): 235-241, 2021; pii:S232245682100030-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj30

ABSTRACT: Chicken Anemia Virus (CAV) is an extremely contagious immunosuppressive disease causing high economic losses in poultry production. In the present study, tissue samples (bone marrow, thymus, and spleen) were collected from 86 different broiler chicken farms located in fourteen governorates in Egypt during 2020. They suffered from retard growth, weakness, and a drop in egg production with an observed mortality rate ranged 5-15%. A total of 26 samples were positive for CAV using PCR in six governorates in Lower Egypt with a 30% incidence rate, especially in Sharkia (78%), Ismailia (62.5%), and Alexandria (60%). The viral protein1 (VP1) gene of CAV was genetically characterized by sequencing of 10 selected viruses in six governorates. revealing that all Egyptian strains were clustered into two groups (A, B) that was distinct from vaccine strains (Del-Ros, Cux-1, and 26PA) which were clustered in group C. The seven Egyptian viruses in this study (A-Egypt-AN1-2020 to A-Egypt-AN7-2020) were clustered with the viruses from Japan, Argentina, and Malaysia in group A, and the other three viruses (A-Egypt-AN8-2020, A-Egypt-AN9-2020, A-Egypt-AN10-2020) were clustered with the viruses from Nigeria, and India in group B. The Egyptian viruses in the current study acquired new specific mutations clustering them into new subgroups (2A, 2B). By mutation analysis comparing with Del-Rose reference strains, V75I, M97L, and K139Q, E144Q were recorded in all viruses in the group A and B. All Egyptian viruses in the current study had specific new mutations at Y13N, H22N. Moreover, mutation at G74E in Egyptian viruses recorded in the current study was related to sub group 2A, I83V in three strains (A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020), and S140A in the hypervariable region was found in four strains (A/Egypt /AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and A/Egypt/AN5/2020) in subgroup 2A. Furthermore, Q139 and Q144 amino acid substitutions, which are important in viral replication, were observed in all viruses. The field viruses in the study were distinct from the vaccinal strains by phylogenetic analysis and A.A. identity. In conclusion, the CAV was continuously circulating in Egypt from different genotypes. It acquired new specific mutations clustering them in a new subgroup, and it was distinct from vaccinal strains. Therefore, it is important to conduct continuous monitoring on the genetic evolution of CAV and further studies on the pathogenicity of the virus and the vaccine efficacy.
Keywords: Chicken Anemia Virus, Egypt, Genetic evolution, Viral protein 1 gene

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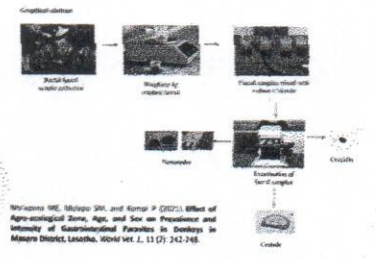
Effect of Agro-ecological Zone, Age, and Sex on Prevalence and Intensity of Gastrointestinal Parasites in Donkeys in Maseru District, Lesotho



Nts'aoana ME, Molapo SM, and Kompi P.

World Vet. J. 11(2): 242-248, 2021; pii:S232245682100031-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj31

ABSTRACT: Gastrointestinal parasites are considered to be silent killers of animals. The objective of the current study was to determine the effect of the agro-ecological zone, age, and sex on the parasite prevalence and fecal egg/oocyst count in donkeys residing in Lesotho. A total number of 720 fecal samples were collected rectally from 120 indigenous donkeys that were randomly selected from the highlands, foothills, and lowlands of Maseru district, Lesotho. The fecal samples were collected every two months for one year and examined using the floatation technique. The overall prevalence for nematodes, coccidia, and cestodes in donkeys were 87.78%, 4.31%, and 1.53%, respectively. The highest nematode prevalence and intensity were detected in the donkeys of highlands. The coccidian infection was lower in the lowlands while cestodes infection was more prevalent in the foothills. Donkey's age had an impact on the nematode fecal egg load but did not affect the prevalence of nematodes in donkeys. Age did not significantly affect the prevalence and fecal egg/oocyst count of cestodes and coccidia. Male donkeys had a higher prevalence and fecal egg count of cestodes. In conclusion, the nematodes were found to be the major gastrointestinal parasites of donkeys in the Maseru district. Therefore, there is a need to design a sustainable strategy aimed at controlling the gastrointestinal parasites in donkeys.
Keywords: Agro-ecological zone, Eimeria, Fecal egg count, Helminth, Prevalence

DOI: https://dx.doi.org/10.54203/scil.2021.wvj39

ABSTRACT: Adjuvant chemotherapy might be indicated in some canine mammary cancer cases due to metastatic potential. In this regard, studies to determine adverse events following chemotherapy protocols are valuable. The purpose of this prospective clinical trial was to evaluate the safety and tolerability of gemcitabine and carboplatin combination in dogs with malignant mammary tumors. For this prospective clinical trial, 21 female dogs mastectomized due to malignant mammary neoplasia underwent adjuvant chemotherapy with gemcitabine (3 mg/kg, 60-minute IV infusion) and carboplatin (10 mg/kg, 20-minute IV infusion) based protocol every 21 days for three cycles. They were monitored periodically for treatment-related adverse events by clinical and laboratory evaluations. A total of 17 (80.9%) dogs developed leukopenia, 10 (47.6%) neutropenia, and 15 (71.4%) thrombocytopenia at least once along with the three chemotherapy cycles. All these hematologic toxicities were grade 1, 2, or 3. Two (9.5%) animals had evidence of gastrointestinal toxicity; however, clinical signs were mild to moderate (grades 1 and 2). No dog had life-threatening adverse events (grade 4) or even died (grade 5) of treatment-related complications. The adjuvant chemotherapy protocol with gemcitabine and carboplatin was well-tolerated and safe in female dogs for mammary cancer treatment with self-limiting hematological and gastrointestinal adverse events.
Keywords: Adverse event, Canine, Mastectomy, Toxicity, Tumor

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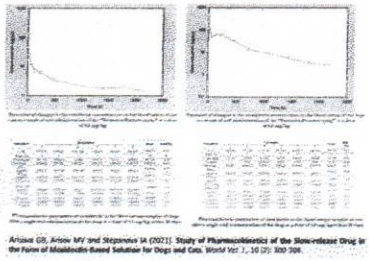
Research Paper

Study of Pharmacokinetics of the Slow-release Drug in the Form of Moxidectin-Based Solution for Dogs and Cats



Arisova GB, Arisov MV and Stepanova IA (2021).

World Vet. J. 11(2): 300-306, 2021; pii:S232245682100040-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj40

ABSTRACT: The pharmacokinetic characteristics of the moxidectin-based drugs have been studied in the blood serum of animals after a single oral administration of the drug at the therapeutic dose in form of syrup. The drug is intended to control parasitic diseases of cats and dogs. The present studies on cats and dogs (drug administration and blood sampling) were conducted in the experimental farm of Kurilovo, Russia, for three months. The study involved six dogs and six cats, half breed, aged one to four years. The samples included six dogs (four male and two female) and six cats (three male and three female), and groups were formed according to the principle of analog groups. The drug, moxidectin, was orally administered once at the dose of 1.5 mg per one kg of animal's weight. The active substance of the drug was identified in the blood serum of animals by High-Performance Liquid Chromatography (HPLC) with fluorescence detection. The result of the current study showed that based on the pharmacokinetics of moxidectin, the concentration of the active substance in the blood serum after three hours reached 134.80-498.09 ng/ml in cats and 479.07-1459.40 ng/ml in dogs. The obtained results indicated that a single administration of the drug at the recommended therapeutic dose could ensure the maintenance of therapeutic concentrations of moxidectin in the blood, and accordingly, the protection of animals from parasites for up to 90 days.
Keywords: Cats, Dogs, Moxidectin, Pharmacokinetics, Solution

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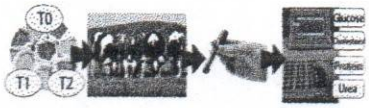
Research Paper

The Effect of Different Dietary Energy and Protein Sources on Blood Profile of Crossbreed Holstein Dairy Cows Raised in Small Stake Holder Farms



Hudaya MF, Sitaresmi PI, and Widayati DT.

World Vet. J. 11(2): 307-312, 2021; pii:S232245682100041-11

DOI: https://dx.doi.org/10.54203/scil.2021.wvj41

ABSTRACT: The study aimed to evaluate the effect of protein and energy supplementation on the biochemical blood parameters in Holstein cows. The effect of energy and protein supplementation used corn and soybean meal was evaluated on biochemical blood profile in three groups of Holstein cows raised in small stakeholder farmers in Yogyakarta from February to May 2020. Thirty multiparous Holstein cows were allocated to three treatment groups, namely T0 in which the cows fed by the basal diet from the local farmer as well as the T1 (3.5% energy and protein supplementation) and T2 (5% energy and protein supplementation), in which the cows were fed by added energy and protein supplementation. The diets designed for the treatment groups were different from the basal diet by adding two additional ingredients which were soybean meal and corn meal in purpose to depress the stress from adaptive feeding. The results showed that the treated cows (T1 and T2) had significantly higher serum concentrations of glucose (T1 = 2.12 ± 0.49 mmol/L, T2 = 1.86 ± 0.40 mmol/L) rather than T0 (0.98 ± 0.48 mmol/L). The total concentration of serum protein and urea in treated cows was significantly lower than those with the basal diet. Total serum protein and urea in T1 were 0.69 ± 1.37 mmol/L and 7.21 ± 1.99 mmol/L, respectively; which they were 0.63 ± 0.06 mmol/L and 7.69 ± 3.07 mmol/L in T2, compared to the T0 which were 0.82 ±0.05 mmol/L and 7.69 ± 3.07 mmol/L, respectively. There was no significant difference in blood cholesterol among all treatment groups. In conclusion, the supplementations that varied in the proportion of energy and protein intake affected some biochemical blood profiles, such as glucose, protein, and blood urea nitrogen.
Keywords: Biochemical blood parameters, Crossbreed Holstein cows, Energy supplementation, Protein supplementation, Traditional farmers

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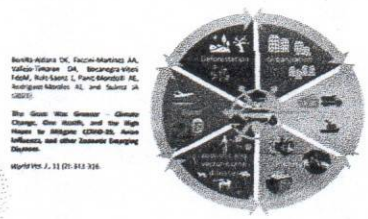
Editorial

The Grass Was Greener - Climate Change, One Health, and the High Hopes to Mitigate COVID-19, Avian Influenza, and other Zoonotic Emerging Diseases



Bonilla-Aldana DK, Faccini-Martínez ÁA, Vallejo-Timaran DA, Bocanegra-Viteri FdeM, Ruiz-Saenz J, Paniz-Mondolfi AE, Rodriguez-Morales AJ, and Suárez JA.

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Role of Elastin Expression in Thickening the Postpartum Vaginal Wall in Virgin and Postpartum Rat Models

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ABSTRACT

Childbirth induces a number of alterations, including ligament weakening and increased vaginal distensibility. The occurrence of vaginal laxity or distensibility is associated with the vaginal wall and introitus overstretching during vaginal parturition while the pathophysiology is due to increased levator dimension and trauma to the levator ani muscle through avulsion (macrotrauma) or overdistension (microtrauma). Elastin is an extracellular matrix protein that confers elastic properties to organs and tissues, particularly those requiring elasticity. Elastin plays a vital role in the functioning of numerous tissues, such as the lungs, blood vessels, heart valves, ligaments, tendons, and skin. It is also a component of the vaginal mucosa. The aim of the present was to evaluate the role of elastin in the thickening of the postpartum vaginal wall composed of epithelial mucosa, and to understand the mechanism underlying vaginal laxity or distensibility within parous and nulliparous animal models. A total of 32 female white rats (*Rattus norvegicus*) were used in the present study. They were divided into two groups, each group consisting of 16 rats. The control group (C0) consisted of virgin nulliparous rats, which were sacrificed on the second day after vaginal parturition. Pregnant rats (group C1) were sacrificed on the second day after vaginal parturition. The median elastin expression in group C1 was higher (3 ± 0.56) than group C0 (2.85 ± 0.75). The mean thickness of the vaginal mucosal epithelium in group C0 (56,8 931µm) was greater than group C1 (44,98 349µm). The comparison of vaginal mucosal epithelium thickness between the two groups indicated a significant difference between groups C0 and C1. Elastin levels were significantly correlated with epithelial thickness. The expression of elastin significantly affects the vaginal wall thickness, which further affects vaginal laxity or vaginal distensibility.

Keywords: Distensibility, Elastin, Vaginal wall, Animals

INTRODUCTION

Pregnancy causes a number of alterations in the biomechanical behavior of both humans and animals, including ligament weakening and increased vaginal distensibility. The pelvic floor muscles in rats and humans support the protective mechanisms against perineal trauma by increasing stiffness and the extent of sarcomere during parturition. During vaginal delivery, the puborectalis muscle is subjected to several extreme stretching with an estimated stretch ratio of 1.5-3.5 cm long. The extent to which the muscle elongates varies is between 25% and 250% (Qureshi et al., 2018; Gachon et al., 2019).

The commonly associated mechanism with parturition involves overstretching of the vaginal wall and introitus during vaginal delivery, and its pathophysiology is related to increased elevator dimension and trauma to levator ani from avulsion (macrotrauma) and overextension (microtrauma), meaning that vaginal laxity or distensibility is linked to pregnancy and childbirth (Kamisan et al., 2015; Dietz et al., 2016; Abdool et al., 2018).

Shek and Dietz (2009) reported an Ultrasound study that examined the dimensional change of the levator hiatus in postpartum women with and without morphological abnormality, which was then associated with their type of childbirth. Vaginal birth could induce hiatal widening, particularly after an avulsion and even without macroscopic levator trauma, thus potentially enable increasing the hiatal distensibility. Vaginal laxity is the most undesirable symptom, reported by approximately 60.7% of women. Moreover, levator avulsion occurred in 15% of women who undergo vaginal delivery. Therefore, It can be inferred that significant changes in the pelvis and distension of the levator hiatus are followed by vaginal delivery (Shek and Dietz, 2009; Abdool et al., 2018).

The pelvic floor muscles play a critical role in female sexual function. A smaller vaginal dimension is linked to sexual dysfunction, particularly dyspareunia. Trauma to the levator ani muscle during childbirth is associated with an increase in vaginal hiatus, which in turn might affect sexual function and vaginal laxity (Roos et al., 2020).

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At the cellular level, vaginal muscles and pelvic supporter are sustained by the integrity of the connective tissue and the attachment between the vagina, sides of the pelvis, and levator ani muscle. The connective tissue as the base of the vagina and its surrounding structures contained collagen, elastin, glycoproteins, hyaluronan, and proteoglycans, which were actively redesigned throughout the woman's life, particularly during hormonal changes (Newman et al., 2018).

Elastin is an extracellular matrix protein that provides elastic properties to organs and tissues, mainly those that require elasticity or are involved in an elongation and shrinkage cycle. Elastinplays a vital role in the functions of numerous tissues, such as the lungs, vessels, heart valves, ligaments, tendons, and skin. Elastin is composed of 90% elastic fibers and forms an inner core that is surrounded by unbranched microfibrils. Elastin accounts for only two to four percent of the dry skin weight of a human, yet it has an important structural function in providing mechanical support and is involved in various cell signaling pathways. The rate of elastogenesis diminishes with age (Mithieux and Weiss, 2005; Rodríguez-Cabello et al., 2018).

A study by Zong et al. (2010) indicated that comparing elastin metabolism in the female vagina with and without Pelvic Organ Prolapse (POP) found increased levels of tropoelastin, mature elastin, pro Matrix Metalloproteinase (MMP) 9, and active MMP-9 in women with prolapse. The metabolism of elastin was altered in the vagina with prolapse. In addition, the shape of the vaginal tissue rapidly changed in response to mechanical stretching. It was also observed that elastin levels peaked in the absence of hormones. Damage to elastin fibers might be due to increased elastin-degradation enzymes, MMP-2, and MMP-9. Therefore, MMP-2 and MMP-9 levels would decrease along with an increase in the elastin levels in the vagina with POP. The thickening of the elastin fibers in the vaginal wall of patients with anterior POP was due to the remodeling of the extracellular matrix. Moreover, the proximal vagina contained more collagen level in total and less elastin level than its distal counterpart. The vagina is mostly composed of type I collagen, which gives it tensile strength (Zong et al., 2010; Zaki et al., 2016; Rynkevic et al., 2017). Accordingly, to understand the mechanism of elastin in the process of vaginal laxity or distensibility in parous and nulliparous animal models, the role of elastin in postpartum vaginal wall thickening was evaluated in the current study, which is composed of vaginal epithelial mucosa.

MATERIALS AND METHODS

The present research was an experimental study involving 32 female white rats (*Rattus norvegicus*). The subjects were allocated into two groups consisting of 16 rats each. Group 1 (C0) or the control group consisted of 16 female rats, nulliparous and virgin, aged 4-5 months with a body weight of 170-200 grams. that were sacrificed on day two, while group 2 (C1) entailed pregnant rats that were sacrificed on the second day following vaginal parturition. Rats were housed in individual rearing cages with dark lighting, monitored air temperature with a flow rate of 5 to 7.5 km/hours (gentle breeze), and local humidity with one-atmosphere pressure by inhalation and oxygen demand of 2.68 ml/gram/hour.

Ethical approval

Ethics approval of the present research project was obtained from the Ethics Committee of the Health Research Faculty of Veterinary Universitas Airlangga, Surabaya, Indonesia, with the certificate number 2.KE.116.12.2020. All research work was completed in the same institute.

Mating and breeding of female rats

Previous observations of the estrous cycles were carried out in female ratas using the vaginal swab. Swabs with small cotton swabs moistened with physiological NaCl were then checked on a glass slide. The cells were then fixed with methanol and stained with 10% Giemsa solution. Observation of the estrous cycle was carried out under a light microscope with a magnification of 100×.

Female rats were injected with Pregnant Mare Serum Gonadotropin (PMSG) to synchronize the estrous cycle and with human Chorionic Gonadotropin (hCG) to induce superovulation. The 10 IU PMSG was administered intraperitoneally, followed by 10 IU hCG after 48 hours. After the hCG administration, the rats were bred with male rats using a monomating method. Mating was confirmed for the next 17 hours using a vaginal plug. The vaginal plug was composed of coagulated gelatinous secretions that prevented spermatozoa from leaking. Successful copulation was assumed in the presence of a vaginal plug, and it was recorded as day 0 of pregnancy.

Vaginal tissue sampling

The rats were sacrificed under general anesthesia with ketamine and xylazine. Anesthetic ketamine n the range of 80-100 mg/kg mixed with xylazine in the range of 5-10 mg/kg. The intraperitoneal injection dose was 0.2 ml of ketamine-xylazine mixture for each rat. The entire vaginal tissue was swiftly dissected after disinfection with 70% alcohol. The

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tissues were then split and fixed in 10% formalin buffer. The collected samples were sent to the pathology laboratory for immunohistochemistry preparation and histopathological examination.

Hematoxylin Eosin staining procedure

Hematoxylin and eosin stains were used to observe and measure the thickness of the vaginal mucosa. The sliced tissue samples were initially deparaffinized. Then, these tissue slices were fixed in methanol at gradually decreasing concentrations (100%, 90%, 80%, 70%, and 30%) then washed in PBS. After immersion in ethanol, tissue slices were placed in hematoxylin for six minutes. After rinsing with water, the slices were consecutively dipped into ammonia and eosin solutions. Sample tissues were then dehydrated and re-fixated in methanol with gradually increasing concentration (80%, 90%, 95%, and ethanol absolute), allowed to dry, and thereafter evaluated under a microscope (Cardiff et al, 2014).

Immunohistochemistry evaluation technique

Immunohistochemical staining was performed to detect elastin expression. The vaginal wall samples were fixed to the object glasses with methanol containing 3% diluted hydrogen peroxide. Dakocytomation (peroxidase-blocking reagent) was applied to sample parts and then incubated at room temperature with primary antibodies against the monoclonal antibody anti-elastin (*monoclonal antibody elastin* (BA-4): sc-58756, Santa Cruz Biotechnology, Inc. (1: 300), California, USA). All samples were incubated with biotin-labeled secondary antibodies (Trekkie Universal Link) and incubated overnight with streptavidin-conjugated peroxidase (Trekavidin-HRP Label) and Diaminobenzidine (DAB) as the chromogen. The elastin was counterstained with Mayer's hematoxylin and eosin (Fedchenko and Reifenrath, 2014).

Measurement of vaginal wall thickness and elastin expression

Vaginal wall thickness was measured using the calibrated Image Raster 3 software. The measurements were performed in 10 microscopy fields at 200× magnification and counted in 10 different fields. The mean quantitative result (µm) of each HE-stained sample was recorded. The evaluation of elastin expression was based on the percentage of the elastin-expressing epithelium of the vaginal mucosa using the Semi-quantitative Immuno_Reactive Score (IRS) method. The mean percentage of the monoclonal antibody-positive vaginal mucosal epithelium was observed under a microscope at 400× magnification in 10 microscopy fields. The Remmele scale index was obtained by multiplying the positive cell percentage score by the color reaction intensity score. A positive cell percentage score was interpreted as score 0 indicating no positive cells, score 1 referring to less than 10% positive cells, score 2 showing 11-50% positive cells, Score 3 accounting for 51-80% positive cells, and Score 4 suggesting more than 80% positive cells. The color reaction intensity score was interpreted as score 0 meaning no color reaction, score 1 referring to low color intensity, score 2 denoting medium color intensity, and score 3 signifying strong color intensity (Fedchenko and Reifenrath, 2014).

Statistical analysis

The obtained data were tested for normality using the Kolmogorov-Smirnov test and analyzed using SPSS software (version 24). Furthermore, non-parametric intervariable data were verified using the Mann-Whitney-U Test to determine the differences in parameters between the groups, and statistical significance was set at $p \leq 0.05$. Parametric data were verified using an independent t-test. The correlation between the two groups was verified using Pearson's correlation test.

RESULTS AND DISCUSSION

The expression of elastin was monitored using immunohistochemistry and the vaginal wall thickness with hematoxylin and eosin staining. There were differences in elastin expression and vaginal wall thickness between the virgin nulliparous and parous rat groups. The data observed between the treated groups are provided in the following tables and figures.

The results of elastin expression in the vaginal mucosal epithelium are shown in Figure 1. Figure 1A represents the elastin expression in vaginal epithelium samples of group C0, exhibiting a score of 2 with the immunoreactive cells being in the range of 10-50% in all microscopic fields with a mean expression of 2.85 ± 0.75. Figure 1B represents the elastin expression in vaginal epithelial samples from group C1 with a score of 3 and around 51-80% immunoreactive cells in all microscopic fields, with a mean expression of 3.0 ± 0.56.

Diagram 1 indicates that the median value of elastin expression in group C1 was higher than that of group C0. Differential tests of both groups with the Mann Whitney U-test indicated that the elastin expression in the control group was significantly different from that of the treatment group C1 ($p = 0.032$; $\alpha < .05$, Table 1).

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Table 1. Mann-Whitney test results of elastin expression in female rats.

Group \ Elastin Expression	n	Median	Interquartile Deviation	Minimum	Maximum	P-*value*
C0	16	2.85	0.75	1.1	6.6	0.032*
C1	16	3.00	0.56	2.0	7.2	

(*) significantat p < .05, group C0: Nulliparous female rats, group C1: Postpartum female rats.

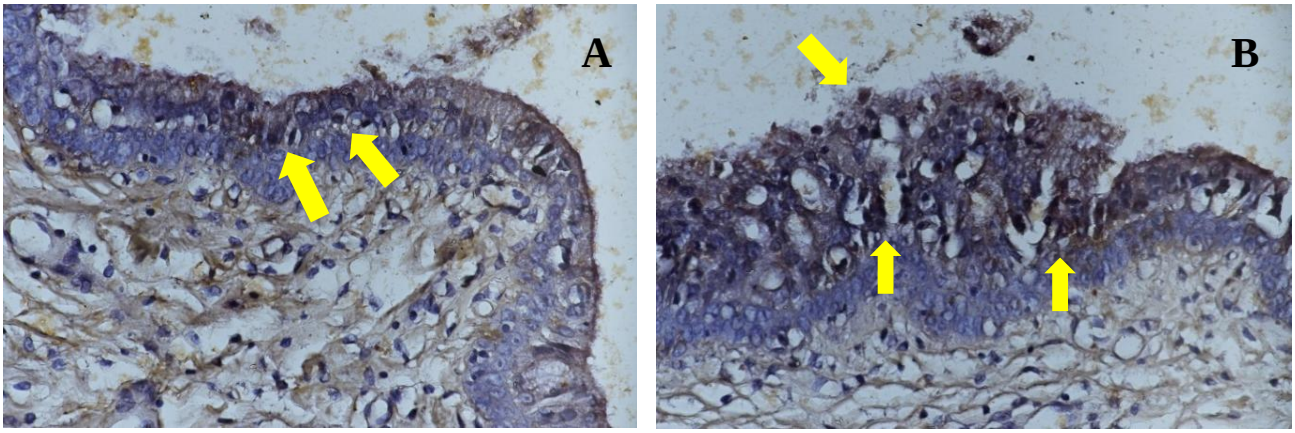

Figure 1. Immunohistochemical results of elastin in female rats. **A:** Expression of elastin with medium intensity on immunoreactive cells (arrows) in group C0, **B:** Positive expression with strong intensity is indicated by a brownish dark color on immunoreactive cells (arrows) in group C1, (Immunocytochemistry test; 400× magnification)

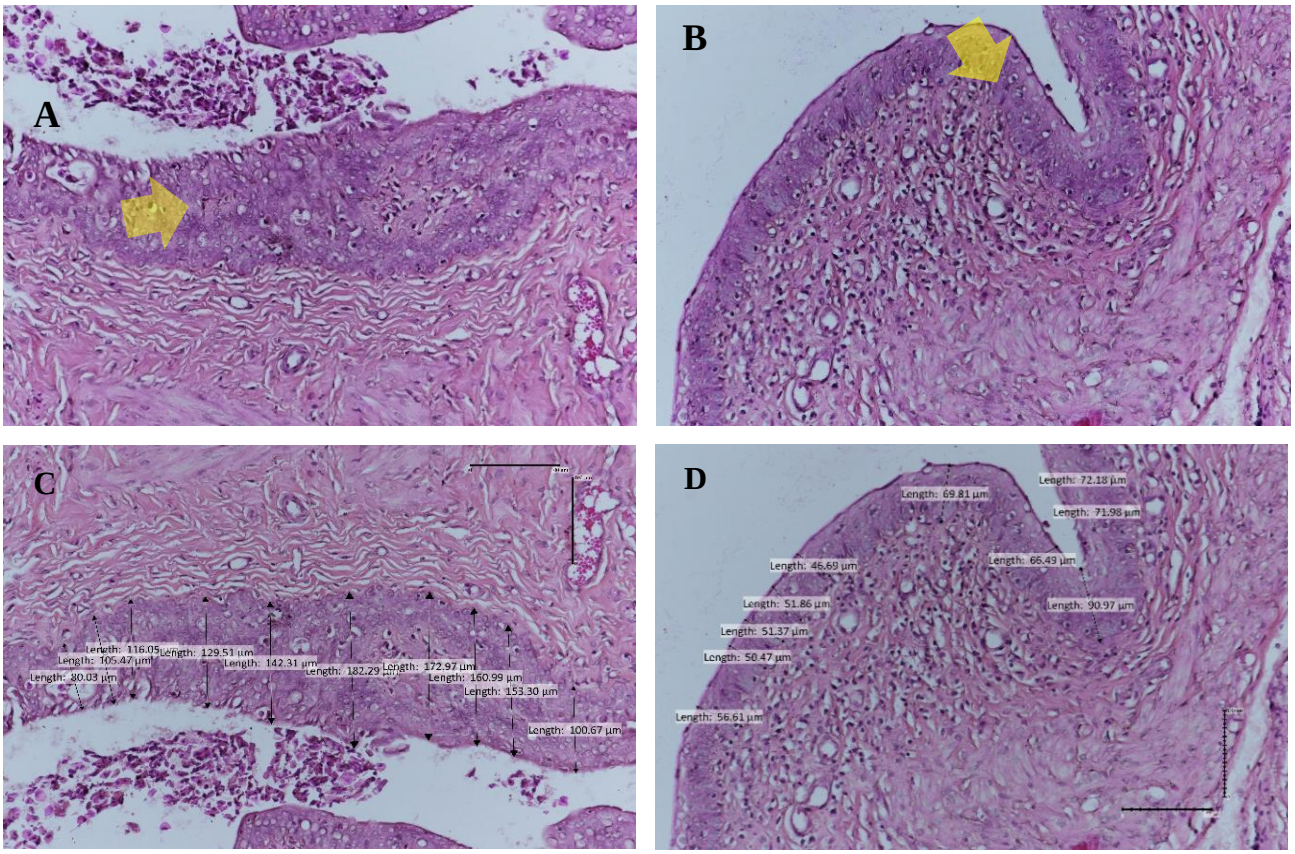

Figure 2. HE-stained samples for vaginal wall/mucosal epithelium thickness measurement. **A:** Vaginal mucosal epithelium layer (arrow) in group C0, **B:** Vaginal mucosal epithelium layer (arrow) in group C1, **C-D:** Measurement of vaginal mucosal epithelium thickness with Image Raster 3 software in groups C0 and C1 (Hematoxylin Eosin; 200× magnification)

Vaginal wall thickness results

Figure 2 shows a histological representation of vaginal wall thickness, indicated by the vaginal mucosal epithelium thickness. Figure 2A presents the thickness of the mucosal epithelium in group C0 while Figure 2B illustrates the thickness of the mucosal epithelium in group C1. Figures 2C and 2D show the measurement of the mucosal epithelial thickness of groups C0 and C1 with Image Raster 3. Remarkably, the majority of the mucosal epithelial thickness in

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group C0 was greater than that in group C1. Diagram 2 indicates that the mean value of vaginal mucosal epithelial thickness was greater in group C0 (56,8 931µm) than in group C1 (44,98349 µm). Table 2 shows a comparison of vaginal mucosal epithelium thickness between the groups using an independent t-test. As can be seen, groups C0 and C1 exhibited significant differences ($p = 0.033$; $\alpha < 0.05$, Table 2).

Table 2. Differentiation test of vaginal density in female rats.

Group	Vaginal Density					
	N	Mean	Standard Deviation	Minimum	Maximum	P
C0	16	56.8931	16.109954	38.205008	87.517398	0.033**
C1	16	44.98349	15.116408	27.482611	77.894783	

* Significant at $p < .05$, group C0: Nulliparous female rats; group C1: Postpartum female rats. N: number of samples. The correlation test between two variables of elastin and epithelial thickness indicated a significant correlation ($p = 0.385$; $\alpha < 0.05$).

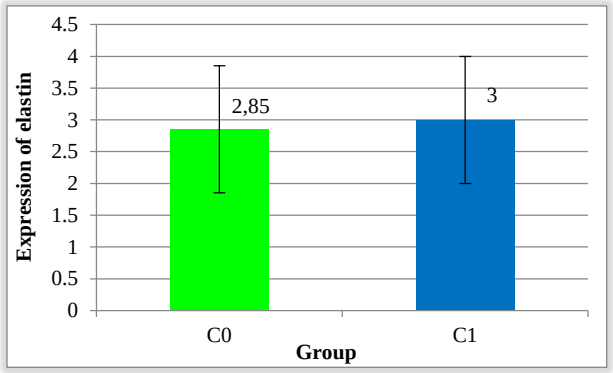

Diagram 1. Median value of expressed of elastin (Group C0: nulliparous female rats; group C1: postpartum female rats).

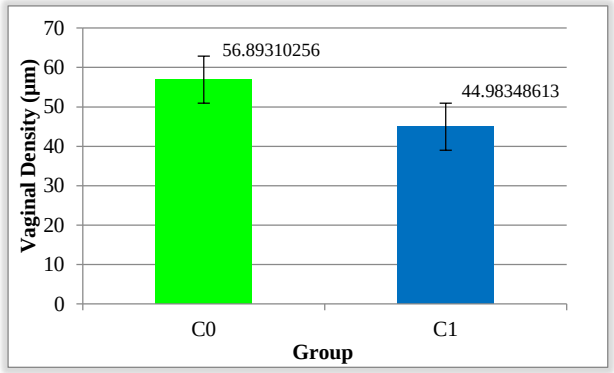

Diagram 2. Mean value of vaginal density (Group C0: nulliparous female rats; group C1: postpartum female rats).

The morphology and physiology of the vulva and vagina change with age, especially during puberty (when the menstrual cycle occurs), pregnancy, and menopause. Remodeling of the vaginal wall and pelvic floor connective tissues leads to a rapid increase in the weight of the uterus, as well as the size, due to the deposition of collagen and elastin. The studies in rats with multiple fetuses have shown that the increase in the weight of the uterus is six to eight-fold compared to non-pregnant uteri, while in humans it is 11-fold. It was then shown that collagen content reduced rapidly after parturition. The wet weight has completed 70% of the involution needed to restore to the baseline value. The collagen removal was 77% completed and elastin removal was 86% completed. The vaginal wall consisted of four layers, including stratified, non-keratinized, squamous epithelium, lamina propria, a dense connective tissue layer rich in fibrillar collagen and elastin, filled with fibroblasts, a muscular layer composed of internal circular and external longitudinal smooth muscle fibers, and tunica adventitia, an elastic tissue layer rich in fiber and collagen that supports the vaginal wall. The lamina propria and muscular layer are the two main layers that confer strength to the vaginal wall (De Landsheere et al., 2013; Dhital et al., 2016; Tadir et al., 2017).

In the present study, elastin was expressed more in the postpartum treatment group than in the nulliparous group, with a significant difference. Elastin was modulated by estrogen of the extracellular matrix and fibroblasts, which were responsible for collagen production. The mucosal epithelium functions according to the estrogen level, which naturally reacts to hormonal fluctuations throughout the woman's life, as well as during the menstrual cycle. Postmenopausal women reported having estrogen, whose estradiol levels averaged 14.1 ± 0.9 pg/ml and estrone levels averaged 27.5 ± 1.2 pg/ml. Physiological estradiol levels in prepubertal children in the range of approximately < 20 pg/ml, adolescent girls 20-300 pg/ml, adult mestrual women 30-800 pg/ml, and postmenopausal women < 20 pg/ml. During pregnancy, the average levels of estradiol in women were up to 20.000pg/ml. The ionized epithelium is rich in glycogen fermented by *Lactobacilli* to decrease vaginal pH levels. The lamina propria mainly consists of collagen fibers and elastin, and contains dense plexuses of small blood vessels, lymphatic vessels, and nerves. This layer is more populous toward the surface and less populous toward the muscular layer. The lamina propria papillae are scarce on the anterior vaginal wall and grow deeper and stronger toward the posterior wall (Tadir et al., 2017).

In the present study, elastin was more notably expressed in the postpartum treatment group than in the nulliparous group, with a significant difference. This result was in line with a study by Jallah et al. (2014), in which elastin production in nulliparous control animals was higher than in the four and eight weeks post-injury, supported by decreased smooth muscle bundles of the vaginal muscularis. Several other studies supported the fact that postpartum and vaginal wall prolapse samples exhibit diminished amounts of smooth muscle cells and their supporting tissues based on

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immunohistochemical evaluation, both quantitative and qualitative decrease in collagen and elastin (Farouk et al., 2013; Jallah et al., 2014; Kerkhof et al., 2014).

At the cellular level, the MMP-9 expression increased in the postpartum period due to its vital role in regulating type I and III collagen and elastin in vaginal elasticity. An imbalance in the proportion of MMP-9 and type I and III collagen carried the risk of prolapse. Furthermore, fibulin-5 expression played a critical role in elastin synthesis. Fibulin-5 was involved in the homeostasis of the extracellular elastin matrix in connective tissues. Moreover, elastin was genetically influenced by the mRNA gene LOX-1. It was also known that elastin synthesis was related to estrogen production. Estrogen participates in modulating the extracellular matrix, smooth muscles, and fibroblasts which are responsible for collagen production. Estrogen production gradually diminishes with age. Estrogen also contributes to childbirth. Elastin production is also vital during parturition to preserve vaginal wall elasticity and maintain structural integrity against the stretching of the vaginal wall. As Word et al. (2009) stated, elastin fibers in humans are synthesized in early life, reach their peak during the third trimester, and gradually decrease by the postnatal period. In a woman's reproductive organs, elastin fiber synthesis is accelerated, particularly during the postpartum period (Word et al., 2009; Downing et al., 2013; Novida, 2013).

In the present study, it has been found that the mean thickness of the vaginal mucosal epithelium was greater in the nulliparous animal model than in the postpartum group. This finding was in accordance with a study conducted by Hamner et al. (2020), who stated that the measured epithelial thickness in the nulliparous group was higher than the postpartum group on the third day. The muscular layer of the vaginal wall was significantly altered during the postpartum period due to uneven thinning of the smooth muscle and smooth muscle bundles. The proliferation of the vaginal mucosal epithelium was hormonally influenced by FBLN5, actionin, and estrogen. Downregulation of ERα correlated with a decreased efficacy of higher doses of estrogen in terms of collagen mRNA, total collagen content, distensibility, and activation of *TGFβ1* gene expression. The estrogen receptor RE-β on the stromal and epithelial layers could govern the mitotic activity of cells to differentiate and increase the mucosal epithelial thickness (Montoya et al., 2015; Hamner et al., 2020).

The thinner postpartum vaginal mucosal epithelium might be due to reduced estrogen levels during the postpartum period as well as mechanical factors. During parturition, intra-abdominal pressure increased sharply as part of the fetal expulsion process. These mechanisms have a direct effect on the vaginal wall. This increased mechanical force caused the connective tissue to stretch, which affected the thickness of the vaginal mucosal epithelium, including its extracellular matrix component. According to Young et al. (2017), pregnancy specifically induced the vagina and its supporting connective tissue causing vaginal distensibility. This distensibility reached its maximum point during vaginal childbirth, making the vaginal wall prone to injury, decreased elasticity, and weakness. Other physiological factors that affect the dilation of the vaginal wall and uterus include fetal size, mechanical contraction, and distension of uteri (Zaki et al., 2016; Young et al., 2017).

CONCLUSION

The tissues of the female reproductive tract are significantly remodeled and altered to allow the fetus to grow and give birth. Elastin production is a vital part of the extracellular matrix in the uterus and vaginal wall, which is remodeled during parturition and at various stages of pregnancy. This study highlights that the expression of elastin significantly influences the vaginal wall thickness, leading to vaginal distensibility that affects collagen structure in pregnancy and parity.

DECLARATIONS

Authors' contribution

Trisniartami Setyaningrum performed research concept and design, wrote the article, and approved the article. M. Yulianto Listiawan performed the collection and/or assembly of data. Brahmana Askandar Tjokroprawiro performed measurements and analyzes experimental data. Widjiati wrote the article and final approval of the article. Budi Santoso performed a critical revision of the article. Cita Rosita Sigit Prakoeswa performed a critical revision of the article. All authors approved the final draft of the manuscript for submission to this journal. Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

Competing interests

The authors have not declared any conflict of interest.

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Ethical considerations

All authors should not submit manuscripts that are concurrently under consideration for publication in another journal or have already been published as a peer-reviewed publication. Duplicate submission and concurrent publication are highly unethical publishing behavior.

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