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Contact

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Section Policies

Publication Frequency

Publication Ethics

Indexing

Article Processing Charge

Peer Reviewers Process

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Home > Archives > Vol 8, No 1 (2019)

VOL 8, NO 1 (2019)

JURNAL OVOZOA : JOURNAL OF ANIMAL REPRODUCTION

TABLE OF CONTENTS

ORIGINAL ARTICLE

KADAR MILK UREA NITROGEN (MUN) PADA SAPI PERANAKAN FRIESIAN HOLSTEIN (PFH) BERDASARKAN CALVING INTERVAL YANG BERBEDA

PDF
1-4

doi 10.20473/ovz.v8i1.2019.1-4

Hanif Sabekti Pratama, Imam Mustofa, Widya Paramita Lokapinrasari, Pudji Srianto, Rimayanti Rimayanti, Suzanita Utama

POTENSI PEMBERIAN EKSTRAK BUAH DELIMA (*Punica granatum* L) TERHADAP KUALITAS SPERMATOZOA TIKUS PUTIH (*Rattus norvegicus*) YANG TERPAPAR PANAS

PDF
5-9

doi 10.20473/ovz.v8i1.2019.5-9

Nucifera Fadhillah Santoso, Budi Utomo, Mirni Lamid

PENGARUH PEMBERIAN MADU TERHADAP STAGING SPERMATOGENESIS DAN SEL LEYDIG PADA MENCIT (*Mus musculus*) YANG DIINFEKSI *Toxoplasma gondii*

PDF
10-16

doi 10.20473/ovz.v8i1.2019.10-16

Paraswita Indah Fitri, Wurlina Wurlina, Sri Chusniati, Lucia Tri Suwanti, Mufasirin Mufasirin, Hani Plumeriastuti

PENGARUH PENEMBAKAN LASERPUNKTUR PADA TITIK REPRODUKSI ITIK CAMPBELL (*Anas platyrhynchos domesticus*) BETINA TERHADAP PRODUKTIVITAS TELUR

PDF
17-21

doi 10.20473/ovz.v8i1.2019.17-21

Sena Sangga Renata, Tri Wahyu Suprayogi, A.T Soelih Estoepangestie, R.T. Santanu Adikara, Benjamin Chr. Tehupuring, Sri Hidanah

EFEK PEMBERIAN L-ARGININ TERHADAP GAMBARAN HISTOLOGI JUMLAH SPERMATOSIT PRIMER PADA MENCIT (*Mus musculus*) SETELAH TERPAPAR SUHU PANAS

PDF
22-25

doi 10.20473/ovz.v8i1.2019.22-25

Dirga Januar Surya Utama, Suherni Susilowati, Tatik Hernawati, Erma safitri, Sri Mulyati, Tri Nurhajati

PROFIL GEN RECEPTOR GROWTH HORMONE (rGH) PADA SAPI MADRASIN

PDF
26-31

doi 10.20473/ovz.v8i1.2019.26-31

Gigih Lesmana Arganata, Budi Utomo S, R.T.S Adikara R.T.S Adikara

POTENSI EKSTRAK BUAH PARE (*Momordica charantia* L.) TERHADAP JUMLAH SEL LEYDIG DAN HORMON TESTOSTERON MENCIT (*Mus musculus*) JANTAN

PDF
32-35

doi 10.20473/ovz.v8i1.2019.32-35

People

Contact

Editorial Team

Reviewers

Instruction for Author

Guide for authors

Online Submission

Download Documents

Journal Policy

Focus and Scope

Section Policies

Publication Frequency

Publication Ethics

Indexing

Article Processing Charge

Peer Reviewers Process

Open Access Statement

Archiving

Plagiarism Screening

History

Copyright

old website

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USER

Username

Password

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JOURNAL CONTENT

Search

Faradillah Hapsari Dwi Putri, Wurlina Wurlina, Benjamin Christoffel Tehupuring

PENGARUH BAHAN PENGECER SARI KACANG KEDELAI (*Glycine max*) TERHADAP VIABILITAS DAN NEKROSIS SPERMATOZOA DOMBA SAPUDI

PDF
36-42

doi 10.20473/ovz.v8i1.2019.36-42

Kicky Hanis Immelda, Suherni Susilowati, Ira Sari Yudaniayanti

PROFIL GEN GROWTH HORMONE (GH) SAPI HASIL PERSILANGAN MADURA DAN LIMOUSIN DENGAN METODE PCR-RFLP

PDF
43-48

doi 10.20473/ovz.v8i1.2019.43-48

Ghea Aquatica Puteri, Budi Utomo S, Roesno Darsono

HUBUNGAN MORFOMETRI DENGAN PRODUKSI SUSU SAPI PERAH PERANAKAN FRIESIAN HOLSTEIN (PFH)

PDF
49-53

doi 10.20473/ovz.v8i1.2019.49-53

Yudhistira Eka Putra, Sri Mulyati, Sri Mumpuni S.

PENGARUH PEMBERIAN JAMUR *Fusarium graminearum* TERHADAP HISTOPATOLOGI TUBULUS SEMINIFERUS MENCIT (*Mus musculus*)

PDF
54-60

doi 10.20473/ovz.v8i1.2019.54-60

Fierda Kabayo, Abdul Samik, Ismudiono Ismudiono, Tjuk Imam Restiadi, Soeharsono Soeharsono, Hani Plumeriastuti

PENGARUH EKSTRAK DAUN PARE (*Momordica charantia*) TERHADAP FOLIKEL OVARIUM TIKUS (*Rattus norvegicus*) DALAM KONDISI HYPERGLYCEMIC

PDF
61-65

doi 10.20473/ovz.v8i1.2019.61-65

Ganeswara Muharam Hazmi Rezady, Hani Plumeriastuti, Rimayanti Rimayanti

CASE REPORT

ANALISIS FAKTOR YANG MEMPENGARUHI KEJADIAN MASTITIS SUBKLINIS DAN KLINIS PADA SAPI PERAH (STUDI KASUS DI KOPERASI AGRIBISNIS DANA MULYA KECAMATAN PACET, KABUPATEN MOJOKERTO)

PDF
66-70

doi 10.20473/ovz.v8i1.2019.66-70

Hefi Choirun Nisa, Bambang Purnomo S., Tita Damayanti L., Mas'ud Hariadi, Romziah Sidik, Nenny Harijani

EFISIENSI REPRODUKSI SAPI POTONG AKSEPTOR INSEMINASI BUATAN (IB) DI KECAMATAN TIKUNG, KABUPATEN LAMONGAN TAHUN 2015 DAN 2016

PDF
71-75

doi 10.20473/ovz.v8i1.2019.71-75

Laili Salisa Masruroh, Widya Paramita Lokapirnasari, Tjuk Imam Restiadi

PENGUKURAN NILAI KONDISI TERNAK (NKT), CONCEPTION RATE (CR), SERVICE PER CONCEPTION (S/C) PADA SAPI CROSSBREED LIMOUSIN DI KECAMATAN BALONGPANGGANG KABUPATEN GRESIK

PDF
76-81

doi 10.20473/ovz.v8i1.2019.76-81

Fajar Septian H, Imam Mustofa, Bambang Sektiari L.

POTENSI TEKNOLOGI INSEMINASI BUATAN PADA PENINGKATAN PRODUKTIVITAS ITIK TURI LAMONGAN

82-88

doi 10.20473/ovz.v8i1.2019.82-88

Tjuk Imam Restiadi, Tatik Hernawati, Dadik Rahardjo, Thomas V. Widiyatno

Search Scope

All

Search

Browse

By Issue

By Author

By Title

Other Journals

FONT SIZE

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PENGARUH EKSTRAK DAUN PARE (*Momordica charantia*) TERHADAP FOLIKEL OVARIUM TIKUS (*Rattus norvegicus*) DALAM KONDISI HYPERGLYCEMIC

INFLUENCE OF PARE LEAVES EXTRACT (*Momordica charantia*) ON FOLICLES OVARY IN RAT (*Rattus norvegicus*) DUE TO THE EFFECT OF HYPERGLYCEMIC CONDITION

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ABSTRACT

The purpose of this study was to prove that the provision of pare leaf extract can reduce the process of follicular atrophy in the ovaries caused by hyperglycemic conditions. The total of this study was completed within 20 days, including the induction of Streptozotocin for 5 days, treatment for 14 days, and 1 day for ovarian collection. The K- group as a negative control without being induced by Streptozotocin and received no treatment. The K+ group as a positive control was only induced by Streptozotocin at a dose of 55 mg / Kg BW and D₁₀ without treatment. The T1 group was induced by Streptozotocin at doses of 55 mg / Kg BW and D₁₀ and treated with pare leaves extract (*Momordica charantia*) at a dose of 1.26 ml / Kg BW. T2 group was induced by Streptozotocin with dose of 55 mg / Kg BW and D₁₀ and was treated with pare leaves extract (*Momordica charantia*) at a dose of 2.52 ml / Kg BW. Induction of Streptozotocin intraperitoneally and once-daily peroral treatment. The result showed that treatment group could make a significant difference with the positive control. Showing that amount of gap could make *Momordica charantia* to be alternative treatment for hyperglycemic condition.

Key word: Hyperglycemic, Follicular Atrophy, Streptozotocin, *Momordica charantia*.

Introduction

Diabetes mellitus is a collection of symptoms that arise in a person caused by an increase in blood glucose levels due to decreased insulin secretion progressively backed by insulin resistance (Suyono, 2011). Diabetes mellitus is a chronic, heterogeneous and life-threatening disease that is usually a metabolic disorder.

Hyperglycaemia can be caused by many things, but most often it is by diabetes mellitus. Hyperglycemia is a medical term for circumstances where blood sugar levels are higher than normal. In hyperglycemia, sugar accumulates in the blood because it fails to enter the cell. Failure occurs due to hormones that help the entry of blood sugar into the cell, the insulin hormone. Failure is common because the amount of the hormone is less or defective function. The

insulin hormone itself is produced by the pancreas.

In rats (*Rattus norvegicus*) normal blood glucose level is 62-175 mg / dl (Malole and Pramono 1999), if the blood glucose level exceeds that number then the mouse can be ascertained in hyperglycemic state. Hyperglycemic can cause metabolism of carbohydrate, protein, and lipid disorders. Metabolic disorders can cause failure in various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Kurniawan 2005, Lumbantobing 2003), as well as on the reproductive organs.

According to Garris (1994) hyperglycemic conditions in Chinese hamsters (*Cricetus griseus*) can cause damage to the endometrial structure resulting in widespread epithelial extension in the lumen and degeneration of the stromal membrane, the occurrence of uterine cell atrophy and lipid

accumulation. Based on research Garris and Garris (2003), in hyperglycemic rats occur atrophy follicle due to the accumulation of lipids. Hyperglycemic conditions in the female reproductive system can affect the function of ovarian follicles. The hyperglycemic state affects glucose transport in the ovary and estrogen production produced by the ovarian follicle so that estrogen levels in the body become low (Cox et al., 1994). In estrogen mice will affect the sensitivity of the vaginal epithelium to be codified as a sign that the mice have entered the estrous stage (Jesionowska et al., 1990). Thus the hyperglycemic state is suspected to affect the ovarian structure and estrus cycle in mice.

Selection of the use of pare (*Momordica charantia*) as a medicine is obtained from empirical evidence conducted in many countries around East Asia, Japan, Korea, and China. In addition, the Amazon community has traditionally used this plant especially the leaves to treat Diabetes Mellitus disease. In general the part used is a water decoction of the leaf (dekoktum). In the leaves of this plant contains momordisina, momordina, carantina, resin, and oil. *Momordica charantia* has antimicrobial activity, antioxidants and cytotoxic effects derived from momordisina and momordina. Antioxidants can form cell defense mechanisms against free radical damage (Manna, Sinha and Sil, 2009).

Materials and Methods

The research conducted in Veterinary Pathology Laboratory of Veterinary Medicine Faculty of Airlangga University Surabaya, for the extraction process of *Momordica charantia* held in "Balai Penelitian dan Konsultasi Industri – Laboratorium Penelitian dan Konsultasi Industri" Surabaya, and at "Kandang Hewan Coba" Laboratory of Veterinary Medicine Faculty of Airlangga University Surabaya for preparation and staining of ovarian tissue, also at Biochemistry Laboratory of Medical Faculty of Airlangga University Surabaya. The study lasted for 1 month, beginning on August 2017 until September 2017.

After 7 days of adaptation of the room, each mouse was weighed. A total of 20 rats

were divided into 4 groups containing 5 mice. The first group becomes a negative control. The second group was a positive control given STZ resulting in a hyperglycemic condition. The third group was given STZ and *M. charantia* extract by 50%. And lastly the fourth group was given STZ and extract *M. charantia* at 100%.

Group 2,3,4 injected STZ 55mg / kg BW as much as 0,1 ml on the same day. The rat tail was soaked in warm water for 1-2 minutes to allow vaso dilation, then the needle was inserted parallel to the tail and a 0.1 ml STZ solution was injected according to 55mg / kg BW through the intraperitoneal for 4 consecutive days, up to 4 days ahead of the addition of D10 dissolved with drinking water. 24 hours after the injection, blood sampling through the tail and blood glucose was examined using a glucostix measuring device (glucostix). Rats were maintained in a hyperglycemic state.

On the 13th day the administration of *Momordica charantia* extract for group 3 with dose 1.26 g / kg BW and for group 4 with dose 2.52 g / kg BW. This treatment was carried out for 14 days. On the 28th day the termination was taken and then the ovaries were taken for the preparation of histology preparations.

Preparation of ovarian preparation was done using paraffin method and hematoxylin-eosin staining. The first step was to do ovarian fixation with 10% PBS formalin solution, followed by dehydration using stratified alcohol and clearing using xylol. After clearing was done paraffin infiltration and embedding in paraffin. After the paraffin blocks were formed the blocks were attached to the holder, trimming, and slicing using rotary microtom. The formed bands were affixed to affixing. The latter process was staining with hematoxylin-eosin dye. The final step was the mounting (Handari, 1993). Observation of ovarian structure was done by counting the number of primary follicular atresi, secondary atresi, tertiary atresi, and corpus luteum on the incvasion of ovarian histology.

Result and Discussion

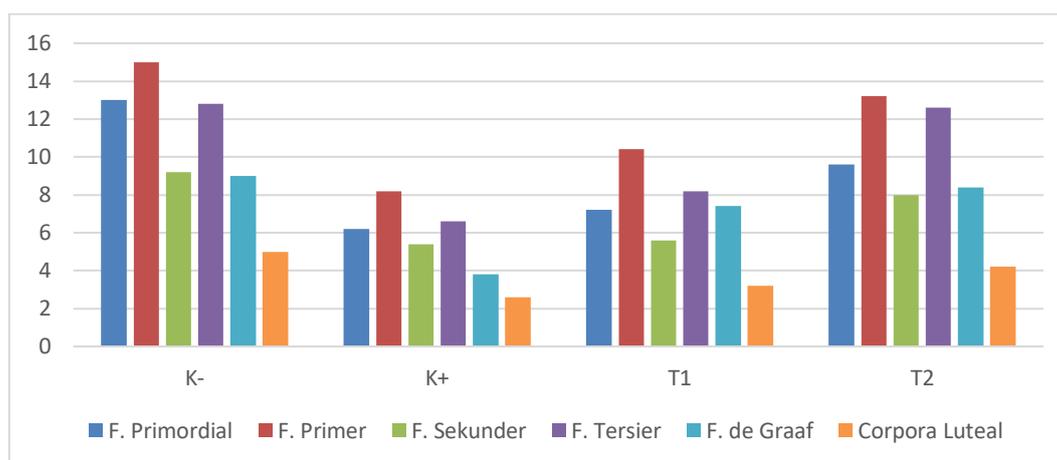


Figure 1. Diagram of average and standard deviation of observation of the number of ovarian follicles

Group K- became a negative control group that was not exposed or treated. For groups of K+, T1, and T2 the experimental animals were induced with Streptozotocin (STZ) at doses of 55 mg / Kg BW intraperitoneally and D10 infused by mixing into drinking water, after which the mice got hyperglycemic conditions. For the K+ group after animals try to be induced by Streptozotocin (STZ) at doses of 55 mg / Kg BW and D10 and became hyperglycemic, animals try didn't get any treatment to make positive control of hyperglycemic conditions. While the T1 and T2 groups after induced by Streptozotocin (STZ) with dose of 55 mg / Kg BW and D10 and became hyperglycemic, the animals try got the treatment of pare leaf extract (*Momordica charantia*) with the dose of 1.26 ml / Kg BW (T1) and 2.52 ml / Kg BW (T2) once a day orally. This treatment was carried out for 2 weeks / 14 days.

Based on figure 1, The results of examination of the number of follicles in white rats after induced Streptozotocin with a dose of 55 mg / kg BW and then given therapy with pare leaf extract with a dose of 1.26 ml / kg BW and 2.52 ml / Kg BW showed a significant effect on the reduction of atrophy white rat follicles.

In the experimental group that was not induced by Streptozotocin and did not receive any therapy of pare leaf extract (K-), showed the mean values of premodial follicles 13, primary follicles 15, secondary follicles 9.2, tertiary follicles 12.8, follicles

de graaf 9, and corpus luteum 5. This value indicated the average number of follicles in white mice under normal conditions.

In the experimental group of Streptozotocin-only animals with a dose of 55 mg / kg BW and D10 without treatment of pare leaf extract (K+), showed the mean values of premodial follicles 6.2, primary follicles 8.2, 5.4 secondary follicles, follicles tertiary 6.6, follicles de graaf 3.8, and corpora luteal 2.6. From the average results obtained showed significant results when compared with normal conditions (K-).

Streptozotocin is a glucosamine-nitrosourea compound. As with other alkylating agents in the nitrosourea class, it is toxic to cells by causing damage to the DNA, though other mechanisms may also contribute. DNA damage induces activation of poly ADP-ribosylation, which is likely more important for diabetes induction than DNA damage itself (Szkudelski T, 2001). Streptozotocin was similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2, but was not recognized by the other glucose transporters. This explained its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2 (Wang Z, Gleichmann H, 1998). Administration of D10 was performed to increase blood sugar levels after induction of Streptozotocin (STZ).

According to Colton et al. (2003) reduced communication between granulosa cells leads to altered paracrine communi-

cation between oocytes and granulosa cells. In addition, follicle cell cells required nutrient supply through the gap junction for oocyte development. The gap junction was a special area of adjacent cell membranes that allow for the communication between these cells (Granot and Dekel, 1998). Gap junctions play an important role in the development of granulosa cells, differentiation and luteinization (Kidder and Mahwi 2002 in Krysko et al., 2004). In follicle development, such communication is also needed to control the meiotic state of the oocyte. The types of protein gap junctions were known as connexin. Granulosa cells are known to express connexin-43 while in the oocytes are connexin-37 (Granot and Dekel 1998) Based on a study of Chang et al. (2005), hyperglycaemia caused decreased expression of connexin-43 protein that affected intracellular communication in granulosa cells and increased apoptosis in these granulosa cells. This is similar to that of Colton et al. (2003) suggesting that high glucose levels simultaneously also increased apoptosis in granulosa cells.

In the experimental group that Streptozotocin-induced dose of 55 mg / Kg BW and D10 treated with pare leaf extract 1.26 ml / Kg BW (T1) and 2.52 ml / Kg BW (T2), showed the mean respectively from the premodial follicles of 7.2 and 9.6, the primary follicles of 10.4 and 13.2, the secondary follicles 5,6 and 8, the tertiary follicles 8.2 and 12.6, the graft follicles of 7.4 and 8, 4, and corpora luteal 3.2 and 4.2. These results indicated that pare leaf extract could reduce the occurrence of atrophy and decreased the blood glucose levels of Streptozotocin-induced white rats but less significant for the group with a dose of 1.26 ml / Kg BW (T1). The results shown in the group were almost the same as those without treatment (K+).

In group T2 the number of follicles in white rats was premodial follicle 9.6, primary follicles 13.2, secondary follicles 8, tertiary follicles 12.6, follicles de graaf 8.4, and corpora luteal 4.2. This was a result of near normal conditions. In the T2 group using a dose of pare leaves extract of 2.52 ml / Kg BW for 14 days, this dose was very effective to reduce the occurrence of atrophy of follicles in white rats that have been induced by Streptozotocin at a dose of

55 mg / Kg BW. It also proved that pare leaf extract could be an alternative therapy for diabetes mellitus.

One of the content of pare leaves was flavonoids. Flavonoids have acarbose-like effects that inhibited the enzyme α -glucosidase that serves to break down polysaccharide compounds into glucose monomers, so that the absorption of glucose in the intestine is reduced and blood glucose levels decrease (Hartika, 2009).

According Ardra (2017), some phytochemical compounds contained in pare leaves are momordina, momordicina, resinic acid, charantine, tricosanin acid, resin, saponin, vitamin A and D, and fat. Saponins can help reduce blood glucose levels because they can form a membrane layer on the surface of the small intestine, thereby inhibiting glucose absorbs (Mills and Kerry, 2000). Limtrakul et al., (2012) also argues that saponins have a polypeptide structure similar to insulin to act as "insulin like" in lowering blood glucose levels.

Conclusion

Based on the results of this study could be concluded that the provision of pare leaf extract (*Momordica charantia*) could reduce blood glucose levels and reduced the atrophy of the white rat's ovarium follicles (*Rattus norvegicus*) induced Streptozotocin (STZ).

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