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Pangkat/Golongan : Pembina (Gol. IV/a)
Jabatan : Wakil Dekan III

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Nama : Dr. Erma Safitri, drh., M.Si
NIP : 196907231999032001
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1	Immunomodulatory Activity of Black Jinten Oil (Nigella sativa) as Macrophage Activator for Salmonella typimurium Infected Rat	2020
2	Screening the Reproductive Tract of Dairy Cattle for Pathogenic Micros	2019
3	Human Chorionic Gonadotropin (hCG) from Urine of Pregnant Women to Manipulate in vivo Ovulation and Pregnancy of Madura Cows	2019
4	Anti Early Embryonic Protein (EEP) for Pregnancy Test by Microtiter Strip in Dairy Cows	2019
5	The Effect of Feeding High Level of Protein on Reproductive Performance of Bali Starling.	2019
6	Antisperm Antibody in Repeat Breeder Friesian Holstein Cows at KPSP Setia Kawan Nongkojajar, Tutur District, Pasuruan, Indonesia.	2019
7	Diagnosis of Single and Twin Pregnancy, and Early Embryo Mortality Through Progesterone Level Test on Local Does.	2019
8	Improvement of Pregnancy Rate in Bali Cows with the Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF2α.	2019
9	Progesterone Profile of Dairy Cows which Experienced the Failure of Pregnancy to Artifical Insemination (AI).	2019
10	Effect of Heat Shock Protein (HSP) in Post Thaw Baluran Bull Semen	2018
11	Potency of Mycotoxin Binders on MDA Level, Expressions of Caspase 9 and Caspase 3 in The Uterus of Mice Exposed to Zearalenone	2017

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12	Polymorphism of Growth Hormone Gene in The Artificial Insemination Result of Madura Cattle with Limousin Semen as a Reference for Genetic Selection	2018
13	Implementation of fotogrametry techniques as body mass estimation of indo-pacific bottle nose dolphin (Tursiops aduncus) in bali dolphin lodge	2020
14	Uji Sensitivitas Kebuntingan Sapi Perah Menggunakan Pregnancy Specific Protein B (PSPB) Microtiter Strip dan Progesteron sebagai Gold Standard	2007
15	Estimation of Equine Chorionic Gonadotropin (eCG) concentrate in the Blood Sera of Pregnant Mare	2014
16	Efek Pemberian L-Arginin Terhadap Gambaran Histologi Jumlah Spermatosit Primer pada Mencit (Mus musculus) Setelah Terpapar Suhu Panas	2019
17	Anti Prolactine Overcomes Heat Stress on Laying Hen.	2008
18	Unnatural Forced Moulting in The Laying Hen as Cause of Zoonosis from Salmonella Enteritidis	2009
19	Case Study: Dystocia on Beef Cattle in Kunir Regency of Lumajang District, East Java, Indonesia in 2015 and 2016	2017
20	Teratogenic Effect of Congenital Toxoplasmosis in Chicken Embryo	2017

Adapun penelitian tersebut layak dilakukan, meskipun belum ada ***Ethical Clearence*** karena menggunakan hewan coba yang minimal dan menghasilkan output yang sangat baik.

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Surabaya, 3 April 2023
Wakil Dekan III,

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Vol. 96 October 2019 No. 10

CONTENTS

GENERAL ARTICLES :

CLINICAL AND FIELD ARTICLES :

Indian Vet. J., October 2019, 96 (10) : 20 - 23

Antisperm Antibody in Repeat Breeder Friesian Holstein Cows at KPSP Setia Kawan Nongkojajar, Tutur District, Pasuruan, Indonesia

FadilaZikri Amanda, Sri PantjaMadyawati, IsnainiFadilah and Erma Safitri[1]

Faculty of Veterinary Medicine, UniversitasAirlangga, Surabaya 60115, Indonesia.

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Abstract

This study presents a laboratory explorative method by using sample of cervical mucus and blood serum from 11 Holstein Friesian (HF) cows, aged 4 to 9 years age, normal estrus cycle and gave birth before. They were divided into 2 groups, 10 dairy cows that have history of repeat breeders (4 times or more AI) (RB) and 1 normal breeder (2 times AI) as a control (C). Samples were examined using indirect-ELISA test method. The results of this study indicate that the antisperm antibody concentration that appears in cervical mucus samples and blood sera of control group has a lower concentration value (0.342 and 54.860 ng/mL) than RB group (233,776 and 944,531 ng/ml).

Key words : Antisperm antibody, Repeat breeder, Holstein Friesian.

One of the cause of repeat breeding is the production of antispermic antibodies (ASA) in mucous membranes the female genital tract due to injury. Immunologic causes of reproductive failure have been reported in humans (Mahdi *et al*., 2011), livestock (Fayemi, 2005; Zraly *et al*., 2003) and in other species like rabbits and horses (Risvanli *et al*., 2005). Antisperm antibodies (ASAs) are antibodies that are developing against spermatozoa which can affect the fertility of spermatozoa, leading to repeat breeding. ASA can be found in blood serum, cervical mucus, oviduct fluid, uterine fluid, and follicular fluid. The presence of ASA may inhibit the movement of spermatozoa through the cervical mucus, preventing changes in membrane fluidity required for capacitance, reducing the ability of spermatozoa to undergo acrosome reactions and disrupting the binding of zonapellucida and fertilization (Fijak and Meinhardt, 2006).

Materials and Methods

Samples of cervical mucus and blood serum were taken from 10 female HF cows with history of repeat breeding (RB) and 1 normal Friesian Holstein cow (C) at KPSP SetiaKawan, Tutur District, Pasuruan, Indonesia (Mayawati *et al*., 2019). Cervical mucus samples were collected using a 10 ml falcon tube followed by a cervical massage, blood samples from the jugular vein in a 10 ml venoject without anticoagulants and kept for 30-60 minutes to separate the serum and then centrifuged for 5-10 minutes at 3000 rpm to obtain of supernatant to perform the ELISA-Indirect method to detect antisperm antibodies.

The Elisa test was performed according to the Antisperm Antibody Manual, *ie* the blood serum sample was inserted into a well-coated antibody pellet originating from the AsAb substrate reagent on a microtiter plate of 100 µL, adding 100 µL PBS (pH 7.0-7.2) in the blanks. Especially for cervical mucus samples, and 10 µL of balance solution was added to 100 µL the sample specimen. 50 µL conjugate was added to each well (except the blank well) and mixed well covered and incubated for 1 hour at 37 ° C. Washed with ELISA-washer. 50 µL Substrate A and 50 µL Substrate B were added to each well including blank, then covered and incubated for 10-15 min at 20-25 ° C (avoid sunlight). 50 µL of Stop Solution was added to every well including blank and mixed well. ELISA reader with wavelength 450 nm was used to read the results.

Results and Discussion

The antisperm antibody examination of

[1]*Corresponding author* : Email : rma_fispro@yahoo.com

Table I. Concentration of Antisperm Antibodies in Cervical Mucus Samples and Blood Serum of Dairy Cow (FH) using Indirect-ELISA.

Cow Code	Cervical Mucus(CM)	Blood Serum(BS)
	(ng/mL)	(ng/mL)
C	0.342	54.860
RB1	10.124	270.479
RB2	12.175	294.124
RB3	10.535	343.725
RB4	58.241	391.814
RB5	39.373	866.947
RB6	70.523	854.884
RB7	132.576	631.008
RB8	146.269	457.719
RB9	170.441	879.452
RB10	233.776	944.531

Note: C : Cow without the history of repeat breeding; RB: Cow with repeat breeding history.

samples were performed using indirect-ELISA testing method on 10 repeat breeder cows (RB) with the history of AI ≥3 times but not pregnant and 1 dairy cow HF with normal estrus cycles, had done AI 2 times and pregnant as a control (C). The results of this study revealed that the antisperm antibody concentration that appears in cervical mucus samples and blood sera of C group has a lower concentration value (0.342 and 54.860 ng/mL) than RB group (233,776 and 944,531 ng/ml) (Table I).

The value of antisperm antibody concentration that emerged after indirect-ELISA test showed that sample of cervical mucus or blood serum in the dairy cow HF control (C) which had artificial insemination (IB) 2 times had a lower concentration value 0.342 and 54.860 ng / ml when compared with the overall sample of cows that have history of repeat breeding. The highest concentration of anti semen antibodies in cervical mucus and serum samples was found in dairy cow HF which had repeat breeder with criteria had been done AI 4 times (RB10), each value 233,776 and 944,531 ng / ml.

More number of insemination leads to high concentrations of antispermic antibody concentrations in cervical mucus samples and blood sera, this is because in general semen containing spermatozoa presents antigens that appear at various stages of development. These antigens, present early on in sperm, adhere to ejaculation and are involved in the maturation and fertilization process, they also act as a protective against the immune system of the female reproductive tract (Vivas *et al*., 2007). One of the basic characteristics of sperm cells is the continuous change in its antigenic structure due to the loss of surface molecules during maturation and insemination. In artificial insemination technology (AI), the antigenic structure of sperm cells changes due to the addition of different diluents, freezing and liquefaction procedures and reduction of seminal plasma volume (Cheema *et al*., 2016).

The reasons were also raised by Risvanli *et al*. (*loc. cit*) the presence of spermatozoa having contact with blood thus leading to development of ASA in animal body. The event is triggered by inflammation, such as metritis and vaginitis or trauma and bleeding that occurs during the process of artificial insemination which ultimately has an important role in the development of ASA. In humans, Thaper *et al*. (2014) argued that cross-reactivity between a particular epitope on the surface of bacteria and spermatozoa, in particular involving the determinants of carbohydrates may be one of the potential trigger mechanisms for the induction of antisperm antibodies in both men and women.

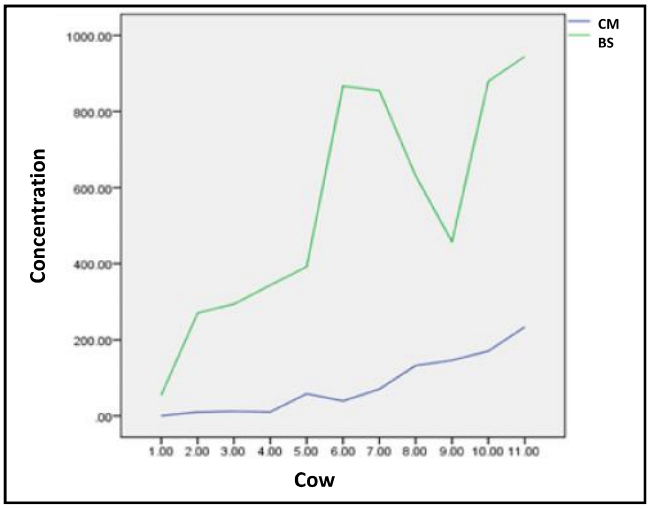

Fig 1. Graph of antispermic antibody concentration of cervical mucus samples and blood sera in normal cattle and repeat breeders.

The variation in concentrations obtained in cow with IB to 4 is caused by recurrent events of more traumatic injury during parturition in high-parity cow (Srivastava *et al*., 2016). As described by Cheema *et al*. (*loc. cit*) that the percentage of cow with IPA titres; IgA and IgG as well as higher ELISA in blood serum and maximal cervical mucus in cows belonging to the second to fourth parity. This suggests that the proportion of cow with higher ELISA titres in blood serum and cervical mucus increases with increasing parity. The proportion of Zebu beef positive sperm antibodies was significantly associated with increased parity. It is also possible that less aseptic gun Ib and plastic sheets are used in relation to hygienic management of gun IB and plastic sheet, so that other bacteria that come along with artificial insemination can be the cause of ASA.

The concentration values obtained in the indirect-ELISA test were found to be higher in blood serum compared with the concentration of cervical mucus because in general the cement had a very heterogeneous antigen content. Because sperm have auto-antigenic (auto-immunization) as well as iso-antigenic (iso-immunization) potential, it is able to induce the production of sperm-reactive T-cells in both men and women, thus opsonized and then targeted by leukocytes (cytotoxic sperm effect) (Bronson, 2011). It is not a single ASA that affects fertility but more likely some ASA causes infertility. Furthermore, it has been postulated that antibodies to a single sperm antigen cannot cause infertility. It has also been reported that not all ASA, whether produced in women or men, affects the potential for fertility because cognitive antigens do not have to be involved in the fertilization process (Sedlackova *et al*., 2010).

Despite differences in concentration levels between cervical mucus and blood serum, the results of the Spearman Correlation test resulted in a significant correlation between cervical mucus and blood serum with a significant significance of 0.001 and a high correlation between the two samples with a correlation coefficient of 0.864. The presence of a significant correlation between cervical mucus and blood serum due to the same percentage that is reactive to sperm in IgG and IgA tests in serum and cervical mucus suggests that the presence of IgG and IgA produced against sperm surface proteins is present in the blood and cervical mucus. So they do play an important role in the production of antibodies in the female reproductive tract (Lazarevic *et al*., 2003).

Summary

Based on the results of the study, we found that the antisperm antibody concentration that emerged after indirect-ELISA assay on control samples, both cervical mucus and blood serum had a lower concentration value and the highest obtained in cow repeat breeder (RB) with criteria had been done artificial insemination 4 times.

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Indian Vet. J., October 2019, 96 (10) : 23 - 25

Economics of Inclusion of Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*) in Broiler Feeds

S.A. Jadhav, S.M. Bhalerao[1], A.V. Khanvilkar, S.D. Changan and L.A. Pangaonkar

Department of Animal Nutrition, Krantisingh Nana Patil College of Veterinary Science, MAFSU, Shirwal-412801, Dist-Satara, Maharashtra.

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Abstract

An experiment was carried out on 240, day-old broiler chicks for a period of six weeks. The chicks were divided randomly into four treatment groups having three replicates of 20 birds each. The four treatments were *viz.* control group (T_0), 0.50 % turmeric powder (T_1), 0.50 % ginger powder (T_2) and combination of turmeric and ginger powder at the level of 0.50 % each (T_3). All the standard managemental practices were followed during the trial period. The experimental feed was fed in three phases as pre-starter, starter and finisher. The economics of broiler production was calculated by considering feed cost and net production cost per bird. The profit was calculated by subtracting the cost of production per bird from sale price of birds on live body weight basis. The profit per kg live body weight was found to be Rs. 19.09, Rs.21.99, Rs.17.72 and Rs.16.00 in T_0, T_1, T_2 and T_3 groups, respectively. The birds in T_1 group having 0.5% supplementation of turmeric powder resulted in more profit than control and other groups.

Key words: Broiler birds, Turmeric supplementation, Economics

Poultry serves as a vital tool to provide nutritional security and supplementary income. As indiscriminate use of antibiotics in poultry industry is reported to be rising, use of herbal and plant derivatives may prove to be a potential alternative for promoting poultry output. Although many plants are reported to have beneficial effects, the rhizome part of turmeric (*Curcuma longa*) containing curcuminoids, zingiberene, turmerone and curlone is reported

[1]*Corresponding author* : Email : sanjaymbhalerao@rediffmail.com

THE INDIAN VETERINARY JOURNAL
Vol. 96 October 2019 No. 10

AUTHOR INDEX

THE INDIAN VETERINARY JOURNAL

Vol. 96 October 2019 No. 10

SUBJECT INDEX

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