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Research Article
Blood Metabolic and Estradiol Level of Repeat Breeder and Fertile in Friesian Holstein Cross Breed Cows in the Tropic

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Abstract

Background and Objective: The repeat breeding becomes the major reproduction problem in tropic area especially in Indonesia. It relates to blood metabolic and hormonal level. This research was conducted to investigate the level of blood metabolic and estradiol between the repeat breeder Friesian Holstein Cross Breed Cows (FHCB) and the fertile FHCB cows. **Materials and Methods:** Twenty FHCB cows in luteal phase on 2nd to 3rd lactation were used in this research. Group I consist of 10 repeat breeder FHCB cows and group II consist of 10 fertile FHCB cows. Blood samples were collected through jugular vein prior to feeding. The level of total protein, phosphorus, glucose, cholesterol and estradiol in serum were calculated. The data were analyzed by using the independent samples t-test for comparing the blood metabolic and estradiol between the repeat breeder FHCB cows and the fertile FHCB cows. **Results:** The results showed that repeat breeder FHCB cows were found to have lower level of all of the parameters of blood metabolic and estradiol descriptively, however, only the serum cholesterol and serum phosphorus had the significant difference ($p<0.05$) between the repeat breeder FHCB cows and the fertile FHCB cows. **Conclusion:** It could be concluded that level of serum cholesterol and serum phosphorus played a role in repeat breeding.

Key words: Estradiol, fertile cows, Friesian Holstein cross breed, repeat breeder cows, serum cholesterol, serum glucose, serum phosphorus, serum protein

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Repeat breeder is the normal cow that fails to conceive after three or more inseminations[1]. Repeat breeding has the adverse impact especially in economical value on the dairy cattle and impacts on the low of fertility such as; in increase in calving interval and increase of culling rates[2]. Some problems that can make the cow become repeat breeder are nutritional imbalance, genetic, skill of the inseminator and the environmental factors[3]. Repeat breeding is the reproduction problem and it correlates with nutritional status[4]. The imbalance of nutrition can alter the activity of ovarium and leads to the hormonal imbalance thus, the cow become repeat breeder[4].

Nutritional status can be reflected by the blood metabolic in dairy cow such as; serum protein, serum phosphorus, serum cholesterol and serum glucose[2,4,5]. Reproduction of the dairy cow is also influenced by the estradiol[6] which has the important role in reproduction function[7]. The dietary proteins play a role in reproduction[8]. The protein are the source of the amino acid where it is required for synthesizing the gonadotropins and gonadal hormones[1]. The cholesterol was the precursor of the steroid hormone[2]. The glucose has the important role in reproduction. The lower of serum glucose can inhibit the production of gonadotropins hormone which has the important role in reproduction[9]. Deficiency of phosphorus influences at the level of pituitary and ovary and may interfere with fertilization causing early embryonic death[5]. The phosphorus had the correlation with the fertility[10,11]. The aim of this study was to investigate the blood metabolic and estradiol on repeat breeder FHCB cows compare to fertile FHCB cows.

MATERIALS AND METHODS

Ethical approval: The protocol were approved by the Faculty of Veterinary, Universitas Gadjah Mada, Indonesia (approval reference number 0014/EC-FKH/ Eks./2019).

Study area: The study was conducted in Samesta Dairy Farms and Yogyakarta Dairy Farms. Blood metabolic was analyzed in Integrated Research and Testing Laboratory, Universitas Gadjah Mada. The serum estradiol was analyzed in Laboratory of Physiology, Faculty of Veterinary, Universitas Gadjah Mada. The nutrient content of feed was analyzed in Laboratory of Animal Feed Technology, Faculty of Animal Science, Universitas Gadjah Mada. The vaginal smear was carried out in Laboratory of Physiology and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada. The entire study was conducted from 1st October, 2018 until 23rd April, 2019.

Materials: Twenty head of cows were used in this research. The cows were in luteal phase on the 2nd to the 3rd lactation and healthy with the Body Condition Score (BCS) 2.5-3. The cows were kept under the freestall system in Samesta Dairy Farms and Yogyakarta Dairy Farms. Micro lab 300 was used for analyzing the concentration of serum protein, serum phosphorus, serum cholesterol and serum glucose. Bovine estradiol enzyme-linked immunosorbent assay (ELISA) kits were used for analyzing estradiol concentration.

Luteal phase identification: The identification of luteal phase (diestrus) was done by using vaginal smear method (Fig. 1)[12]. The sample of vaginal epithelium was collected before the blood collecting. The characteristic of luteal phase based on vaginal smear were the parabasal cell which would be found as the major cell [12].

Nutritional status: The cows were fed with Samesta commercial concentrated feed, Yogyakarta Dairy Farms concentrated feed *Panicum muticum*, rice straw, corn straw and king grass (Table 1).

Blood sampling: Three milliliters of blood were collected from jugular vein prior to feeding to avoid the changes in blood metabolic. The blood collected then was put into Ethylene Diamine Tetra Acetic acid (EDTA tube)-coated tube. The tube was transported in an ice container to the laboratory. The tube was centrifuged at 3000 rpm for 15 min to obtain the serum. Serum was stored at -20°C. The serum then was used for estimating the serum protein, serum phosphorus, serum cholesterol, serum glucose and estradiol.

Chemical analyzing: Serum protein, serum phosphorus, serum cholesterol and serum glucose were measured by using Micro lab 300 spectrophotometer. Serum protein was estimated by using the biuret method (Biuret, Colorimetric kit). Serum phosphorus was measured by using photometric method. The serum cholesterol was measured by using cholesterol oxidase-para amino phenazone (CHOD-PAP) method. Serum glucose was measured by using the glucose oxidase-peroxidase aminoantipyrine (GOD-PAP, Liquid kit) method. Estradiol was measured by using enzyme-linked immunosorbent assay (ELISA) method.

Reproduction performance: A cow was considered a Repeat Breeder (RB) if it had at least 3 Artificial Insemination (AI) and no subsequent calving or more than 3 AI irrespective of subsequent calving or not with cows at risk being those with at least one AI[13].

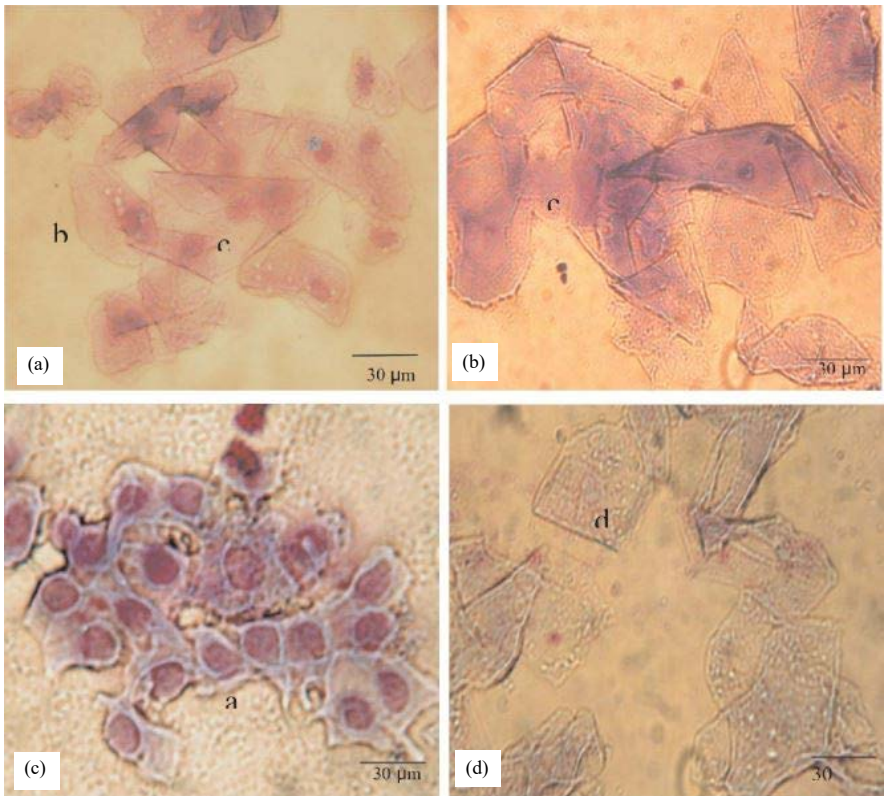

Fig. 1(a-d): Morphology of vaginal epithelium (a) Parabasal cell, (b) Intermediate cell, (c) Cornificated superficial cell and (d) Keratinised superficial cell

Table 1: Nutrient content of Friesian Holstein cross breed cow

	Nutrient content (%)							
Type of feed	DM	OM	CP	EE	CF	NFE	TDN	P
Samesta commercial concentrated feed	85.53	84.09	5.65	2.47	11.86	80.02	86.06	0.6
Yogyakarta dairy farm concentrated feed	86.73	86.62	7.46	0.92	19.09	72.53	76.32	0.7
Panicum muticum	22.66	80.48	5.33	3.07	22.77	68.83	70.03	0.45
Rice straw	33.06	77.35	3.40	1.57	27.63	67.40	65.59	0.12
Corn straw	33.20	89.87	7.26	1.31	22.94	68.49	71.29	0.32
King grass	20.58	92.33	2.76	1.30	37.29	58.65	56.83	0.2

RB: Repeat breeders, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, TDN: Total digestible nutrient, P: Phosphorus

Statistical analysis: The data were analyzed by using the independent samples t-test for comparing the blood metabolic and estradiol level between the repeat breeder and the fertile cows. All statistical analysis was performed by using the Statistical Program for Social Science (SPSS) version 16.

RESULTS

Luteal phase identification: The result of vaginal smear method was shown in Fig 2. The parabasal cells were found as the major cell in both of repeat breeder cows and fertile cows. It indicated that all of the cows used in this study were in the luteal phase (Fig. 2).

Nutrient status: The nutrient content of feeds and dry matters intake were shown in Table 1 and 2, respectively. The feed used in this research were Samesta commercial concentrated feed, Yogyakarta Dairy Farm concentrated feed, *Panicum muticum,* rice straw, corn straw and king grass. The most important finding of Table 1 was about the information of *Panicum muticum*. The *Panicum muticum* had the highest

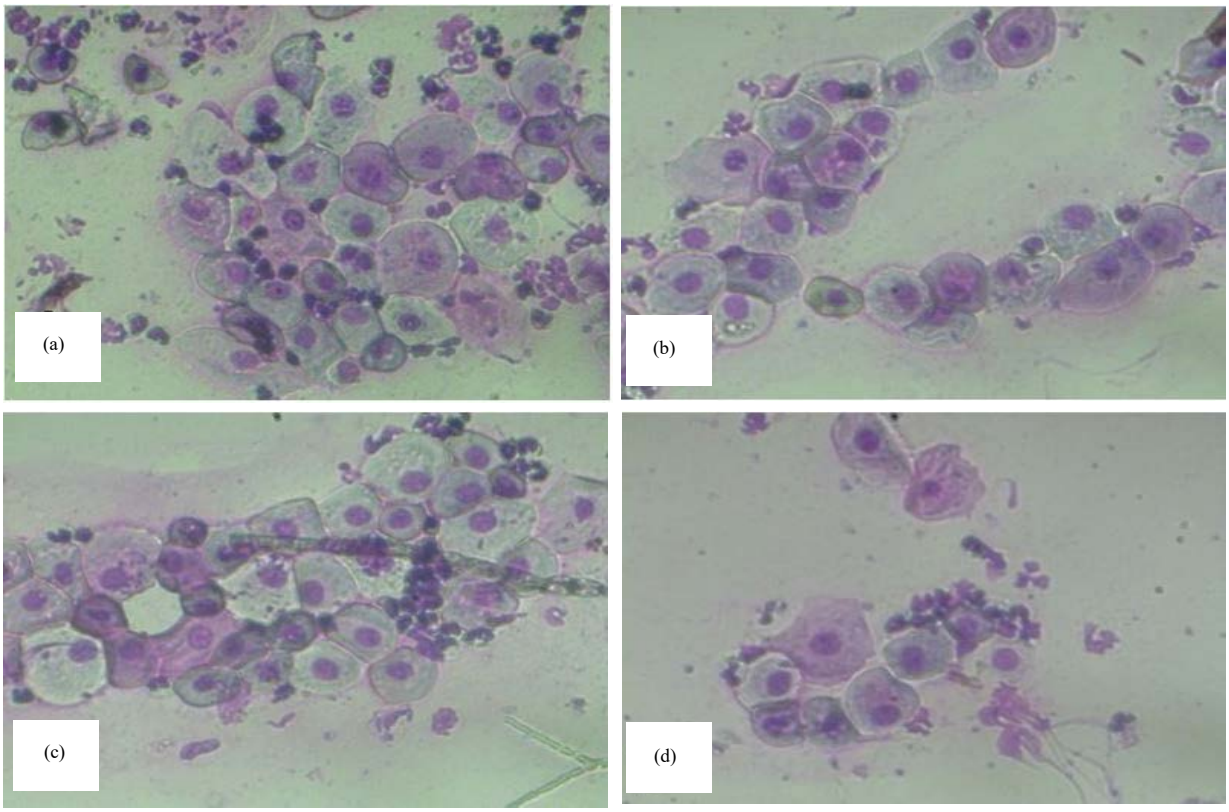

Fig. 2(a-d): Vaginal epithelium on repeat breeder cows and fertile cows, (a-b) Repeat breeder cows and (c-d) Fertile cows

Table 2: Dry matters intake of Friesian Holstein cross breed cow

	Type of cow	
Type of feed (kg)	Repeat breeder cows	Fertile cows
Samesta commercial concentrated feed	38.49±10.50	52.69±9.0
Yogyakarta Dairy Farm concentrated feed	28.55±11.70	13.01±8.7
Panicum muticum	8.57±2.80	18.04±3.2*
Rice straw	0.28±0.1	1.85±0.7
Corn straw	10.53±4.2	13.18±3.0
King grass	18.01±7.3	10.74±7.6

*p<0.05

Table 3: Nutrient intake of Friesian Holstein cross breed cow

	Type	
Nutrient intake (kg)	Repeat breeder cows	Fertile cows
DM	14.92±2.0	15.65±1.4
OM	12.91±2.0	13.38±1.4
CP	0.86±0.1	0.89±0.1
EE	0.27±0.1	0.33±0.1
CF	3.03±1.1	2.92±0.9
NFE	10.76±1.1	11.51±1.0
TDN	11.26±1.1	12.09±1.1

RB: Repeat breeders, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fiber, NFE: Nitrogen free extract, TDN: Total digestible nutrient

content of extract ether than the other feeds. It also had the highest content of phosphorus than the other forages.

Table 2 presented only the dry matters intake of *Panicum muticum* that showed significantly lower ($p<0.05$) in repeat breeder cows than the fertile cows. Since, *Panicum muticum* had the high content of extract ether and phosphorus while, the dry matters of it had found to be higher in fertile cows than the repeat breeders cows, it indicated that *Panicum muticum* played a role in this current study.

The nutrient intake of the cows was shown in Table 3. All of the nutrient intake parameters tended to be higher in the fertile cows than in the repeat breeder cows except for the crude fiber. The consumption of crude fiber had found to be higher in repeat breeders than the fertile cows. The crude fiber was the nutrient contained of cellulose and hemicellulose which the cows need more time to degrade it to be useful for the energy source. It indicated that the fertile cows consumed more nutrient than the repeat breeder cows. Even there was no significant difference of nutrient intake between both of the repeat breeder cows and the fertile cows, this tendency correlated with the result of blood metabolic and estradiol level.

Table 4: Level of different parameters in repeat breeder and the fertile Friesian Holstein cross breed cow

Parameters	Concentration	
	Repeat breeder cows (n =10)	Fertile cows (n =10)
Serum protein (g dL^{-1})	7.30±10.02	7.50±00.65
Serum phosphorus (mg dL^{-1})	2.03±00.41	2.46±00.44*
Serum cholesterol (mg dL^{-1})	126.08±17.50	152.72±25.58*
Serum glucose (mg dL^{-1})	56.77±60.39	59.31±30.95
Serum estradiol (pg dL^{-1})	5.13±10.96	5.89±10.88

*p<0.05

Blood metabolic and estradiol level: The average of blood metabolic and estradiol of the repeat breeders and the fertile cows were shown in Table 4. The blood metabolic was represented by serum protein, serum phosphorus, serum cholesterol and serum glucose. The result showed that repeat breeder cows were found to have lower level of all of the parameters of blood metabolic and estradiol descriptively. It was found that significant differences (p<0.05) on serum phosphorus and serum cholesterol, but no significant differences on serum protein, serum glucose and serum estradiol (Table 4).

DISCUSSION

The serum phosphorus was significant lower (p<0.05) in repeat breeder cows than the fertile cows in this study. Lower serum phosphorus concentration in repeat breeder animals has also been reported in many other studies[5,14,15]. Phosphorus is essential for transferring of biological energy specially through Adenosine Tri Phosphate (ATP)[16]. While, the ATP itself has the important role as the precursor of cyclic Adenosine Mono Phosphate (cAMP). Cyclic adenosine monophosphate was the original 2nd messenger promoted by adenylyl cyclase activation after ligation of G protein-coupled receptors by ligands including reproduction hormones[17] such as; Follicle Stimulating Hormone (FSH) and Luteinizing Hormone[2] (LH). Low level of phosphorus causes on some reproductive diseases such as; prolonged of estrous cycle, low conception and long of calving interval[18]. Blood phosphorus level dropped significantly in cows with anestrous and repeat breeding[19]. From Table 1, *Panicum mucitum* had a high level of phosphorus content. Furthermore from Table 2, dry matter intake of *Panicum muticum* was significant higher (p<0.05) in fertile cows than the repeat breeders cows. It indicated that *Panicum muticum* played a role in this study.

This study showed that serum cholesterol was significant lower (p<0.05) in repeat breeder cows than the fertile cows[4,15]. Cholesterol is a form of lipid or fat circulating in the bloodstream. Cholesterol is essential for building and maintaining the cells and also making several essential hormones[20]. Cholesterol is crucial for the biosynthesis of the steroid hormone that plays a role in reproduction such as; androstenedione, progesterone and also estradiol under the influence of LH[2,21]. The low level of nutrient such as; cholesterol can alter the endocrine system, furthermore it leads to reproduction failure[9], silent estrus, sub-estrus and lowering the conception rate[4] since, the cholesterol was the precursor of steroid hormone[22]. From Table 1, *Panicum mucitum* had the highest level of ether extract content from all of the type of feeds. Meanwhile, from Table 2, dry matter intake *Panicum muticum* was significant higher (p<0.05) in fertile cows than the repeat breeders cows. It indicated that *Panicum muticum* once again played a role in this study.

Current study showed that there was no significant different on serum protein between the repeat breeder cows and the fertile cows[4,5,14,20]. It can be explained with the nutritional status between both of them from the Table 3.

From Table 3, there was no significant different on crude protein intake between repeat breeders and the fertile one. There was other factor that probably played a role in this result. The serum protein had the positive correlation with Blood Urea Nitrogen (BUN)[23,24]. The infertile cows had the higher level of BUN than the fertile cows[2]. Furthermore, BUN can alter the environment of uterine by lowering pH so, it had the harmful effects on oocytes or embryos[25,26].

Glucose is one of the essential nutrient in reproduction by synthesizing the gonadotropin releasing hormone (GnRH) from hypothalamus[2]. This current study also showed that there was no significant difference on glucose between the repeat breeder cows and the fertile cows[14]. From previous study there was another factor that probably influence the result. Serum glucose has a positive correlation with malondialdehyde. The MDA is one of oxidative stress as a causative factor and had affected the fertility[27]. Malondialdehyde is the last product of lipid peroxidation. The changes of MDA concentrations can be used as a biomarker of oxidative stress[28].

Estradiol is the reproduction hormone produced by granulosa cells and theca sell of follicle ovarian. It is essential in reproduction of the female vertebrates[29]. Steroid hormones like an estradiol plays a central role in the regulation of uterine functions including morphological changes in the endometrium during the estrous cycle[30]. The relationship between the repeat breeder and the level of luteal estradiol has not yet been fully elucidated. Even there was no the significant different on estradiol level between the repeat breeder cows and the fertile cows, this study showed that the

repeat breeding related to the low level of estradiol since, the estradiol level tended to be lower in repeat breeders than the fertile cows and it could be the information for the next research.

CONCLUSION

Repeat breeder FHCB cows were found to have lower level of all of the parameters of blood metabolic and estradiol descriptively, however, only the serum cholesterol and serum phosphorus had the significant difference ($p<0.05$) between the repeat breeder FHCB cows and the fertile FHCB cows. It implicated that of serum cholesterol and serum phosphorus played a role in repeat breeding.

SIGNIFICANCE STATEMENT

This study discovered the possible different level of blood metabolic and estradiol between the repeat breeder cows and the fertile cows. It provided the nutrient data as the novelty for this study and it used for the supporting information that could be the justification of the result. It also provided the novel information about the luteal estradiol of repeat breeder cows and the fertile cows. This study can be beneficial for the dairy farmers in tropic area for running the management of reproduction. This study will help the researcher to identify the critical areas of cause of repeat breeding where the other researchers were not able to explore.

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