http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2019.356.360

Research Article
Cortisol and Blood Urea Nitrogen Profiles in Fertile and Repeat-breeder Holstein-friesian Crossbred Cows

[1]Diah Tri Widayati, [2]Adiarto, [2]Budi Prasetyo Widyobroto and [2]Yustina Yuni Suranindyah

[1]Laboratory of Animal Physiology and Reproduction, Department of Animal Genetic and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, 55281 Bulaksumur, Yogyakarta, Indonesia
[2]Laboratory of Dairy Science and Milk Industry, Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, 55281 Bulaksumur, Yogyakarta, Indonesia

Abstract

Background and Objective: Repeat breeding, in which conception fails after repeated inseminations is a major problem in the dairy industry that leads to economic losses. To examine the role of stress under this condition, this study was conducted to evaluate cortisol and blood urea nitrogen (BUN) levels in fertile and repeat-breeder Holstein-friesian crossbred cows in smallholder farms. **Materials and Methods:** Thirty cows were divided into two groups: Fertile and repeat-breeder cows, with 15 cows per group. Blood samples were collected from the caudal vein at night, 8 h after feeding, once in the oestrus phase during two oestrus cycles and cortisol levels were analyzed by using an enzyme-linked immunosorbent assay with a commercial kit. The BUN was analyzed by the urease and glutamate dehydrogenase method. **Results:** Significant differences in cortisol and BUN levels were observed between fertile and repeat-breeder cows. Cortisol levels were higher in the repeat-breeder group (6.860±1.427 ng mL^{-1}) compared to those in the fertile group (3.145±1.103 ng mL^{-1}). Furthermore, BUN levels were also higher in repeat-breeder cows (31.45±4.70 mg dL^{-1}) than in fertile cows (27.30±2.69 mg dL^{-1}). **Conclusion:** Cortisol and BUN levels above the normal range may contribute to repeat breeding in Holstein-friesian crossbred cows.

Key words: Cortisol, repeat breeding, blood urea nitrogen, holstein-friesian crossbred cows, repeat-breeder cows

Citation: Diah Tri Widayati, Adiarto, Budi Prasetyo Widyobroto and Yustina Yuni Suranindyah, 2019. Cortisol and blood urea nitrogen profiles in fertile and repeat-breeder holstein-friesian crossbred cows. Pak. J. Biol. Sci., 22: 356-360.

Corresponding Author: Diah Tri Widayati, Laboratory of Animal Physiology and Reproduction, Department of Animal Genetic and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, 55281 Bulaksumur, Yogyakarta, Indonesia Tel: +62 (274) 513363

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

Low fertility of repeat-breeder cows leads to substantial economic losses in the cattle industry, along with reduced milk production, increased calving intervals and also increased culling rates and reduction in livestock fertility has been partially caused by stress[1,2]. Previous studies have reported that an elevation in the level of glucocorticoids, such as; cortisol titres associated with chronic stress, can lead to reproductive failure[3,4]. As such, stress is considered to be related to the reproductive efficiency in cattle and other types of livestock. In response to stress, the hypothalamic-pituitary-adrenal (HPA) is activated. Neurological responses in the brain are then transmitted and hormonal responses from the hypothalamus are stimulated[4]. Corticotropin-releasing factor (CRF) has been shown to release from the hypothalamus, stimulating the pituitary to release adrenocorticotropic hormone (ACTH) into the blood, which in turn stimulates the adrenal gland to synthesize cortisol[4]. Cows secrete glucocorticoid hormones, such as; cortisol and corticosterone, from the adrenal gland in response to stress. Such excessive cortisol production has been found to have an adverse effect on ovum development and ovulation[3,5]. In addition, glucocorticoids could suppress the response of luteinizing hormone (LH) to gonadotropin releasing hormone (GnRH), thereby suppressing the production of oestrogen and progestin, resulting in ovulation inhibition (obstructed ovulation)[6].

A nutritional imbalance or deficiency has been proposed as a cause of repeat breeding, which can be detected by blood metabolite analysis. Blood plasma urea is a common indicator that can be used to evaluate rumen nitrogen metabolism. Blood plasma urea in ruminants is often used to determine protein availability for detecting potential protein level imbalances that could disrupt the secretion of gonadotropin[6,7]. The blood urea limit for normal reproduction has been reported to be 19 mg dL^{-1}; cows with blood urea levels greater than 20 mg dL^{-1} and milk urea concentrations of more than 18 mg dL^{-1} have been associated with pregnancy failures after artificial insemination[4,8]. Increased urea from high-protein feeds could directly reduce fertility by disrupting progesterone in micro-uterine conditions[9-12].

Holstein-friesian crossbred cows are an important for source of animal protein and an importance source of income for farmers. These cows are docile and adaptable to environment. Thus, the objective of the present study were to measure the cortisol and blood urea nitrogen (BUN) concentrations in repeat-breeder and fertile Holstein-friesian crossbred cows, to evaluate their utility as a marker for the clinical diagnosis of repeat breeding.

MATERIALS AND METHODS

Fertile and repeat-breeder cows: The study design was approved by the Animal Ethics Committee of Universitas Gadjah Mada (Yogyakarta, Indonesia). The research subjects included 30 head cows in healthy condition weighing 400-450 kg that were divided into 2 equal groups: Fertile or repeat-breeder cows. Blood samples of the cows were obtained from smallholder farmers in several areas of Sleman district, Yogyakarta, Indonesia. Cortisol and BUN analysis were carried out at the Integrated Research Laboratory, Universitas Gadjah Mada and the entire study was conducted between May and November, 2018. The cows received feed comprising 40-45 kg of grass and 6-8 kg of concentrate per head. Forage was provided to the animals in the morning and evening, while the concentrate was provided in the afternoon. Dietary nutrients were listed in Table 1.

Blood sampling: Blood samples were taken once in the oestrus phase during two oestrus cycles. Blood samples were collected through the dairy caudal vein using a vacuum holder that was attached to a 21G venoject needle (Terumo, Tokyo, Japan). Fresh blood samples were then centrifuged at 3500 rpm (1372 g) for 15 min to obtain blood plasma. The plasma was then transferred to a microtube using a 1 mL syringe. Blood plasma samples were analyzed for hormones using a commercial enzyme-linked immunosorbent assay cortisol kit (DRG, Germany). The BUN level analysis was conducted with reference to diaSys clinical chemical procedures (DiaSys Diagnostic Systems, Holzheim, Germany) using the urease and glutamate dehydrogenase method (Urease-GLDH, Kinetic UV kit).

Data analysis: Cortisol and BUN were expressed as Mean±standard deviation and analyzed by using a one-way analysis of variance (ANOVA) to compare means between the 2 groups. All statistical analyses were performed by using the Statistical Program for the Social Sciences (SPSS) version 16.0 (IBM, Chicago, IL, USA). Probability at $p \leq 0.05$ was considered statistically significant.

RESULTS

Significant differences in cortisol and BUN profiles were observed between repeat-breeder and fertile Holstein-friesian crossbred cows (Table 2). Cortisol levels were significantly higher ($p \leq 0.05$) in the repeat-breeder group compared to

Table 1: Nutrient contents of Holstein-friesian crossbred diets

	Dry matter		
Type of feed	Crude protein (%)	Ether extract (%)	Crude fiber (%)
Elephant grass	13.05	3.84	30.62
Concentrate	12.76	5.37	17.11
King grass	11.68	1.70	32.49
Corn stalk	9.20	2.30	25.70
Rice straw	4.04	0.53	31.62

Table 2: Cortisol and blood urea nitrogen levels (mean±standard deviation) for fertile and repeat-breeder Holstein-friesian crossbred cows

Cows	Cortisol hormone (ng mL^{-1})	Blood urea nitrogen (mg dL^{-1})
Fertile	3.145±1.103[a]	27.30±2.69[a]
Repeated breeder	6.860±1.427[b]	31.45±4.70[b]

[a,b]Means with different superscripts within a row differ significantly ($p<0.05$)

those in the fertile group. In addition, BUN levels were significantly higher ($p\leq0.05$) in repeat-breeder cows than in fertile cows. Furthermore, BUN levels were high in both fertile and repeat-breeder Holstein-friesian crossbred cows.

DISCUSSION

Cortisol hormone levels in repeat-breeder cows were higher than those of fertile cows. These results were found to exceed the normal range (3.8-4.4 ng mL^{-1})[13] indicated that cortisol likely had an effect on reproduction failure and repeat-breeder events[6]. Repeat-breeding in cows might be caused by several factors such as; fertilization failure or early embryo death. Fertilization failure could be caused by a damaged ovum, delayed ovulation, poor-quality semen or abnormalities in the reproductive organs. Early embryo death occurred when the embryo has cytogenetic abnormalities, unfavourable uterine conditions due to an imbalance in the maternal hormonal system, environmental stress or due to immunological factors[14,15].

Stress releases adrenocorticotropic hormone (ACTH) from the anterior pituitary that stimulated the release of cortisol from the adrenal cortex, thereby inhibiting the release of luteinizing hormone (LH), which can delay or prevent ovulation[16,17]. Specifically, LH has been shown to play a role in releasing eggs from the follicle and LH deficiency has been shown to cause follicles to fail ovulation, resulting in larger-sized follicles than the normal[18,19]. Repeat-breeder cows that experience stress during oestrus exhibit increased secretion of ACTH, which could affect LH hormonewaves[15,18]. In addition, high cortisol concentrations affect GnRH in the hypothalamus, leading to a decline in LH production in the follicular phase[17,18], ultimately preventing ovulation in the oestrus cycle[19,20]. In addition, high levels of cortisol could decrease the production and performance of progesterone from the adrenal gland[4,20-22], which could lead to repeat-breeding events in livestock due to the inability of the body to maintain pregnancy, leading to the death of the growing embryo.

In cows, elevated concentrations of plasma urea and ammonia negatively affect the quality of the uterine fluid due to increased ammonia concentrations, which, along with urea have direct and toxic effects on the endometrium and adverse effects on the mRNA expression levels of endometrial fertility-related genes[9,10]. A high ammonia content in the uterine fluid results from amino acid metabolism and in particular, the utilization of glutamine for energy[11]. Ammonia that enters the uterine lumen increased the luteal phase and exacerbated the negative effects of urea on pH and other secretions[12], leading to reduced fertility.

Urea that circulates in blood vessels can be measured as urea nitrogen in a fraction of blood plasma or serum and is often expressed as the BUN value[12]. In the present study, BUN levels in repeat-breeder dairy cows were higher than those in fertile cows suggested a likely influence of BUN on the incidence of repeat breeding[23]. A previous study showed that a high concentration of urea was associated with impaired fertility, decreased available energy, environmental pollution and economic losses[22]. In addition, blood urea levels of more than 19 mg dL^{-1} led to a 20% reduction in pregnancy rates[12]. The BUN levels in this study were higher than normal for both the fertile and repeat-breeder groups. High levels of urea in the blood could enter the reproductive tract of the uterus and affect uterine pH, thereby altering uterine micro-conditions and decreasing fertility rates and ultimately leading to increased likelihood of repeat breeding[1,8,10]. Furthermore, Butler[24] found that cows with elevated blood urea levels greater than 20 mg dL^{-1} had increased follicular fluid and oocytes of virgin dairy cows with high ammonia-to-urea ratios showed lower cleavages than those of low-yielding ammonia cows.

Cows fed diets high in protein are likely to have increased blood urea concentrations and a high urea content in the uterine fluid. The high blood urea content could lead to a change in uterine pH, greatly influencing the hormones oestradiol and progesterone. Moreover, high urea in the uterine fluid had shown to increase the secretion of PGF2α and PGE2 hormones, which hinders the creation of optimal conditions for embryonic development[24]. The findings of this study serve as a baseline reference for future investigations with repeat-breeder Holstein-friesian crossbred cows and demonstrated that cortisol and urea

nitrogen concentrations can serve as useful markers for identifying repeat-breeder cows. Future research must be focus on these two aspects in order to obtain more comprehensive information about repeat-breeder cases in Holstein-friesian cows.

CONCLUSION

Cortisol and blood urea nitrogen levels above the normal range contribute to repeat-breeder behaviour in Holstein-friesian crossbred cows.

SIGNIFICANCE STATEMENT

This study discovered that cortisol and blood urea nitrogen levels differed significantly between repeat-breeder and fertile cows, demonstrating their feasibility as useful markers for evaluating the fertility of cattle and other livestock. This study demonstrates that cortisol and urea nitrogen levels above the normal range likely contribute to repeat-breeder occurrence in Holstein-friesian crossbred cows. This study will help researchers to uncover the critical areas of repeat breeding that have not been explored to date. Thus, cortisol and urea nitrogen concentrations profiling could be used as a diagnostic tool for repeat breeding, establishing a new paradigm in the field.

ACKNOWLEDGMENT

We would like to thank the Faculty of Animal Science, Universitas Gadjah Mada, for partially sponsoring the research through the HibahPasca Grant (2360/J01.1.25/PASCA/2018).

REFERENCES

1. Gustafsson, H. and U. Emanuelson, 2002. Characterisation of the repeat breeding syndrome in Swedish dairy cattle. Acta Vet. Scand., 43: 115-125.
2. Tsigos, C., I. Kyrou, E. Kassi and G.P. Chrousos, 2016. Stress, Endocrine Physiology and Pathophysiology. In: Endotext, Feingold, K.R., B. Anawalt, A. Boyce, G. Chrousos and K. Dungan *et al.* (Eds.), MDText.com, Inc., South Dartmouth (MA).
3. Prasad, S., M. Tiwari, A.N. Pandey, T.G. Shrivastav and S.K. Chaube, 2016. Impact of stress on oocyte quality and reproductive outcome. J. B iomed. Sci., 23: 36-40.
4. Lucassen, P.J., J. Pruessner, N. Sousa, O.F. Almeida and A.M. van Dam *et al.*, 2014. Neuropathology of stress. Acta Neuropathol., 127: 109-135.
5. Shugaba, A.I., J.O. Hombola and S.A. Ojo, 2010. The effects of induced physical and oxidative stress on the cortisol levels of female wistar rat. J. Med. Trop., 12: 72-75.
6. Anwar, R., 2005. Fungsi kelenjar adrenal dan kelainannya. Subbagian Fertilitas Dan Endokrinologi Reproduksi Bagian Obstetri Dan Ginekologi Fakultas Kedokteran Universitas Padjajaran, Bandung.
7. Budiasa, M.K. and T.G.O. Pemayun, 2015. The blood glucose profile and plasma urea in pospartum anestrus of Bali cattle. Buletin Vet. Udayana, 7: 48-52.
8. Hwang, S.Y., M.J. Lee and P.W.S. Chiou, 2000. Monitoring nutritional status of dairy cows in Taiwan using milk protein and milk urea nitrogen. Asian-Aust. J. Anim. Sci., 13: 1667-1673.
9. Tilbrook, A.J., A.I. Turner and I.J. Clarke, 2002. Stress and reproduction: Central mechanisms and sex differences in non-rodent species. Stress: Int. J. Biol. Stress, 5: 83-100.
10. Gunaretnam, I., T. Pretheeban and R. Rajamahendran, 2013. Effects of ammonia and urea *in vitro* on mRNA of candidate bovine endometrial genes. J. Anim. Reprod. Sci., 141: 42-51.
11. Weiner, D., W.E. Mitch and J.M. Sands, 2015. Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin. J. Soc. Neprol., 10: 1444-1458.
12. Butler, W.R., 2005. Relationships of dietary protein and fertility. Adv. Dairy Technol., 17: 159-168.
13. Yoshida, C. and T. Nakao, 2005. Response of plasma cortisol and progesterone after ACTH challenge in ovariectomized lactating dairy cows. J. Reprod. Dev., 51: 99-107.
14. Karunakaran, M., S.K. Das, E.B Chakurkar and N.P. Singh, 2012. Anoestrus and repeat breeding in dairy cows causes and management. Extension Folder No. 49. ICAR Research Complex for Goa, India.
15. Singh, B., F. Saravia, R. Bage and H. Rodriguez-Martinez, 2005. Pregnancy rates in repeat-breeder heifers following multiple artificial inseminations during spontaneous oestrus. Acta Vet. Scand., 46: 1-12.
16. Etim, N.A.N., E.E.A. Offiong, M.A.D. Udo, M.E. Williams and E.I. Evans, 2013. Physiological relationship between stress and reproductive efficiency. Agric. Biol. J. North Am., 4: 600-604.

17. Von Borell, E., H. Dobson and A. Prunier, 2007. Stress, behaviour and reproductive performance in female cattle and pigs. Hormones Behav., 52: 130-138.
18. Silvia W.J., A.S. McGinnis and T.B. Hatler, 2005. A comparison of adrenal gland function in lactating dairy cows with or without ovarian follicular cysts. J. Reprod. Biol., 5: 19-29.
19. Tuasikal, B.T., T. Totti and K. Ratnawati, 2004. Studi gangguan reproduksi sapi perah dengan teknik Radio Immunoassay (RIA) progesteron. Proceeding of the Risalah Seminar Ilmiah Penelitian dan Pengembangan Aplikasi Isotop dan Radiasi, February 17-18, 2004, Jakarta, pp: 1-7.
20. Whirledge, S. and J.A. Cidlowski, 2010. Glucocorticoids, stress and fertility. Minerva Endocrinol., 35: 109-125.
21. Bage, R., H. Gustafsson, B. Larsson, M. Forsberg and H. Rodriguez-Martinez, 2002. Repeat breeding in dairy heifers: Follicular dynamics and estrous cycle characteristics in relation to sexual hormone patterns. Theriogenology, 57: 2257-2269.
22. Setiadi, A., B.P. Widyobroto and B. Rustamaji, 2003. Blood plasma glucose and blood plasma urea concentartion on Friesian Holstein crossbreed cows were given by diets of rumen's undegradable protein level. J. Indon. Trop. Anim. Agric., 28: 211-217.
23. Widayati, D.T., S. Bintara, I. Natawihardja and D. Maharani, 2018. Blood biochemical profile in fertile and repeat breeder ongole cross breed cows. Pak. J. Biol. Sci., 21: 166-170.
24. Butler, W.R., 1998. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci., 81: 2533-2539.