



Haematological parameters, endocrinological profiles, antioxidant and oxidative stress profiles of repeat breeding crossbred cows in Andaman and Nicobar Islands

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ABSTRACT

The present study was carried out to assess the effect of repeat breeding syndrome (RBS) on haematological, antioxidant, oxidative stress and endocrinological profiles in crossbred cows under tropical island ecosystem of Andaman and Nicobar Islands. Each of the twelve number of cows was selected (which were affected with repeat breeding syndrome) as Group 2 and normal cyclic breeding cows as Group 1. These cows were maintained under similar type of managemental conditions. Haematological profiles, antioxidant and hormone profiles were estimated by automatic blood analyser and commercial diagnostic kits, respectively. The result revealed that the crossbred cows with RBS suffered severe anaemia. The RBS affected animals showed significantly lower level of antioxidant profiles and significantly higher malondialdehyde (MDA) than the unaffected animal groups. Similarly, endocrinological profiles revealed that the RBS affected animals had significantly higher level of cortisol, prolactin and lower level of 17 β -estradiol (E2), progesterone (P4), follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroxine (T4) and insulin like growth factor-1 (IGF-1) than the unaffected crossbred cows. The study concluded that the RBS was due to anaemia, lack of antioxidants, overproduction of free radicals and disturbances of endocrinological profiles in crossbred cows of Andaman and Nicobar Islands.

Keywords: Andaman and Nicobar islands, Antioxidants, Blood, Crossbred cows, Hormone, Island ecosystem, Repeat breeding syndrome

Repeat breeder cow is defined as that cow which does not become pregnant even for more than third or subsequent breeding or remain infertile after numerous services. Repeat breeding is generally characterised by long calving interval (18–24 months) (Purohit 2008), low conception rate (<40%) and high service per conception (>3) (Rustamadji *et al.* 2007). Repeat breeding syndrome is one of the most important problems, significantly affecting the reproductive efficiency and is a main cause of economic loss to the dairy herds (Yusuf *et al.* 2010) in terms of wastage of semen, increasing intervals to conception, insemination costs, increased culling and replacement costs, and loss of genetic gain through increased generation intervals and reduced fertility rate (Garcia-Ispuerto *et al.* 2007). Other potential causes of RBS are poor quality of semen and inadequate insemination technique (Hallap *et al.* 2006), uterine and/or cervical/vaginal infections (Moss *et al.* 2002), endocrine disorders (Lopez-Gatius *et al.* 2004), ovulation failures

(Silvia 1994), obstructed oviducts, defective ova, anatomical defects of the reproductive tract (Silvia 1994) and early embryonic death (Bage *et al.* 2002).

Blood is one of the important constituents in the body, which maintains the physiological equilibrium. Thus any disturbance in this equilibrium maintenance will lead to disease or pathological condition, which in turn can be known from the changed haematological, endocrinological and biochemical parameters. Kumar *et al.* (1991) carried out haematological studies to correlate with reproductive performance of dairy cattle. Kumar and Sharma (1991) has reported lower values of TRBC, Hb and PCV in repeat breeder animals. It was also reported that RBS cows have increased level of MDA and nitric oxide, and decreased catalase (CAT), superoxide dismutase (SOD), ascorbic acid, reduced glutathione (GSH) and total antioxidant capacity (TAC) (Ahmed *et al.* 2010).

Follicle stimulating hormone (FSH) is an important hormone in initiating reproductive activity. FSH stimulates the growth, development and function of the follicle (Dierich *et al.* 1998). A linear decrease in serum LH occurred in cows throughout the onset of pregnancy, whereas serum LH remained unaffected in non-pregnant

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cows (Jordan and Swanson 1979). LH is responsible for causing the follicle to rupture (ovulate) by acting on the ovary which will release the egg. Following ovulation, the LH also stimulates the development of the corpus luteum (CL). Progesterone is a steroid hormone produced by the CL on the ovary (Bulman and Lamming 1978). High levels of progesterone are produced which helps to maintain pregnancy while cows become pregnant by inhibition of GnRH release. If the cow does not become pregnant, the CL begins to degenerate and the level of progesterone abruptly decreases on days 18 or 19 and allowing the cow to return back to estrus on day 21–23 (Ulberg and Lindley 1960). Estrogen (Estradiol) plays a critical role in most metabolic, behavioural and morphological requirements, which are essential in reproduction of the female animals (Henricks *et al.* 1972). Therefore, knowledge on haematological, endocrinological and biochemical values is useful in diagnosing various pathological, endocrinological and metabolic disorders, which can adversely affect the productive and reproductive performance of cows, which in turn leads to economic loss to the dairy farmers (Perumal *et al.* 2013a, b). There is no information regarding the haematological, endocrinological, and antioxidant and oxidative profiles in repeat breeding cows in comparison with normal cyclic cows at oestrous stage in the crossbred cows of Andaman and Nicobar Islands. Therefore, the present study has been designed to analyze various haematological, endocrinological, and antioxidant and oxidative profiles in repeat breeding cows at stage of oestrous in comparison with normal cyclic crossbred cows of Andaman and Nicobar Islands with an idea to understand the abnormal condition of reproduction, RBS and to rectify the same.

MATERIALS AND METHODS

Location: Present study was conducted at Guptapara, Indira Nagar and Chouldhuri of South Andaman district, Andaman and Nicobar Islands. Average maximum and minimum temperature were 30.1 and 23°C, respectively. Relative humidity was in range of 82–94% and annual rainfall is >3100 mm spread over 8 months. Crossbred cows were maintained in the semi-intensive system where they were allowed for grazing from 07:00 to 12:00 h.

Experimental animals: Twenty four apparently healthy adult crossbred cows of 4–6 years of age with good body condition (score 5–6) were selected from the cattle herd in these villages. They were maintained under hygienic managemental conditions. Prophylactic measures like deworming and vaccination were done as per the farm schedule.

Experimental procedure: Twelve crossbred cows with RBS and twelve normal cyclic cows were selected on the basis of their reproductive history obtained from their records. Crossbred cows that did not become pregnant even for more than three insemination or breeding were selected in repeat breeding syndrome (RBS) and animals that were coming regularly to heat and conceived within 1–2 cycle

were selected as normal cyclic to conduct the study. Current status of reproductive organs of all animals in the study was also examined and verified by per rectal examination.

Hematological analysis: Approximately 10 mL of jugular blood sample was collected from each experimental animal in 15 mL sterile polypropylene centrifuge tube containing heparin (20 IU/mL of blood) as anticoagulant. Hematology was carried out using automatic veterinary scan hematological analyzer directly after the samples were received by the research laboratory within 30 min after samples were collected. Hematological profiles such as total red blood cells (TRBC), haemoglobin (Hb), and erythrocytic sedimentation rate (ESR), packed cell volume (PCV), total white blood cell (TWBC), lymphocytes, monocytes, neutrophils, eosinophils and platelets were estimated.

Biochemical analysis: Plasma was separated in refrigerated centrifuge at 3500×g at 4°C for 10 min and stored in aliquots at –20°C until analysis. Separated plasma was aliquoted into small aliquots in micro cryo-tubes and placed frozen at –20°C until further analysis of biochemical and antioxidant profiles with commercial diagnostic kits. Antioxidant profiles such as TAC, GSH, SOD and CAT were estimated with commercial available kit (Cayman, USA) and oxidant profile such as MDA were estimated as per method described by Shah *et al.* (1989). Endocrinological profiles such as FSH, LH, E2, P4, cortisol, T4, IGF-1 and prolactin were measured by commercial available diagnostic kits (Cayman, USA). Protocol for these assays was performed as per manufacturer's instructions.

Statistical analysis: Statistical analysis of the data was done as per standard procedures. Student t-test was conducted to assess the significant difference between the with RBS affected and normal cyclic crossbred cows (Statistical Analysis System for Windows, SPSS (Version 10) Inc., Chicago, Illinois, USA). Graphs presented the non-transformed data. Pearson's correlation coefficient was estimated between the experimental parameters. Differences with values of $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

RBS affected animals had significantly lower TRBC, Hb, ESR, PCV and TWBC ($p < 0.05$) @ 13.83, 15.57, 23.54, 15.56 and 12.95%, respectively than in normal cyclic non-repeater cows (Fig. 1). Similarly neutrophil, lymphocyte, monocyte, eosinophil and platelet (unaffected: 323.76 ± 8.64 ; affected: 386.95 ± 9.32) were significantly higher in RBS affected than in unaffected cows @ 20.63, 11.29, 26.77, 23.34 and 8.67%, respectively (Fig. 2). Endocrinological profiles revealed that FSH, LH, E2, P4, IGF-1 and T4 was lower and cortisol and prolactin were higher significantly ($p < 0.05$) in RBS animals than in normal cyclic non-repeater cows @ 28.71, 62.64, 53.64, 22.83, 15.80, 16.56 and 24.26 and 31.80%, respectively (Fig. 3). Similarly antioxidant profiles such as TAC, CAT, GSH and SOD were lower and MDA concentration was higher significantly ($p < 0.05$) in

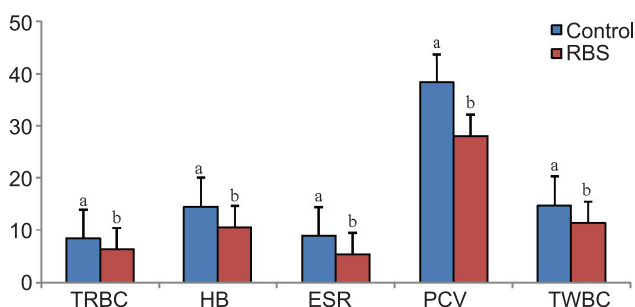


Fig. 1. Hematological profiles in repeat breeding syndrome (RBS) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($p < 0.05$) difference between the control and RBS affected cows. TRBC, Total red blood cell ($\times 10^6/\text{mm}^3$); HB, haemoglobin (g/dl); ESR, erythrocyte sedimentation rate (mm/hr); PCV, packed cell volume (%) and TWBC, total white blood cell ($\times 10^3/\text{mm}^3$). $n = 6$ cows for control and RBS affected cows.

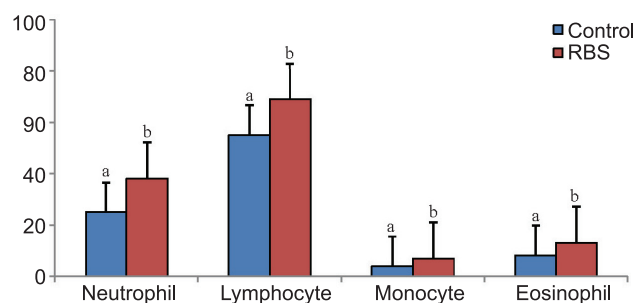


Fig. 2. Differential leukocyte count in repeat breeding syndrome (RBS) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($p < 0.05$) difference between the control and RBS affected cows.

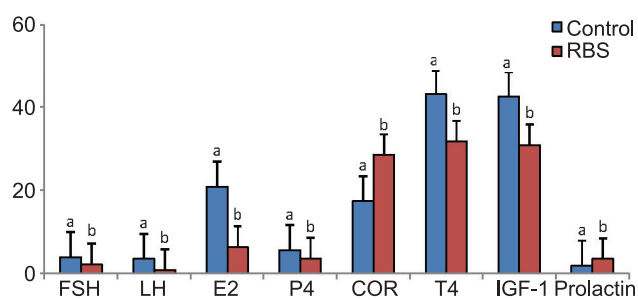


Fig. 3. Endocrinological profiles in repeat breeding syndrome (RBS) affected animals (mean±SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($p < 0.05$) difference between the control and RBS affected cows. FSH, Follicle stimulating hormone (mIU/ml); LH, luteinizing hormone (mIU/ml); E2, 17 β -estradiol (pg/ml); P4, progesterone (ng/ml); COR, cortisol (nmol/L) and T4, thyroxine (nmol/L); IGF-1, insulin like growth factor-1 and prolactin. $n = 6$ cows for control and RBS affected cows.

RBS cows than in cyclic non-repeater cows @ 11.08, 11.03, 16.67, 16.12 and 10.67%, respectively (Fig. 4). These hematological parameters showed significant ($p < 0.05$) positive correlation with FSH, LH, E2, P4, T4, IGF-1 and antioxidant profiles whereas significant ($p < 0.05$) negative

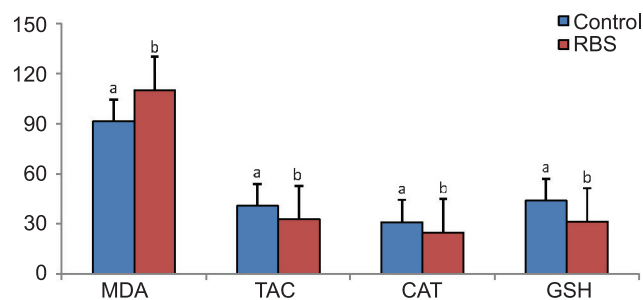


Fig. 4. Biochemical profiles in repeat breeding syndrome (RBS) affected animals (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($p < 0.05$) difference between the control and RBS affected cows. MDA, Malondialdehyde (nmol/L); TAC, total antioxidant capacity (nmol/ μ L); CAT, catalase (nmol/min/L); GSH, glutathione (nmol/min/L). $n = 6$ cows for control and RBS affected cows.

correlation was observed with MDA, cortisol and prolactin.

Significantly higher blood profiles in normal cyclic than in repeat breeder cows at oestrous is due to the fact that RBC is responsible for transport of O_2 to the tissues leading to increased consumption of O_2 in reproductive tract, which in turn, help in better energy utilization and contraction of smooth muscle that leads to successful fertilization and higher conception rate in normal cyclic cows. TRBC was higher in oestrous cow which is due to hyperactivity and excitement, arising out of high level of estrogen from graffian follicle (Coles 1986). Similar observation was noted in buffaloes (Kumar *et al.* 1991) and heifers (Kumar *et al.* 1992) where blood profiles were higher in normal cyclic than in repeat breeder cows. Kumar and Sharma (1991) reported significantly lower TRBC, Hb and PCV in repeat breeder animals than in normal animals. Ganong (2001) studied that high concentration of Hb, PCV and RBC were desirable physiological characters for efficient transport of O_2 and CO_2 . Thus, cows with higher concentration of Hb, PCV and RBC were more economical as a result of improved reproductive efficiency. In fertile oestrous, level of Hb was higher as compared to non-fertile oestrous (Kumar and Sharma 1991, Perumal *et al.* 2013a, Perumal *et al.* 2013b). The ESR value was significantly higher ($p < 0.05$) in normal cyclic oestrous as compared to repeat breeding oestrous cows and this indicated that the normal oestrous cows are having higher RBC count as compared to repeat breeder oestrous cows and have very rapid sedimentation rate than in repeat breeder cows. TWBC was significantly higher in normal cyclic than in repeat breeder cows as it may be due to movement of excessive amount of leucocytes into uterus at the time of oestrous for phagocytosis process. In states of excitement, exercise and strange surroundings, there is also leukocytosis (neutrophilia), since adrenaline liberated during these states which mobilizes the marginal neutrophil pool cells (Sastri 1989). A wide variety of microbes have been isolated from repeat breeding cows including *Salmonella*, *Staphylococcus*, *Corynebacteria*, *Pseudomonas* and *Escherichia coli* (Prajapati *et al.* 2006). Often these

organisms are present collectively with anaerobic bacteria like *Fusiformis necrophorus* and Bacteroids species (Azawi 2010) and rarely fungi are also reported. Therefore, the differential leucocyte differential count has been increased in the RBS affected cows in the present study.

Higher E2 stimulated higher LH, which is needed for normal cycle and increased the conception rate whereas in the RBS cows, short E2 exposure and low LH before its peak was reported and this indicated the RBS cows express short proestrus (Evans *et al.* 1997). Furthermore, a low pre-surge LH concentration is also a reason for a slightly longer estrus-to-ovulation interval in the RBS cows than in the normal cycling cows (31.5 vs 28.9 h) (Sood *et al.* 2015). Moreover, the cows with higher milk yield had a lower serum E2 concentration on the day of estrus and greatly reduced estrus duration compared with those with lower yields (Lopez *et al.* 2004). Similar result was observed in the present study that the RBS cows had significantly lower level of FSH, LH, P4 and E2.

Increased cholesterol is required for steroidogenesis under influence of LH surge and its level was lower in RBS cows than in normally cycling cows (Madumeet Singh and Pant 1998) which in turn affect the biosynthesis of androstenedione, progesterone and estrogen. Thus the level of these steroid hormones was lower in RBS cows. It was also reported that RBS cows have significantly lower total protein concentration than in normally cycling cows (Shiraz Khan *et al.* 2010) which leads to deficiency of certain amino acids required for the biosynthesis of gonadotropins and gonadal hormones (Vohra *et al.* 1995). Therefore, peptide or protein hormone level was lower in the RBS affected cow which also induces disturbances in the reproductive hormones leading to inactive ovaries (Roberts 1971). Dietary energy restriction may decrease IGF-I concentrations, causing altered follicular growth and development of a sub-functional CL (Burns *et al.* 1997). In RBS, cows may suffer deficiency of nutrients or unable intake or unable to utilize the nutrients that lead to loss of energy leads to decreased level of IGF-1 in the RBS cows.

Lower plasma progesterone profile was reported in RBS cows even under all the effective synchronization protocols which could be due to ovulatory failure and/or luteal insufficiency and RBS cow carries irregular concentration of progesterone during different stages of the estrous cycle which prevents the conception and development of embryo (Prajapati *et al.* 2018).

One of the factors influencing cattle fertility is stress. Cortisol is a hormone secreted by adrenal glands in response to stress. Hollenstein *et al.* (2006) reported that stress increases the blood cortisol level and is able to delay or block LH spikes, affecting estrous expression with reduced level of blood estradiol. The high cortisol production may affect the reproductive system of female cows leading to impaired ovum and ovulation development (Shubaga *et al.* 2010). Therefore, it is necessary to observe cortisol hormone in the female cows which is suffering from RBS. Moreover, cortisol in cattle with RBS was higher and it inhibits the

secretion of GnRH and LH from the hypothalamic-pituitary system, resulting in the inhibited growth of follicles as well as reduced level of estrogen and progesterone. Stress in the form of heat, pregnancy and milk production increased the formation of reactive oxygen and nitrogen species (ROS and RNS). These ROS and RNS increases the lipid peroxidation and apoptosis and infertility (Agrawal *et al.* 2003) and the biological outcome of infertility is due to these ROS and RNS affecting the folliculogenesis and steroidogenesis (Agrawal *et al.* 2005).

Oxidant/antioxidant markers in serum of repeat breeding cows showed increased MDA and nitric oxide (NO) and decreased concentration of CAT, SOD, ascorbic acid, GSH and TAC (Ahmed *et al.* 2010). MDA is a byproduct of lipid peroxidation and used as an index of the rate of tissue reaction chain and also MDA is used as an indicator of oxidative stress (OS) in cells and tissues. It is well known that healthy animal has a well balanced reactive oxygen species (ROS) and antioxidants and when the balance is disrupted towards an overproduction of ROS leads to occurrence of OS. ROS have ability to induce the pathological processes in the female gonads, accessory glands and reproductive tract which affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy leading to longer calving to conception interval, poor conception rate and fertility failure (Kumar *et al.* 2012). Repeat breeding syndrome has considerably increased due to increased MDA and NO and decreased CAT, SOD, ASCA, GSH and TAC in dairy animals (Pande *et al.* 2013). SOD is present in the ovarian tissue which induces transcription factor for steroidogenesis (Suzuki *et al.* 1999). Similarly, in the present study, significantly lower concentration of antioxidant profiles and significantly increased level of oxidative stress profile were observed in RBS cows than in normal cyclical cows.

Repeat breeding syndrome is one of the most prevalent reproductive disorders in Andaman and Nicobar Islands. Biochemical parameters and hematological profiles in RBS affected cows are characterized by significant decrease in RBC, Hb, PCV, FSH, LH, P4, E2, T4, IGF-1 and antioxidants whereas there was an increase in TWBC, neutrophil, eosinophil, lymphocyte, ROS, cortisol and prolactin.

REFERENCES

- Agarwal A, Gupta S and Sharma R. 2005. Oxidative stress and its implications in female infertility—A clinician's perspective. *Reproductive BioMedicine* **11**(5): 641–50.
- Agrawal A, Sahel R A and Bedaiwy M A. 2003. Role of reactive oxygen species in the pathology of human reproduction. *Fertility and Sterility* **79**: 829–43.
- Ahmed W M, El-khadrawy H H, Emtenan M, Ali A H and Shalaby S A. 2010. Clinical perspective of repeat breeding syndrome in buffaloes. *Journal of American Science* **6**: 11.
- Azawi A O. 2010. Uterine infections in buffalo cows: A review. *Buffalo Bulletin* **29**: 154–71.
- Bage R, Gustafsson H, Larsson B, Forsberg M and Rodriguez-Martinez H. 2002. Repeat breeding in dairy heifers: follicular

- dynamics and estrous cycle characteristics in relation to sexual hormone patterns. *Theriogenology* **57**: 2257–69.
- Bulman D C and Lamming G E. 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. *Journal of Reproduction and Fertility* **54**(2): 447–58.
- Burns P D, Spitzer J C and Henricks D M. 1997. Effect of dietary energy restriction on follicular development and luteal function in non-lactating beef cows. *Journal of Animal Science* **75**: 1078–86.
- Coles E H. 1986. *Veterinary Clinical Pathology*. 4th Edn. W. B. Saunders Company, London, U.K.
- Dierich A, Sairam M R, Monaco L, Fimia G M, Gansmuller A, LeMeur M and Sassone-Corsi P. 1998. Impairing follicle stimulating hormone (FSH) signaling *in vivo*: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. *Proceedings of the National Academy of Sciences* **95**(23): 13612–17.
- Evans N P, Dahl G E, Padmanabhan V, Thrun L A and Karsch F J. 1997. Estradiol requirements for induction and maintenance of the gonadotropin releasing hormone surge: Implications for neuroendocrine processing of the estradiol signal. *Endocrinology* **138**: 5408–14.
- Garcia-Ispuerto I, Lopez-Gatius F, Santolaria P, Yaniz J L, Nogareda C and Lopez-Bejar M. 2007. Factors affecting the fertility of high producing dairy herds in northeastern Spain. *Theriogenology* **6**: 632–38.
- Hallap T, Nagy S, Jaakma U, Johannisson A and Rodriguez-Martinez H. 2006. Usefulness of a triple fluorochromes combination Merocyanine 540/Yo-Pro 1/Hoechst 33342 in assessing membrane stability of viable frozen-thawed spermatozoa from Estonian Holstein AI bulls. *Theriogenology* **65**: 1122–36.
- Henricks D M, Dickey J F, Hill J R and Johnston W E. 1972. Plasma estrogen and progesterone levels after mating, and during late pregnancy and postpartum in cows. *Endocrinology* **90**(5): 1336–42.
- Hollenstein K, Janett F, Bleul U, H Assig M, K Ahn W and Thun R. 2006. Influence of estradiol on adrenal activity in ovariectomized cows during acute stress. *Animal Reproduction Science* **93**: 292–302.
- Jordan E R and Swanson L V. 1979. Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. *Journal of Animal Science* **48**(5): 1154–58.
- Kumar A K, Dangi S S, Mahla A S, Rajoriya J S and Purohit G N. 2012. Role of nitric oxide in animal reproduction. *Theriogenology Insight* **2**: 137–44.
- Kumar R, Jindal R and Rattan P J S. 1991. Plasma hormonal profile during estrus cycle of Murrah buffalo-heifers. *Indian Journal of Animal Sciences* **61**(4): 382–85.
- Kumar R, Sharma T P and Rattan P J S. 1992. Hematological studies during estrous cycle in Murrah buffalo-heifers. *Indian Veterinary Journal* **69**: 894–897.
- Kumar S and Sharma M C. 1991. Level of haemoglobin and certain serum biochemical constituents in rural cows during fertile and non fertile oestrus. *Indian Veterinary Journal* **68**: 361–64.
- Lopez H, Satter L D and Wiltbank M C. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. *Animal Reproduction Science* **81**: 209–23.
- Lopez-Gatius F, Yaniz J L, Santolaria P, Murugavel K, Guijarro R, Calvo E and Lopez-Bejar M. 2004. Reproductive performance of lactating dairy cows treated with cloprostenol at the time of insemination. *Theriogenology* **62**: 677–89.
- Madumeet Singh and Pant H C. 1998. Blood biochemical profile of normal and repeat breeding cows in Himachal Pradesh. *Indian Journal of Animal Reproduction* **19**: 156–57.
- Moss N, Lean I J, Reid S W J and Hodgson D R. 2002. Risk factors for repeat-breeder syndrome in New South Wales dairy cows. *Preventive Veterinary Medicine* **54**: 91–103.
- Pande M, Das G K and Khan F A. 2013. Endometritis impairs luteal development, functions and nitric oxide and ascorbic acid concentrations in buffalo (*Bubalus bubalis*). *Tropical Animal Health and Production* **45**: 805–10.
- Perumal P, Chamuah J K, Krishanappa B, Vupru K and Khate K. 2013a. Retention of placenta in mithun crossbred cow (Phre) – A case report. *Veterinary World* **6**(13): 171.
- Perumal P, Vupru K, Khate K and Rajkhowa C. 2013b. Retention of placenta in mithun (*Bos frontalis*) cow – A case report. *International Journal of Livestock Research* **3**(2): 185–90.
- Prajapati A R, Dhama A J, Hadiya K K and Patel J A. 2018. Impact of different ovulation synchronization protocols on plasma profile of progesterone, protein and cholesterol in cyclic repeat breeder crossbred cows. *International Journal of Livestock Research* **8**(7): 91–100.
- Prajapati S B, Ghodasara D J, Prajapati K S, Purohit J H and Rani V R. 2006. Microbiological study of endometritis and its antibiotic spectra in repeat breeder buffaloes. *Indian Journal of Animal Reproduction* **27**: 69–71.
- Purohit G. 2008. Recent development in the diagnosis and therapy of repeat breeding cows and buffaloes. *CAB Rev Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **3**(63): 1–33.
- Roberts S J. 1971. *Veterinary Obstetrics and Genital Diseases*. 2nd Edn. CBS Publishers and Distributors New Delhi. pp 105
- Rustamadi B, Ahmadi K and Sutarno T. 2007. Kinerja usaha peternakan sapi perah rakyat sebagai tulang punggung pembangunan persusuan Nasional. Yogyakarta: Paper disampaikan pada Lokakarya Persusuan Nasional.
- Shah J K and Walker's A M. 1989. Quantitive determination of MDA. *Biochemistry and Biophysics Acta* **11**: 207–11.
- Shiraz Khan, Thangavel A and Selvasubramanian S. 2010. Blood biochemical profile in repeat breeding cows. *Tamilnadu Journal of Veterinary and Animal Sciences* **6**(2): 75–80.
- Shugaba A I, Hombola J O and Ojo S A. 2010. The effects of induced physical and oxidative stress on the cortisol levels of female Wister rats. *Journal of Medicine in Tropics* **12**: 72–75.
- Silvia W J. 1994. Embryonic mortality and repeat breeder cows. *Proceedings of the National Reproduction Symposium*, 27th Annual Conference of the American Association of Bovine Practitioners, Pittsburgh, PA, 151–160.
- Sood P, Zachut M, Dube H and Moallem U. 2015. Behavioral and hormonal pattern of repeat breeder cows around estrus. *Reproduction* **149**: 545–54.
- Ulberg L C and Lindley C E. 1960. Use of progesterone and estrogen in the control of reproductive activities in beef cattle 1. *Journal of Animal Science* **19**(4): 1132–42.
- Vohra S C, Dindorkar C V and Kaikini A S. 1995. Studies on blood serum levels of certain biochemical constituents in normal cycling and anestrus crossbred cows. *Indian Journal of Animal Reproduction* **16**: 85–87.
- Yusuf M, Rahardja D P and dan A L Toleng. 2015. Prospect of nutrition in-utero on improvement of reproductive performance in Bali cows kept under smallholder farms. *Journal of Advance Agricultural Technology* **22**(2): 151–55.