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## AN UNUSUAL CASE OF BUFFALO WITH ONLY TWO FUNCTIONAL TEATS IN ANDAMAN ISLANDS

S. Jeyakumar, Z. George, Kuntola Roy and A. Kundu

### ABSTRACT

A non-descript buffalo aged 4 years in the first lactation with one male calf of South Andaman, Andaman and Nicobar Islands was found to have only two teats with two well developed free quarters with the complete absence of the other two quarters and corresponding teats. The congenital anomaly observed only in the buffalo not, in her dam.

**Keywords:** Andaman, non-descript, buffalo, teats, abnormality

### INTRODUCTION

The normal buffalo udder usually has four quarters with four teats projecting ventrally from the two halves of the udder. The fore and hind quarters of each half are seldom equal in Indian buffaloes (Bhalerao, 1985). Congenital abnormalities in the bovine udder include many structural defects viz. fusion of front and hind teats, very small short teats, improperly placed teats, cut-up udders and supernumerary teats. In buffalo, the occurrence of congenital anomalies of the udder is rarely reported. In this present communication, a non-descript buffalo with two functional teats is discussed.

### CASE HISTORY, CLINICAL EXAMINATION AND DISCUSSION

In the present case, a non-descript buffalo aged 4 years in the first lactation with one male calf belonging to a farmer of South Andaman, Andaman and Nicobar Islands was found to have only two teats with two well developed free quarters with the complete absence of the other two quarters and corresponding teats. On clinical examination it was found that the two halves of the udder were of equal size and each half constituted a quarter. Each quarter had well developed ventrally projecting teats which were of equal size and shape and morphologically they were long and tubular in shape (Figures 1 and 2). The milk yield from each quarter / teat was almost equal, amounting to 1.5 Litre per day from each quarter. Pathan *et al.* (2007) reported a similar condition in a Marathadwadi buffalo; they found that in both the dam and the dead female calf the two hind quarters and corresponding teats were absent, indicating the congenital and hereditary nature of the condition. However, in the present case, the congenital anomaly was observed only in the buffalo, not in her dam. Vidya Sagar (2009) reported complete absence of teats in a Murrah buffalo from Gudiwada.

Each mammary gland is a separate entity draining by its own duct system, storage cistern and teat. The differentiation of the udder takes place



Figure 1. A she buffalo with two functional teats (Lateral View).



Figure 2. A she buffalo with two functional teats (Rear view).

in early embryonic period by the interruption of ventral milk lines of ectoderm forming mammary buds that develops into a separate mammary gland. This present condition is a very rare phenomenon which might be attributed to a congenital condition in which of only a pair of mammary glands are retained during embryonic development.

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ULTRASONOGRAPHY AND COMPUTERIZED RADIOGRAPHY AIDED DIAGNOSIS  
OF OESOPHAGEAL FOREIGN BODY OBSTRUCTION  
AND ITS TREATMENT IN A BUFFALO

**D.K. Tiwari, Mehraj u din Dar, S.K. Jhala, Aarti Pitroda, Nisha Joy,  
D.B. Patil, P.V. Parikh, B.G. Prajapati and C.L. Badgajar**

### INTRODUCTION

Oesophageal disorders are relatively uncommon in ruminants (Haven, 1990). Intraluminal blockade of the oesophagus, popularly known as choke, is the most common esophageal disease in large animals. Most ruminants which suffer from this condition are greedy feeders or have nutritional deficiencies, which makes them eat foreign bodies like pieces of leather, wool balls, polythene and rubber sheets, leading to oesophageal obstruction (Tyagi and Singh, 2004).

In ruminants, obstruction occurs mostly in the cervical region, and obstruction of thoracic oesophagus is rare (Tyagi and Singh, 2004). Many oesophageal foreign bodies are radiopaque and can be seen on plain radiographs, while radiolucent foreign bodies are difficult to diagnose. Oesophageal ultrasonography in buffaloes has been rarely reported in the literature. We have assessed the potential value of ultrasonography for the diagnosis of oesophageal foreign body obstruction in a buffalo. This article deals with the diagnosis and surgical management of cervical oesophageal obstruction in a buffalo thought to be associated with ingestion of a foreign body.

### CASE HISTORY

A Murrah-cross buffalo was presented to the department with a history of anorexia and inappetance for the previous ten days. The animal was unable to swallow feed but could drink water with discomfort. Urination was normal, and the consistency of faeces changed from normal to watery with a dark brown colouration. Clinical examination revealed slightly pale mucous membrane, congested conjunctiva with mild toxemic signs and dry muzzle. The rectal temperature was slightly elevated whereas pulse was weak and respiration slow. The foreign body was felt in the mid cervical oesophagus on the left side upon palpation.

### COMPUTERISED RADIOGRAPHY AND ULTRASONOGRAPHY

The computerized plain radiography of the cervical region of the neck was performed in the standing position. A Lateral radiograph revealed a regional intra-luminal mass due to the presence of a radiolucent foreign body occluding the whole lumen of the mid cervical oesophagus.

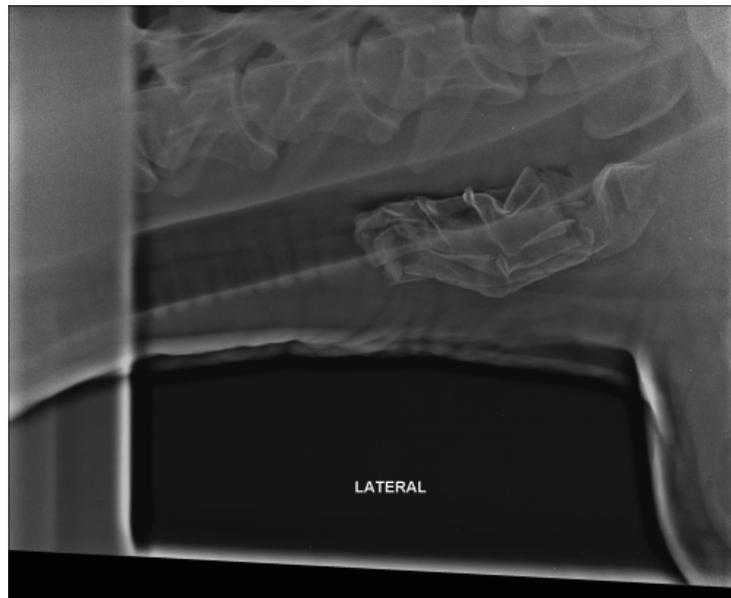


Figure 1. Lateral radiograph showing intra-luminal mass in the mid cervical oesophagus.



Figure 2. Ultrasonogram showing an echogenic posterior shadowing, non anatomic structure in the lumen of oesophagus.

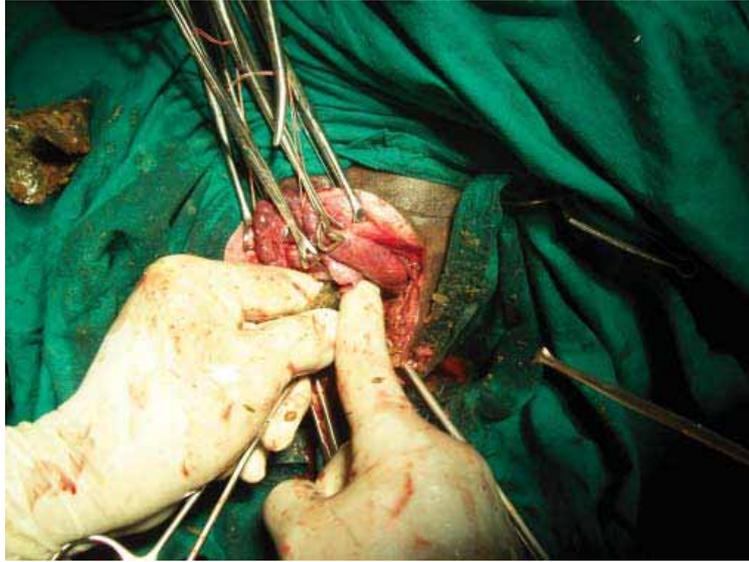


Figure 3. Photograph showing removal of foreign body from the oesophagus.



Figure 4. Photograph showing lather piece removed from the oesophagus.

Following the radiographic examination, B mode ultrasonography of left and right ventral neck regions were performed using 15 and 18 MHz linear probes. The oesophagus was visualized from the left jugular furrow in a longitudinal plane with a slight dorso-median or horizontal scanning. Transverse views were made from the left or right cranially or ventrally in the caudal portion. The normal portion of the oesophagus appeared as a band-shaped structure in longitudinal section, with a hyperechoic centre associated with intraluminal mucus and air. The mucosa and submucosa appeared moderately echogenic and were difficult to differentiate. An echogenic posterior shadowing, non-anatomic structure was seen in the lumen of the oesophagus and the oesophageal wall was oedematous in the obstructed portion. No abnormal blood flow or mural was found in the oesophagus wall, with the hypoechoic foreign body lodged in the lumen of the oesophagus. The oesophagus was medial to the left common carotid artery and thyroid gland. In the upper and middle parts of the neck, the external jugular vein appeared hypoechoic, beneath the skin.

### TREATMENTS

Cervical oesophagotomy was planned immediately. The animal was anaesthetized with xylazine 0.1 mg/kg b. wt. I/V and placed in right lateral recumbency. After aseptic preparation skin incision was given above the obstruction. The muscles were dissected and separated bluntly and the oesophagus was lifted to the level of skin incision. The oesophageal lumen was blocked by placing the intestinal clamps on either side of the obstruction and incision was given over the occluding mass. The foreign body, which was a large, wrinkled piece of leather was procured from

the oesophagus with the help of artery forceps. The oesophageal incision was closed with vicryl no. 1-0 by cushioning pattern and leakage was checked before the closure of muscles. The underlying muscles were sutured with vicryl no. 2 using simple interrupted pattern. The skin was sutured in the routine manner. The animal was administered 1.5 litres of DNS and Melonex 0.5 mg/kg b. wt. during the surgical procedure. Post operative care included antiseptic dressing of the wound using betadine and administration of Oxytetracycline 10 mg/kg b. wt. daily I/M for 5 days. The skin sutures were removed on 9th post operative day. The owner was advised to withhold food and water for 48 h after the surgery. The animal recovered uneventfully.

Ultrasonography aided in ascertaining the length and position of incision on oesophagus. Also, it eliminates the stress of restraint and radiation exposure to the animal and attendants.

### CONCLUSION

Computerized radiography is helpful in diagnosing the radiolucent foreign body in oesophagus, whereas ultrasonography is an ideal diagnostic tool for investigating oesophageal obstruction as well as gastrointestinal disorders in buffaloes. It can also be considered to be a valuable supplementary aid to clinical examination and, in many cases, can facilitate diagnosis.

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## PARATUBERCULOSIS (JOHNE'S DISEASE) IN A BUFFALO: A CASE REPORT

**H.C. Chauhan, A.I. Dadawala, S.S. Patel, P.B. Ranaware, K.M. Jadhav, K.G. Patel,  
N.M. Shah and B.S. Chandel**

**INTRODUCTION**

Paratuberculosis is a chronic, contagious, invariably fatal enteritis which can affect domestic and wild ruminants. The etiological agent, *M. avium subsp. paratuberculosis*, is an acid-fast organism formally known as *M. paratuberculosis*. Uncertainty exists regarding an association between infection with *M. avium subsp. paratuberculosis* and Cron's disease, a chronic enteritis in humans (Thompson, 1994). Cattle, sheep, goats and other wild and domestic ruminants are affected, including deer, camels and llamas. Annual death losses within an infected herd may reach 10%.

Animals are infected by ingestion of food and water contaminated by feces. The incidence of subclinical cases shedding organisms intermittently may be as high as 15%.

In cattle, there is chronic enteritis, often with severe diarrhea. The incubation period may be a year or more. Calves are susceptible but do not show signs until adult-hood. The disease is usually progressive, leading to emaciation and death. Mortality is caused in large part by the malabsorption of amino acids and the loss of protein into the intestine (protein losing enteropathy) (Tripathi *et al.*, 2002). The ileum and colon are usually involved and the infection may extend to the rectum in advanced cases. The mucus membrane becomes corrugated and thickened because of

epithelioid and giant cells, both of which contain many organisms. Large numbers of organisms may be shed in the feces. (Narang and Gurpreet, 2007).

**CASE HISTORY**

A buffalo about 4 years of age was presented to the college clinics, having a history of chronic diarrhea for 3 months and not responding to treatment with antibiotics and other therapy. The animal was emaciated, debilitated (Figure 1) and weak showing watery art diarrhea (Figure 2).

**DIAGNOSIS**

Diarrheic fluid and rectal pinch smear collected and examined after staining with the acid-fast staining method. The smear revealed acid-fast bacteria morphologically resembling *M. avium subsp. paratuberculosis*.

**CONCLUSION**

On the basis of history, clinical symptoms and laboratory examination it was concluded that this was a case of J.D.

### ACKNOWLEDGEMENT

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Figure 1. Emaciated, debilitated buffalo.



Figure 2. buffalo showing fluid diarrhea.

## G-TYPING OF BOVINE ROTAVIRUSES BY USING VP7 GENE SPECIFIC NESTED RT-PCR FROM DIARRHOEIC CALF FAECAL SAMPLES

T.C. Singh and M.K. Jhala

### ABSTRACT

Determination of the G genotypes of group A bovine rotaviruses from 53 diarrhoeic faecal samples were collected from both organized and unorganized farms in and around the Anand area including the Livestock Research Station, Anand, Gujarat. Rotavirus ribonucleic acid (RNA) was extracted from nine faecal samples of diarrheic calves positive for group A rotavirus by polyacrylamide gel electrophoresis (PAGE) followed by silver staining were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) to generate the near full length VP7 gene. Only eight samples yielded the desired product. The amplified products were subjected to G-typing by PCR using a cocktail of G6, G8 and G10 typing primers. Thus, among eight RT-PCR positive samples, G10 was the predominant G type (75%) followed by the type G6 (25%). The G8 type was not detected in any of the samples.

**Keywords:** bovine rotavirus, RNA, RT-PCR

### INTRODUCTION

Livestock farming plays an important role in the rural development programs in Gujarat state, India. The future of any dairy operation depends

upon a successful program of raising calves. However, it has been observed that a large number of calves die at an early age. The calf crop being the future livestock, diarrhoea affecting the neonates is an important disease in the conditions which affect the herd health and economy of the country (Singh and Singh, 1971).

Group A rotaviruses have been identified worldwide as a major cause of diarrhea in the young of many species, including humans (Bellinzoni *et al.*, 1989). Two rotavirus outer capsid proteins, VP4 and VP7, are independently involved in virus neutralization (Hoshino *et al.*, 1985). Group A rotaviruses are classified into G serotypes on the basis of the outer capsid glycoprotein VP7 (Estes and Cohen, 1989). At least, 15 G types and 26 P types have been recognized so far (Kapikian *et al.*, 2001). Although G types 1, 3, 5-8, 10 and 15 have been described in cattle, only G6, G8 and G10 are the most common group A rotaviruses of cattle (Adah *et al.*, 2003; Garaicoechea *et al.*, 2006). Genotyping has been preferred to serotyping due to its good correlation with serotypes, high sensitivity and use of synthetic reagents (Gouvea *et al.*, 1990). Bovine retroviruses (BRV) are very important for preventive veterinary medicine and more specifically, for the development of a vaccine. They are also important from the point of view of ecology and public health because interspecies

transmission from cattle to humans and from humans to cattle have been reported (Fukai *et al.*, 1998). Laboratory diagnosis of rotavirus infection in calves has been based on the identification of viral particles, antigens, or nucleic acids in faecal samples. Antigen capture enzyme linked immunosorbent assay (ELISA), latex agglutination (LA) and reverse transcription-PCR (RT-PCR) have become more standard methods for the diagnosis of bovine rotavirus infections in recent years. Latex agglutination test is more rapid than standard ELISAs and is easy to perform (Zvizdic *et al.*, 2004).

The aim of the present study was to identify the distribution of bovine rotaviruses in Gujarat state calves in recent years by using polymerase chain reaction- (PCR) based typing assays.

## MATERIALS AND METHODS

A total of 53 faecal samples were collected from 9 buffalo calves and 44 cattle calves of 0-8 weeks of age from both organized and unorganized farms in and around Anand area including Livestock Research Station, Anand, Gujarat.

### Extraction of double-stranded ribonucleic acid

A 10% faecal suspension of each sample prepared in phosphate-buffered saline and clarified by centrifugation at 10,000 rpm for 30 minutes. at 4°C was used as the basis for extraction of rotavirus ribonucleic acid (RNA). RNA was extracted from 10% (v/v) faecal suspensions using TRI-Reagent (Sigma, USA), following the manufacture's instructions. To the 250 µl clarified samples, 750 µl of TRI reagent was added, vortexed and incubated

for 15-30 minutes at 15-30°C to allow the complete dissociation of nucleoprotein complex. Chloroform (0.2 volume) was added, vortexed and kept at room temperature for 10-15 minutes and centrifuged at 13,000 rpm for 10 minutes at 4°C. The resultant aqueous phase was transferred to other Eppendorf tubes, one ml of isopropanol was added, and the tubes inverted 4-5 times and kept at -20°C overnight. The RNA was pelleted by centrifugation at 13,000 rpm for 10 minutes, and the pellet washed with pre-chilled 70% ethanol. The pellet was air dried, dissolved in nuclease free water (NFW) and stored at -20°C till further use. The RNA thus extracted was used for c- DNA preparation during RT-PCR.

The specificities of the selected primers used in the present study for the G- typing assays have been evaluated previously (Isegawa *et al.*, 1993). The upstream generic primer on the VP7 gene and three specific G-typing primers (Table 1) were used in a second round of PCR amplification for the characterization of the G6, G8, and G10 serotypes.

### RT-PCR of full-length VP7 and partial-length VP4 genes

Single RNA preparations were centrifuged at 13,000 rpm for 10 minutes at 4°C, and the pellets were washed with 70% ethanol. The ethanol was removed, and the pellets were dissolved in 30 µl of RNase-free water and used as templates for RT-PCR amplification. A total of 2 µl of genomic dsRNA was transferred to a 0.6-ml Eppendorf tube with 1.5 µl (15pmol) of each of the generic G primers and 11.0 µl of nuclease free water (NFW), denatured at 96°C for 5 minutes, and immediately chilled on ice. The denatured dsRNA was then added to the reaction mixture, consisting of 2 µl

of a deoxynucleoside triphosphate (dNTP) mixture (each dNTP at a concentration of 0.8 mM), 1 µl of Ribolock (10 U/µl), 1 µl of reverse transcriptase from MMLV-RT (200 U/µl) and 5.0 µl of 5X RT buffer in a final volume of 25 µl. The sample was preincubated in a thermal cycler (model 480; Perkin-Elmer Europe B.V., Monza, Italy) at 42°C for 60 minutes. The first round of amplification of full-length VP7 consisted of 35 cycles of 1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C, followed by a final incubation at 72°C for 10 minutes.

### Determination of G genotype

RT-PCR products were submitted to a second round of amplification by a modification of a previously described method. An aliquot of 2 µl of (150ng) DNA products was added to a reaction mixture consisting of 12.5 µl of 2X PCR Master Mix, 1 µl (10 pmol) of VP7 upstream primer, 1 µl (10 pmol) of each primer specific for the G6, G8, and G10 genotypes, nuclease free water to a final volume of 25 µl. sample was preincubated at 94°C for 5 minutes to allow activation of the AmpliTaq Gold. The second

round of amplification consisted of 30 cycles of 1 minute at 94°C, 2 minutes at 56°C, and 1 minute at 72°C, followed by a final incubation at 72°C for 10 minutes.

**Analysis and detection of products.** PCR products were analyzed on a 1.5% agarose gel containing ethidium bromide (0.5 mg/ml) in Tris-borate-EDTA buffer.

## RESULTS

During this study, all the nine PAGE positive faecal samples of calves were subjected to RT PCR assay. PCR was used to amplify the the BRV VP7 gene to identify specific genotypes of the BRV strains. The simplified procedure of RNA extraction and the use of a second amplification which allowed the simultaneous detection of different genotypes were adopted.

As shown in Plate1, full length (1062bp) amplification of VP7 gene of BRV was obtained after cDNA synthesis by reverse transcription using Bov9Com5 and Bov9Com3 primers. Out of nine samples, eight samples (88.89%) yielded a specific amplicon of 1062bp (Plate 1).

**Table 1.** Oligo-primers used in RT-PCR and G typing of BRV.

Primers	Sequences (5'-3')	Location	Expected product size
Bov9Com5	GGCTTTAAAAGAGAGAATTTCCGTTTGG	1-28	1062 bp
Bov9Com3	GGTCACATCATACTACTAATCT	1039-1062	
G6	CTAGTTCCTGTGTAGAATC	499-481	500 bp
G8	CGGTTCCGGATTAGACAC	273-256	274 bp
G10	TTCAGCCGTTGCGACTTC	714-697	715 bp

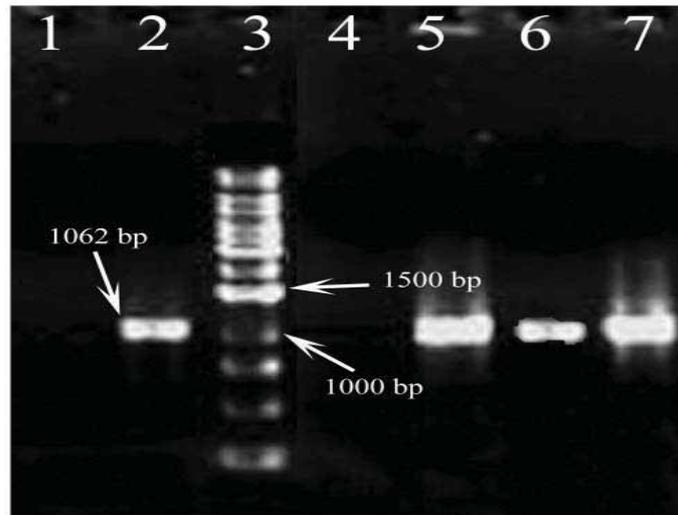


Plate 1. Full length BRV VP7 gene amplification by RT-PCR.  
Lanes 1, 4: showing negative control  
Lanes 2, 5, 6, 7: showing 1062 bp products  
Lane 3: -1 kb plus DNA ladder

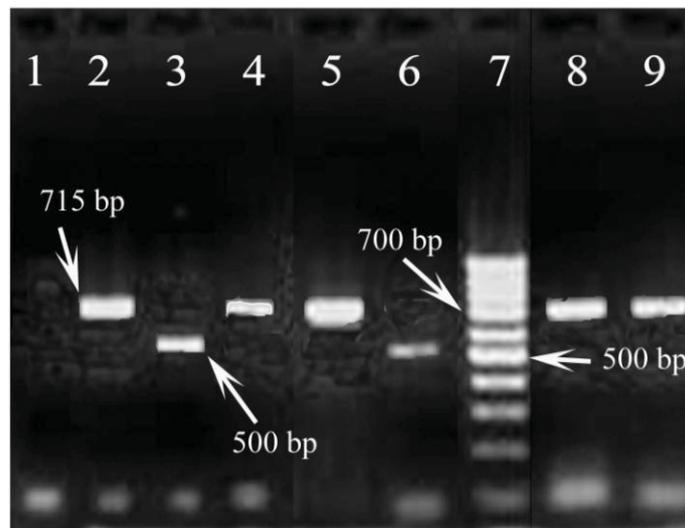


Plate 2. VP7 based G genotyping of BRV by heminested PCR.  
Lane 1: Negative control  
Lanes 2, 4, 5, 8, 9: Showing the type G10 (715 bp product)  
Lanes 3, 6: Showing the type G6 (500 bp product)  
Lane 7: 100 bp DNA ladder

Genotyping of circulating BRV strains was done by heminested PCR of 1062bp amplification product of the VP7 gene. This was achieved by using a common forward primer Bov9Com5 and a pool of typing (reverse) primers (G6, G8 and G10) as suggested by Falcone *et al.* (1999). The expected size of the amplified products from G6, G8 and G10 primers were 500bp, 274 bp and 715bp, respectively. Two samples yielded the products of 500bp and six samples yielded 715bp products indicating prevalence of G6 and G10 types (Plate 2). Thus, among eight RT-PCR positive samples, G10 was the predominant G type (75%) followed by the type G6 (25%). The G8 type was not detected in any of the samples.

Bovine G8 strains have been reported to be rare in some areas while they are detected more frequently in other reports (Reidy *et al.*, 2006). In this study, no sample was found to be positive for the type G8. A bovine G8 strain was first identified in Scotland (Snodgrass *et al.*, 1990) and then North America (Parwani *et al.*, 1993), Thailand (Taniguchi *et al.*, 1991) and Japan (Fukai *et al.*, 1998, 1999). Reports have indicated that G8 is one of the less common serotypes among bovine rotaviruses. However, it could be considered a diarrhoeal pathogen detected with lower frequency in calves.

## DISCUSSION

The serotype specificities of field isolates and, consequently, the availability of reliable diagnostic tools have, nowadays, become of primary relevance to the development of appropriate systems of epidemiological surveillance and control of BRV infection.

In this context, the limitations of existing

serological assays are well recognized, while molecular techniques, such as PCR assays, have become widely accepted as the assay of choice for the fast and complete characterization of field isolates (Taniguchi *et al.*, 1993). As further proof of these points, in the present study, PCR was used to amplify the BRV VP7 directly from the feces of infected calves and to identify specific genotypes of the BRV strains. The simplified procedure of RNA extraction and the use of a second amplification, which allowed the simultaneous detection of different genotypes, resulted in increased sensitivity and in the rapid characterization of the isolates in a large number of field samples.

The higher prevalence of the G10 genotype found in our study is in agreement with reports from many parts of the world (de Verdier Klingenberg *et al.*, 1999; Reidy *et al.*, 2006). Among the Indian bovine population, Balvinder *et al.* (2008), Wani *et al.* (2004), Minakshi (1999) and Gulati *et al.* (1999) have also reported G10 to be the predominant genotype of bovine rotavirus.

These results were in contrast with the distribution of BRV genotypes elsewhere in the world, where low prevalence rate of the G10 genotype and higher prevalence of the G6 genotype have been reported by Snodgrass *et al.* (1990), Chang *et al.* (1996), Falcone *et al.* (1999), Alfieri *et al.* (2004), Pisanelli *et al.* (2005), Reidy *et al.* (2006), Mayameii *et al.* (2007), Monini *et al.* (2008) and Howe *et al.* (2008). Thus, these findings suggest that the G6 and the G10 types, predominance may be restricted as per geographical location, and as far as India is concerned, G10 type appears to be predominant. The present finding was also the first time a study conducted in Gujarat state endorsed similar studies undertaken in other parts of the country. Although interpretation of this study was limited by the small sample size and sample

collection only in restricted regions in Gujarat, this finding was in sharp contrast to the distribution of BRV genotypes elsewhere in the world. The minimum number of rotavirus-positive samples obtained in our study may be due to seasonal distribution, as reported by Davidson *et al.* (1975), since most samples were collected during winter months. The minimal rotavirus infection may also be due to presence of colostral antibodies, which protect the calves from exposure to rotaviral infection, as reported by Paul and Lyoo (1993).

In conclusion, heminested-multiplex PCR, which was an alternative to genotyping for identification and typing of rotavirus genotypes, facilitated the studies on the occurrence and distribution of G genotypes of the bovine population in Gujarat state.

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## EFFECT OF NON-GENETIC FACTORS ON REPRODUCTIVE DISORDERS IN MURRAH BUFFALOES

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### ABSTRACT

Data pertaining to 1055 calving records of 427 Murrah buffaloes from 2000 to 2007 at an organized farm were analyzed to assess the influence of various factors *viz.*, parity, season and period of calving on incidences of reproductive disorders. The results revealed that overall incidences of abortion, still birth, dystocia, retention of foetal membranes (RFM), metritis, endometritis, repeat breeder, anoestrous, pyometra and prolapse in Murrah buffaloes were 7.1, 1.04, 1.23, 12.80, 14.60, 11.85, 10.62, 16.97, 1.52 and 1.23%, respectively. Statistical analysis showed that only parity had a significant ( $P<0.05$ ) influence on incidence of abortion among parturition associated problems and pyometra among post-parturient complications. Period of calving had a significant effect ( $P<0.05$ ) on endometritis among post-parturient complications. Season of calving had no-significant effect on the incidence of reproductive disorders, *viz.* parturition associated problems, postparturient complications and other reproductive problems. In Murrah buffaloes the

winter season seems to be the favourable season compared to the summer season for decreased incidence of peripartum reproductive disorders.

**Keywords:** buffalo, non-genetic, peripartum, reproductive disorders

### INTRODUCTION

Non-reproductive or general health problems along with complex and multi-factorial reproductive disorders have a bearing on reproductive performance depending on their nature and severity. Reproductive problems may be broadly categorized into two groups-periparturient disorders (pre-parturient disorders, parturition associated disorders and post parturient complications) and general disorders (not associated with parturition). Among them periparturient disorders have been recognized as the most important factors affecting fertility (Wilde 2006). Parturition-associated problems include dystocia, still birth, abortions, retained placenta;

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among them, the first two have been documented as the most important factors compromising the future reproductive life of an animal. They increase the chances of developing metritis and retained placenta (Correa *et al.*, 1993). During dystocia, exertion and exhaustion can frequently cause delayed uterine involution and predispose to secondary infections and abnormalities in the resumption of ovarian cyclicity. Jadon *et al.* (2005) reported that parturition associated complications increased susceptibility to for uterine infections in buffaloes. Cases of retained foetal membranes (RFM) have been reported in 2.73 to 9.72% of buffaloes (Tomar and Tripathi, 1992; 1994; Murugeppa and Dubey, 1997; Rahman *et al.*, 1997; Murugeppa, 1998; Prasad and Prasad, 1998; Taraphder, 2002; Tomar *et al.*, 2002). RFM delays uterine involution, predispose cows to endometritis or metritis and decrease fertility (Grohn and Rajala-Schultz, 2000; Maizon *et al.*, 2004). Any lacuna or mishandling during veterinary assistance in case of parturition related problem has a fatal effect on future reproductive performance due to development of postpartum reproductive problems. The present study was conceived to obtain an overview of the parturition- associated problems at an organized farm to optimize the managerial interventions for improving the reproductive performance.

## MATERIALS AND METHODS

Data pertaining to 1055 calving records of 427 Murrah buffaloes from 2000 to 2007 at an organized farm were analyzed to study the incidences and influences of non-genetic factors, *viz.*, parity, season and period of calving on incidences of parturition associated problems over a period of time. The incidences of various parturition

associated complications were calculated as per parity, period and season. They were calculated by taking the proportion of calvings affected in the herd.

The incidence as percent was calculated as follows:

$$\text{Incidence (\%)} = \frac{\text{Number of animals affected by a particular disorder during the period}}{\text{Number of breedable females available in the herd during the period}}$$

The influence of various non-genetic factors, *viz.* parity, period and season of calving on incidence of various parturition associated problems were analyzed by using the Chi-square method (Snedecor and Cochran, 1989).

## RESULTS AND DISCUSSION

The parity, season and period wise details pertaining to the total number of records, the number of calvings/lactations affected and percent incidence of various reproduction disorders have been presented in Table 1. The present study revealed that overall incidence of abortion, still birth, dystocia and RFM in the Murrah buffaloes over a period of eight years was 7.1, 1.04, 1.23 and 12.80%, respectively.

### Parturition associated problems

#### *Abortion, Still Birth and Dystocia*

The overall incidence of abortion, still birth and dystocia (Table 1) in the Murrah buffaloes was

found to be 7.1, 1.04 and 1.23%, respectively. The incidence of abnormal calvings reported in literature in buffaloes ranges from 4.66% to 12.66%, which is in agreement with our findings (Tomar and Ram, 1993; Tomar and Tripathi, 1995; Rahman *et al.*, 1997; Prasad and Prasad, 1998; Taraphder, 2002). These incidences have been comparatively higher in Murrah buffalo than those reported in Surti buffalo (Kulkarni, 1995; Murugeppa, 1998; Kumar and Jain, 2000).

The present study (Table 1) revealed that the third period had the highest incidence of abortion in Murrah buffaloes (12.12%); the incidence varied from 4.46 to 12.12% with the minimum incidence in the fourth period. Better performance in terms of reduced abortions in the fourth period could be due to better management adopted, better nutrition, congenial environmental factors and other unknown causes. The incidence of abortion varied from 6.02 to 8.97% among the different seasons. The lowest incidence was found during the rainy season and highest incidence during the winter season in Murrah buffaloes. The highest incidence of abortion in winter may be predominantly due to deficiency of nutrients available through feed and fodder during this dry period, which is a lean, period and animals are supplied only silage as fodder. Across different parities incidence varied from 5.11 to 10.60%. The minimum incidence was found in first parity and highest incidence in second parity in Murrah buffaloes. Statistical analysis showed that only parity among the non-genetic factors had significant ( $P < 0.05$ ) influence on incidence of abortion. Similarly Kulkarni (1995); Murugeppa and Dubey (1998) observed a significant effect of parity on abnormal calving. Contrary to present finding Tomar and Verma (1987); Tomar and Ram (1993); Tomar and Tripathi (1995); Kumar and Jain (2000) did not find any significant effect of parity

on abnormal calving.

On appraisal of the data presented (Table 1) the highest incidence of still birth was recorded during second period (1.89%) and lowest during fourth period (0.64%) in these Murrah buffaloes. Across the seasons no incidence of stillbirth was recorded during the summer season whereas highest incidence was recorded during the winter season (1.92%). The lowest incidence was recorded during fourth and above parities (0.43%) and the highest incidence during third parity (1.60%) in these Murrah buffaloes. Statistically, the incidence of stillbirth was not affected by any of the non-genetic factors. However, contrary to the present findings Kaikini *et al.* (1976) observed a significant effect of parity on abnormal calving, being highest in younger buffaloes.

The results obtained from the present study (Table 1) showed that the incidence of dystocia was highest in the second period (2.83%) and lowest in the third period (0.34%) in these Murrah buffaloes. The findings indicated that incidence of dystocia in these Murrah buffaloes across different seasons varied from 0.64 to 2.07% with the highest incidence recorded during the autumn season and lowest in the winter season. The findings are in agreement with Kaushik and Mathur (2005), who also recorded the maximum incidence of difficult calvings in autumn season but reported higher values than the present findings. Across different parities incidence varied from 0.85 to 2.66% with the maximum incidence recorded for third parity and lowest for first parity. Statistically, there was no effect of non-genetic parameters on dystocia.

#### ***Retention of Foetal Membranes***

The incidence of RFM in Murrah buffaloes was 12.80% (Table 1), which is in close agreement with the incidence obtained in an earlier study by

Table 1. Incidence of parturition associated complications in Murrah buffaloes.

Effects	Total calving	Abortions		Stillbirth		Dystocia		RFM	
		1	2	1	2	1	2	1	2
Overall	1055	75	7.1	11	1.04	13	1.23	135	12.8
Parity									
Parity 1	352	18	5.11	4	1.14	3	0.85	36	10.23
Parity 2	283	30	10.6	3	1.06	3	1.06	35	12.37
Parity 3	188	15	7.98	3	1.6	5	2.66	30	15.96
Parity 4 & above	232	12	5.17	1	0.43	2	0.86	34	14.66
$\chi^2$ -value	8.88*				1.42		3.89		4.53
Period									
2000-01	232	12	5.17	3	1.29	4	1.72	29	12.5
2002-03	212	13	6.13	4	1.89	6	2.83	33	15.57
2004-05	297	36	12.12	2	0.67	1	0.34	45	15.15
2006-07	314	14	4.46	2	0.64	2	0.64	28	8.92
$\chi^2$ -value	1.63				2.5		7.78		7.19
Season									
Winter	312	28	8.97	6	1.92	2	0.64	37	11.86
Summer	168	12	7.14	0	0	3	1.79	28	16.67
Rainy	382	23	6.02	4	1.05	4	1.05	47	12.3
Autumn	193	12	6.22	1	0.52	4	2.07	23	11.92
$\chi^2$ -value	2.56				4.63		1.32		2.72

\* - Significant (P&lt;0.05)

\*\*- Significant (P&lt;0.01)

1-Number of animals affected

2-Percentage of animals affected

Tomar *et al.* (2002), whereas other workers have reported lower incidences between 2.73 and 9.72% in buffaloes (Rawal and Singh 1991; Tomar and Tripathi, 1992; 1994; Murugeppa and Dubey, 1997; Rahman *et al.*, 1997; Prasad and Prasad, 1998; Murugeppa, 1998; Taraphder, 2002). Because of the large herd size no special periparturient feeding management might have been practiced in the herd during the reporting period, and this may be the reason for higher incidences of RFM cases. (In field condition special feeding management to the periparturient animals are generally followed, which may be reason for very low incidences of RFM cases.)

The present study (Table 1) revealed that the incidence of RFM was highest in the second period (15.57%) and the lowest incidence was in the fourth period (8.92%) in these Murrah buffaloes. Results revealed a progressive improvement in the pre- and post-partum management of the buffaloes. Analysis indicated that the period of calving had a non-significant effect on the incidence of retention of foetal membranes in these Murrah buffaloes. Bhalaru *et al.* (1983) also reported a non-significant effect of period on retention of placenta. However, Tomar and Tripathi (1994); Taraphder (2002) reported significant effects of period of calving on retention of placenta in buffaloes.

The incidence of RFM was found to be highest among summer season calvers (16.67%) and lowest in winter season calvers (11.86%) in these Murrah buffaloes (Table 1). This could be attributed to environmental, managerial, nutritional and other unknown causes. Whereas, higher incidence in the summer season could be due to the inclement weather and stress conditions, which invariably reduce DMI and immunity of animals. When the differences across different seasons were analyzed, it was found that season

of calving had no significant effect on incidence of retention of foetal membranes. Rawal and Singh (1991); Tomar and Tripathi (1994) also reported non-significant effects of season of calving on this trait. However, Bhalaru *et al.* (1983); Prasad and Prasad (1998) reported significant effects of season of calving on this trait.

When viewed across the parities the incidence of RFM was found to be the highest in third parity (15.96%) and the lowest in first parity (10.23%). However, the differences were not statistically significant. Rawal and Singh (1991); Tomar and Tripathi (1994); Tarafder (2002) also did not observe any effect of parity on retention of placenta. Contrary to this, Pandit *et al.* (1982); Prasad and Prasad (1998); Murugeppa and Dubey (1997) reported significant effects of parity on retention of placenta.

## CONCLUSION

The parturition associated problems are the most severe reproduction disorders which need to be addressed in Murrah buffaloes for improving their reproductive efficiency and keeping the buffalo production system economical. These problems need to be evaluated in view of managerial practices followed and remedial measures to be adopted with particular attention towards plane of nutrition.

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COMPARISON OF HORMONAL<sup>A</sup> AND HOMEOPATHIC<sup>B</sup> COMPLEXES FOR TREATMENT OF TRUE ANESTROUS IN POST PARTUM BUFFALOES DURING THE SUMMER

Raman Gupta, M.S. Thakur, O.P. Shrivastava and Nishi Pandey

ABSTRACT

The study was conducted on 45 buffaloes not expressing estrus signs for more than 120 days post partum. Confirmation of true anestrus was done by finding smooth ovaries at rectal examination. Out of the 45, 15 animals were given a hormonal treatment, *viz.*, a single dose of GnRH analogue (Receptal) 5 ml (0.042 mg/ml) I/M; ten of them came into heat (66.67%) within an interval of  $14.0 \pm 1.08$  days and eight animals conceived at estrus with natural breeding. Another 15 animals were treated with a homeopathic complex (Hit-o-gen) 2 boluses orally on days 1 and 4 of treatment; 10 animals (66.67%) expressed estrus at an interval of  $9.10 \pm 0.95$  days, and eight of them conceived. The remaining 15 animals served as controls for both groups and no treatment was given to them. The result shows that both hormonal therapy and homeopathic complex were equally effective for the treatment of true anestrus in buffaloes during the summer. When the two treatments are compared for cost on single animal, the homeopathic is more cost effective as compared to the hormonal.

**Keywords:** post partum, true anestrus, hormonal, homeopathic complex, summer

A. Hormonal (GnRH analogue Buserelin), Receptal (intervet)

B. Homeopathic complex (Alettris farinose 1M, folliculinum 1M, oophorium 1M, pituitary gland 1M), HIT-O-GEN (Goel homoeo pharma)

INTRODUCTION

Estrus is the period of sexual receptivity associated with certain cyclical changes in the reproductive system; thus, estrus is the first stage in the series of processes involved in reproduction. Major contributing factors to cyclicity failure include poor nutrition, seasonality, climatic stress, failure in detection, ignorance of farmers about signs, suppressed hypothalamic pituitary function and post-partum uterine pathology. Non-cyclic animals can be managed by subjecting them to estrus induction therapies. Obviously, the success of any therapy will greatly depend upon the accuracy of identification of etiological factors. Anestrus is the most common and costly infertility problem of buffaloes and results in poor calf crop and milk production losses. It has been suggested that during the summer, when ambient temperature and photoperiod are at their maximum, prolactin level is highest (Kaker *et al.*, 1982) and plasma progesterone levels are lowest, and this in turn will suppress the pulsatile secretion of gonadotropin from anterior pituitary which leads to anestrus condition in buffaloes.

Gonadotropin releasing hormone (GnRH) is a decapeptide produced by the hypothalamus

in a pulsatile manner and affects the release of LH and FSH from the pituitary. Recent advances have greatly contributed in establishing the clinical efficacy of GnRH analogues as estrus inducers and ovulation inducing agents.(Lofsted *et al.*, 1988; Jagger *et al.*, 1987) Rajkumar *et al.* (2006) reported 100% success in estrous induction with the use of a homeopathic drug complex in cows.

Hence, this study was conducted with the aim of comparing homeopathic complex and hormonal treatments in true anestrus buffaloes and evaluate them in terms of estrus induction, fertility and cost of treatment.

## MATERIALS AND METHODS

The study was conducted on 45 non-pregnant buffaloes aged between 4-12 years which had not expressed estrus signs for more than 120 days post partum. Confirmation of anestrus was done on the basis of history and the finding of smooth ovaries without persistent corpus luteum and no uterine pathology on rectal examination twice an interval of 11 days. All the animals housed in cemented sheds with optimum floor space and stall fed as per the standard schedule, the management being identical throughout the study. The 45 experimental animals were randomly divided into three groups (15 animals per group) and each group was subjected to the treatment as indicated in Table 1 below. After the treatment all the animals were observed for expression of estrus, which was detected by parading buffalo bull daily in the morning and evening. Further confirmation of estrus was done by rectal examination. The duration of expression of estrus following the treatment along with its intensity was recorded as intense, moderate and weak on the basis of score

card devised by Shrivastava (1997). The breeding of animals in all the groups was done by fertile bull semen at estrus. Fertility responses of the different groups were evaluated by confirming pregnancy two months after breeding.

## RESULTS AND DISCUSSION

After the treatment the animals were regularly examined for the following observations

Out of the 15 animals from each of Groups 1 and 2, 10 came into estrus with an average post treatment interval of  $14.00 \pm 1.09$  and  $9.56 \pm 3.18$  days. While in control group, only two animals came into estrus with an post treatment interval of  $15.50 \pm 2.50$  days. The percentages of animals showing intense, moderate and weak estrus in Group 1 were 70, 20 and 10% while, in Group 2 the percentages were 70, 30 and zero percent, whereas, in the control group, intense and moderate estrus expression was 50% only. The average post treatment interval of estrus expression was earlier in Group 2 as compared to Group 1 and equal numbers of animals (10) came into heat in both the groups.

The results on induction of estrus as a result of GnRH analogue treatment are in close agreement with Markandeya and Patil (2003); Rathore (2004), who reported that in GnRH treated buffaloes, 66.6% exhibited estrus. Maximum induction of estrus and ovulation has also been reported by Singh *et al.* (2003) in 20 anestrus buffaloes when treated with 5 ml receptal, estrus was established in 80% animals with an average period of  $16.06 \pm 0.65$  days of treatment and out of them, 70% of the buffaloes ovulated. However, Gupta (2008) reported only 33.33% success in induction of estrus with a duration of  $9.56 \pm 3.18$  (5-14) days of treatment.

Table 1. Group wise distribution of animals.

<b>Group I (anestrous)</b>	Single dose of Receptal 5 ml (0.0042 mg/ml) I/M.
<b>Group II (anestrous)</b>	Hit-o-gen 2 Bolus-Orally, one morning and one evening on days 1 and 4 of treatment.
<b>Group III</b>	Control (no treatment)

Table 2. The observations with regard to estrus induction with percent intensity in different groups are summarized.

Group	No. of animals	Total no. of animals respond to treatment	Nature of estrus expression (%)			Mean intervals from withdrawal of treatment to estrus expression
			Intense	Moderate	Weak	
1.	15	10	7/10 =70	2/10 =20	1/10 =10	14.00±1.09
2.	15	10	7/10 = 70	3/10 =30	-	9.10±0.96
3.	15	2	1/2 = 50	1/2 = 50	-	15.50±2.50

Table 3. Containing the data pertaining to fertility rate of buffaloes when naturally served at induced estrus.

Groups	No .of buffaloes	Total no. of buffaloes respond to treatment	No. of buffaloes naturally served	No of buffaloes conceived	Fertility rate
1.	15	10	10	8	9/15 =53.3
2.	15	10	10	8	9/15 =53.3
3.	15	2	2	2	2/15 =13.3

Successful induction of estrus has also been reported with homeopathic drug complex by some workers. Rajkumar *et al.* (2006) treated six anestrus cows with a homeopathic complex, (calcarea phosphorica 30c, aletris farinose 30c, pulsatiella 30c, aurum muriaticum natronatum 30c, sepia 30c and phosphorus 30c) in equal proportion; 15 pills twice daily orally for 10 days treatment was 100% effective in inducing estrus in anestrus cows, which is higher as compared to the present study. Similarly, Chandel *et al.* (2009) treated 140 anestrus buffaloes with Hit-o-gen as a homeopathic drug, and out of them, 100 animals (71.42%) exhibited estrus at an average post treatment interval of 8.36 days, which is earlier as compared to present study.

Although both treatments were equally effective in induction of estrus in post partum anestrus buffaloes, weak heat intensity was observed in Group 1 while in Group 2, heat intensity was intense and moderate. Rathour (2004) used a GnRH analogue and reported the same magnitude of estrus as in the present study. It, therefore, appears that GnRH plays a key role in regulation of reproductive processes in animals. A pulsatile pattern of GnRH may be the only strategy involved to maintain a certain level of FSH and LH secretion and same in the case of homeopathic complex to release FSH and LH from the anterior pituitary gland.

The pregnancy diagnosis was conducted after two months by rectal examination revealed that eight animals (53.33%) were pregnant in both the treated Groups 1 and 2, while only two animals (13.33%) in control group were found pregnant. Satisfactory conception rates due to the use of GnRH analogues and homeopathic complexes has been reported in cattle Shah *et al.* (2003); Singh *et al.* (2003). The present study supports the results of

Rathour (2004) who reported equal conception rate when treating true anestrus buffaloes. However, Pattabiraman *et al.* (1986) recorded low fertility rate in anestrus buffaloes when treated with GnRH analogue. The good fertility obtained in the treated groups may be due to the fact that the maximum number of animals showed intense heat.

It may, therefore could be concluded that both hormonal and homeopathic complexes are equally effective for the treatment of true anestrus in buffaloes during the summer. For treating a single animal, hormonal therapy costs about eight times as much compared to the costs of homeopathic complex. Hence, homeopathic treatment is cost effective as compared to the hormonal therapy and can be effectively used for management of true anestrus during the summer.

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## EFFECT OF A HIGH-PRESSURE FOGGER SYSTEM ON BODY COMFORT AND MILK YIELD IN MURRAH BUFFALOES DURING THE SUMMER

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### ABSTRACT

The high temperature coupled with high humidity places greater thermal stress on buffaloes. The thermal stress is responsible for the variation in the physiological reactions, milk yield, feed intake and reproductive performance. It is a well-known fact that there is a significant drop in milk production during the summer months due to summer stress. Therefore here an attempt has been made to investigate the effect of a high pressure fogger System (HPFF) as a summer management tool.

The present study was conducted on a private dairy farm where a HPFE system was installed. It was found that the HPFF system had highly significant ( $P < 0.01$ ) effects on the body temperature and respiration rate in Murrah buffaloes. The body temperature and respiration rate of Murrah buffaloes were significantly lower under the HPFF system ( $37.52^{\circ}\text{C}$  and 21.61 counts/minute resp.), respectively, than those of the control group ( $37.83^{\circ}\text{C}$ , 23.01 counts/minute resp.).

There was highly significant ( $P < 0.01$ ) diurnal effect on physiological responses in Murrah buffaloes. The body temperature and respiration rate were significantly higher in afternoon hours than morning hours. The body temperature and respiration rate at 6 am, 10 am, 2 pm and 6 pm

were  $36.58^{\circ}\text{C}$ ,  $37.77^{\circ}\text{C}$ ,  $38.21^{\circ}\text{C}$  and  $38.14^{\circ}\text{C}$  and 20.37, 22.10, 25.22 and 21.55 counts/minute, respectively.

The HPFF system also had a significant ( $P < 0.05$ ) effect on test day milk yield. The milk yield of Murrah buffaloes maintained under the HPFF system ( $5.630 \pm 0.129$  kg) was higher than that of the control group ( $5.321 \pm 0.123$  kg). The HPFF system enhanced the daily milk yield by 0.309 kg/day/animal.

The HPFF system played an important role in the body comfort of the animals which is evident from the body temperature and respiration rate of the buffaloes under this system, which were lower by  $1.24^{\circ}\text{C}$  and 4.01 counts/minute, respectively, at the peak hot period (at 2 pm). The HPFF system yielded a significantly higher milk of 0.309 kg per animal/day. It is concluded that the HPFF system was beneficial in terms of the body comfort of the animals and the milk yield in Murrah buffaloes.

**Keywords:** high pressure fogger system on, body comfort, test day milk yield

### INTRODUCTION

Although buffaloes are found in greatest numbers in the tropics and sub tropics, they

have poor heat resistance. The high atmospheric temperature coupled with high humidity places greater thermal stress on buffaloes. The thermal stress is responsible for variations in physiological reactions, milk yield, feed intake and reproductive performance. There is a significant drop in milk production in the summer months due to summer stress.

Access to water or shade is essential for the well-being of buffaloes. Buffaloes have developed the habit of wallowing to cool their bodies for the normal functioning of organs. During the summer, wetting buffaloes can effectively reduce a rise in body temperature through evaporative cooling. Wetting can be achieved by splashing water, showering, sprinkling, mud plastering and some modern technologies like high-pressure fogger systems. The high-pressure fogger system consists of nozzles in a line placed 8 to 9 feet above the floor. This system disperses very fine water droplets and cools the air while raising the relative humidity. Here an attempt has been made to investigate the effect of this as a summer management tool.

## MATERIALS AND METHODS

A batch of 20 lactating Murrah buffaloes of similar age group, parity, stage of lactation i.e. mid-lactation was selected for the present investigation. All the buffaloes were maintained under standard feeding and managerial conditions. The buffaloes were housed in the head out manner in a conventional barn under stall fed conditions. The buffaloes selected were subjected to two different summer managerial practices; the same animals were utilized for both the treatments. Data which were recorded on the days on which the high

pressure fogger system with fans was running was treated as the treatment group while data recorded on days on which no cooling system was functioning were treated as the control group.

1. High pressure fogger system with fan (HPFF) and
2. Without any cooling system as control group.

### 1. The high pressure fogger with fan (HPFF) system

The selected buffaloes were subjected to evaporative cooling by a high pressure fogger system with fans. This system consisted of nozzles in a line placed 8 to 9 feet above floor. The system dispersed very fine water droplets and cooled the air while raising the relative humidity. Curtains placed on the side of the barn facing the prevailing wind kept the mist in the area where the buffaloes were laying or standing. As fog droplets were emitted, they were immediately dispersed into the fan's air stream where they soon evaporated, and the cooled air was blown over the animals' bodies. The foggers were operated on selected days during daylight hours for 4 h from 11.00 to 15.00 h, i.e. during the hours of very high atmospheric temperature.

The variations in body temperature and respiration rate in response to the high pressure fogger system during the summer were recorded weekly at 06.00, 10.00, 14.00 and 18.00 hours to assess the body comfort. The body temperature was measured with the help of a Raytech Mini Temp temperature tester. The body temperature was measured by triggering the Temp temperature tester beneath the tail of animal. This method was used to record the body temperature of buffaloes so as to minimize the discomfort to the animals, as taking rectal temperature causes much discomfort and also takes longer time. The respiration rate was

recorded as count per minute with the help of a stop watch, by the visual observation of ribs while standing one meter away from buffalo and without disturbing the animal. The milk yield of selected lactating Murrah buffaloes was recorded as test day milk yield during the milking hours, at 5.00 am in morning and at 5.00 pm in the evening.

## RESULTS AND DISCUSSION

### Body Temperature

The analysis of variance (Table 1) indicates that the Murrah buffaloes maintained under the high pressure fogger showed significantly ( $P < 0.01$ ) lower body temperature ( $37.52^{\circ}\text{C}$ ) than the buffaloes maintained without any cooling system ( $37.83^{\circ}\text{C}$ ). The HPFF had reduced the body temperature significantly by  $0.31^{\circ}\text{C}$ ; this suggested that the high pressure fogger system was helpful in thermoregulation.

Similar results were obtained by Huang *et al.* (1987); Patel *et al.* (1990); Verma *et al.* (1990); Chen *et al.* (1993); Vijay Kumar *et al.* (1998) and Tarazon-Herrera *et al.* (1999). However Wang *et al.* (1993) reported that a fogging and movable fan system had little effect on rectal temperature in cows.

The significantly lowest body temperature,  $36.82^{\circ}\text{C}$ , was observed in the first week of the experiment during the month of April and the highest body temperature,  $38.18^{\circ}\text{C}$ , was recorded in the fourth week in the month of May. This suggested that the atmospheric temperature had influenced the thermoregulation mechanism of the

Murrah buffaloes significantly. The present findings are in agreement with those reported by Roussel *et al.* (1970b).

It was observed (Table 1) that the significantly lowest body temperature,  $36.58^{\circ}\text{C}$ , was recorded at 6 am, then the temperature gradually but significantly ( $P < 0.01$ ) increased to  $37.77^{\circ}\text{C}$  at 10 am, and then it achieved the significantly highest level,  $38.21^{\circ}\text{C}$ , at 2 pm, and at 6 pm, it had maintained this significant higher level i.e.  $38.14^{\circ}\text{C}$ . However, the body temperatures at 2 pm and 6 pm did not differ significantly (Figure 2). There was an increment in body temperature to the extent of  $1.63^{\circ}\text{C}$  in the afternoon hours over the morning hours, indicating discomfort to the animals.

This suggests the necessity of cooling devices during the afternoon hours to keep the buffaloes' environment comfortable. The present findings are in agreement with Bempong and Gupta (1989); Verma and Hussain (1991); Vijay Kumar *et al.* (1998).

It was also observed that there was a non-significant difference in body temperature at 6 am and 10 am between both the groups, but at 2 pm and 6 pm the body temperature varied significantly ( $P < 0.01$ ) between the groups. The body temperature of buffaloes under the HPFF system was significantly lower than that of the control group. This explains the usefulness of the high pressure fogger system in controlling the body temperature of Murrah buffaloes in the afternoon hours.

Similar findings were obtained by Patel and Dave (1990); Minett (1997); Omar *et al.* (1997); Vijay Kumar *et al.* (1998) However, Sastry *et al.* (1973) reported similar rectal temperature at 3 pm in Murrah heifers in both groups.

### Respiration rate

The average respiration rate (Table 2) in the Murrah buffaloes maintained under the high pressure fogger was 21.61 counts/minute, which was significantly ( $P < 0.01$ ) lower than that of the buffaloes maintained without any cooling devices (23.01 counts/minute). This suggested that the high pressure fogger system has a beneficial effect on the body comfort of Murrah buffaloes.

The present findings are in accordance with Sastry *et al.* (1973); Aii *et al.* (1989); Patel and Dave (1990); Verma *et al.* (1990); Taylor *et al.* (1991); Chen *et al.* (1993); Vijay *et al.* (1998); Tarazon Herrera *et al.* (1999).

The lowest respiration rate (21.61 counts/minute) was observed during first week in the month of April and highest respiration rate (22.70 counts/minute) was observed during fourth week in the month of May during the experimental period (Table 2). This suggests that the respiratory mechanism of buffaloes was influenced by the climatic conditions during the summer months. The present findings are in agreement with those reported by Roussel *et al.* (1970b).

The respiration rate varied significantly ( $P < 0.01$ ) due to time (Table 2). The lowest respiration rate (20.37, counts/minute) was observed at 6 am. The respiration rate then significantly ( $P < 0.01$ ) increased to 22.10 counts/minute at 10 am and it achieved the significantly highest level, 25.22 counts/minute, at 2 pm. Then, it gradually decreased to 21.55 counts/minute at 6 pm. The change in respiration rate from am to pm indicated that the respiration rate of the Murrah buffaloes was influenced by rise in ambient temperature in both groups. The present findings are in agreement with

the previous workers, Bempong and Gupta (1989), Verma and Hussain (1991); Vijay *et al.* (1998).

The respiration rate at 6 am did not vary significantly for both the groups. The respiration rate at 10 am, 2 pm and 6 pm varied significantly ( $P < 0.01$ ) between the groups. The HPFF system reduced the respiration rate in the Murrah buffaloes by 4.01 counts/minute as compared to control group. This explains the necessity of cooling system during the afternoon hours of the day to maintain animals under comfortable conditions (Figure 1). Similar results were also obtained by Sastry *et al.* (1973, Patel and Dave (1990); Omar *et al.* (1997) However, Vijay *et al.* (1998) observed no significant difference for respiration rate at 2.00 pm for both groups in Murrah buffaloes.

### Milk Yield

The average milk yield of the Murrah buffaloes (Table 3) maintained under the high pressure fogger with fan was  $5.630 \pm 0.129$  kg. This was significantly ( $P < 0.05$ ) higher as compared to the milk yield of the Murrah buffaloes maintained without any cooling devices ( $5.321 \pm 0.123$  kg). The high pressure fogger with fan system increased the daily milk yield by 0.309 kg/day/animal. (Table 3) The present findings are similar to those of Roussel *et al.* (1970a); Fuquay *et al.* (1979); Igono *et al.* (1985); Aii *et al.* (1989); Verma *et al.* (1990); Ryan and Boland (1992); Wang *et al.* (1993); Srivastava *et al.* (2001); Senthil Kumar *et al.* (2003).

It suggested that the high pressure fogger with fan helped in reducing the heat stress and thereby in augmenting milk production in the Murrah buffaloes significantly.

Table 1. Week wise means for body temperature (°C) in Murrah buffaloes.

Weeks	Control					High pressure fogger					Pooled				
	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.
1	35.30	36.71	37.10	37.30	36.61	35.50	37.20	37.60	38.00	37.08 <sup>a</sup>	35.40	36.95	37.35	37.65	36.84
2	36.30	37.40	37.90	38.30	37.43 <sup>b</sup>	36.90	38.20	38.30	38.40	37.95 <sup>ef</sup>	36.60	37.80	38.10	38.35	37.71 <sup>a</sup>
3	35.70	37.60	39.20	38.40	37.73 <sup>de</sup>	36.60	37.80	36.20	38.10	37.16 <sup>a</sup>	36.15	37.70	37.70	38.25	37.44 <sup>bc</sup>
4	37.90	38.70	40.10	38.80	38.87	36.60	38.40	36.10	39.00	37.49 <sup>bcd</sup>	37.25	38.55	38.10	38.90	38.18 <sup>e</sup>
5	36.70	38.00	39.40	38.50	38.14 <sup>fg</sup>	37.50	38.20	38.70	38.20	38.14 <sup>fg</sup>	37.10	38.10	39.05	38.35	38.14 <sup>e</sup>
6	37.10	38.40	39.50	38.30	38.32 <sup>g</sup>	36.60	38.10	38.30	38.00	37.73 <sup>de</sup>	36.85	38.25	38.90	38.15	38.02 <sup>de</sup>
7	36.50	37.90	39.10	38.50	38.01 <sup>f</sup>	36.70	38.10	38.00	38.10	37.69 <sup>cd</sup>	36.60	38.00	38.55	38.30	37.85 <sup>d</sup>
8	36.70	37.20	38.90	38.10	37.70 <sup>cde</sup>	36.90	37.30	37.60	38.00	37.45 <sup>bc</sup>	36.80	37.25	38.25	38.05	37.58 <sup>c</sup>
9	36.70	37.60	38.50	37.10	37.61 <sup>bd</sup>	36.50	37.20	37.40	37.10	37.02 <sup>a</sup>	36.60	37.40	37.95	37.40	37.31 <sup>ab</sup>
<b>Average</b>	<b>36.54<sup>a</sup></b>	<b>37.72<sup>bc</sup></b>	<b>38.86</b>	<b>38.19<sup>d</sup></b>		<b>36.63<sup>a</sup></b>	<b>37.81<sup>c</sup></b>	<b>37.57<sup>b</sup></b>	<b>38.08<sup>d</sup></b>		<b>36.58</b>	<b>37.77</b>	<b>38.21<sup>a</sup></b>	<b>38.14<sup>a</sup></b>	
<b>Average</b>					<b>37.83</b>					<b>37.52</b>					<b>37.68</b>

Means bearing same superscripts do not differ significantly

Table 2. Week wise means for respiration rate (Counts/minute) in Murrah buffaloes.

Weeks	Control				High pressure fogger				Pooled						
	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.
1	19.85	21.80	26.10	21.80	22.39	19.65	20.80	22.20	20.70	20.84	19.75	21.30	24.15	21.25	21.61 <sup>a</sup>
2	20.45	22.40	27.55	22.05	23.11	20.20	21.61	23.65	21.45	21.73	20.33	22.00	25.60	21.75	22.42 <sup>b</sup>
3	20.25	22.65	28.25	22.25	23.35	20.40	21.80	23.80	21.50	21.88	20.33	22.23	26.03	21.88	22.61 <sup>b</sup>
4	20.70	22.20	28.20	21.80	23.23	20.85	22.25	23.80	21.80	22.18	20.78	22.23	26.00	21.80	22.70 <sup>b</sup>
5	20.45	22.30	29.15	22.15	23.51	20.30	22.35	23.55	21.15	21.84	20.38	22.33	26.35	21.65	22.68 <sup>b</sup>
6	20.30	22.35	28.00	22.10	23.19	20.50	22.10	23.65	21.35	21.90	20.40	22.23	25.83	21.73	22.54 <sup>b</sup>
7	20.75	22.70	26.95	22.00	23.10	20.75	22.45	22.60	21.25	22.01	20.75	22.58	25.28	21.63	22.56 <sup>b</sup>
8	20.40	22.60	25.85	21.95	22.70	20.05	21.65	22.40	20.55	21.16	20.23	22.13	24.13	21.25	21.93 <sup>a</sup>
9	20.70	22.35	25.05	21.80	22.48	20.05	21.40	22.20	20.30	20.99	20.38	21.88	23.63	21.05	21.73 <sup>a</sup>
<b>Average</b>	<b>20.43<sup>a</sup></b>	<b>22.37<sup>c</sup></b>	<b>27.23</b>	<b>21.99<sup>bc</sup></b>		<b>20.31<sup>a</sup></b>	<b>21.82<sup>b</sup></b>	<b>23.21</b>	<b>21.12</b>		<b>20.37<sup>a</sup></b>	<b>22.10<sup>b</sup></b>	<b>25.22<sup>c</sup></b>	<b>21.55<sup>d</sup></b>	
<b>Average</b>					<b>23.01</b>					<b>21.61</b>					<b>22.31</b>

Means bearing same superscripts do not differ significantly

Table 3. Week wise means and standard error for milk yield in Murrah buffaloes.

Weeks	Control	High pressure fogger	Pooled
1	5.311	6.105	5.708 <sup>bcd</sup>
2	5.614	6.095	5.854 <sup>d</sup>
3	5.568	5.916	5.742 <sup>cd</sup>
4	5.689	5.743	5.716 <sup>cd</sup>
5	5.595	5.482	5.538 <sup>ac</sup>
6	5.170	5.365	5.268 <sup>a</sup>
7	5.355	5.572	5.463 <sup>ab</sup>
8	4.521	4.883	4.702
9	5.067	5.509	5.288 <sup>a</sup>
<b>Average</b>	<b>5.321 ± 0.123</b>	<b>5.630 ± 0.129</b>	<b>5.476 ± 0.118</b>

Means bearing same superscripts do not differ significantly



Figure 1.

## CONCLUSION

The HPFF system played important role in body comfort of animal which is evident from the lower body temperature and respiration rate of buffaloes under this system by 1.24°C and 4.01 counts/minute respectively, at the peak hot period (2 pm). The HPFF system group yielded significantly more milk, 0.309 kg per animal/day. Therefore, it is concluded that the HPFF system was beneficial in terms of body comfort of the animals as well as increasing milk yield in Murrah buffaloes.

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## INFLUENCE OF SUMMER MANAGEMENTAL PRACTICES ON PHYSIOLOGICAL RESPONSES AND TEMPERAMENT IN MURRAH BUFFALOES

**P.B. Rahangdale, D.R. Ambulkar\* and R.D. Somnathe**

### ABSTRACT

The present study was conducted at Udgirkar Dairy farm, Dongargaon, Tal. Akola, Dist. Akola, (M.S.). The wallowing and splashing had highly significant effects ( $P < 0.01$ ) on respiration rate. The overall mean respiration rate of Murrah buffaloes under wallowing, splashing and control group was 28.78 counts/minute, 28.49 counts/minute, and 29.68 counts/minute, respectively. However, its effect on body temperature and temperament score was non-significant.

The time had also highly significant ( $P < 0.01$ ) effect on respiration rate and body temperature. The respiration rate being lowest (24.44 counts/minute) at 6 am and highest (33.54 counts/minute) at 2 pm. A similar trend was observed for body temperature; the lowest body temperature (98.93°F) and the highest (100.54°F) were recorded at 6 am and 2 pm, respectively. But the time of milking had non-significant effect on temperament score.

Eighty percent of the buffaloes in the control group (without any cooling practice) exhibited slightly restless temperament but when these buffaloes were subjected to wallowing and splashing, 20 and 33.33 percent of buffaloes expressed docile temperament, respectively. The average temperament score for Murrah buffaloes

maintained under wallowing, splashing, control group and pooled population was found to be  $2.13 \pm 0.08$ ,  $2.01 \pm 0.05$ ,  $2.17 \pm 0.07$  and  $2.11 \pm 0.06$ , respectively.

**Keywords:** respiration rate, body temperature, temperament score

### INTRODUCTION

It is well known that adequate heat dissipation is essential for the maintenance of normal body temperature and normal functioning of the organs (Verma and Hussain, 1991). High ambient temperature and humidity causes great discomfort to buffaloes. These may affect dairy temperament and physiological responses in buffaloes. During the summer, wetting the buffaloes can effectively reduce the rise in body temperature, respiration rate, and temperament scores, through cooling, which can be achieved by wallowing, splashing, showering, sprinkling, mud plastering. Access to water or shade is essential for the well being of buffaloes. Buffaloes developed the habit of wallowing to cool their bodies for the normal functioning of their organs. It is an established fact that the atmospheric temperature and humidity are responsible for the variation in the physiological

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reactions, milk yield, feed intake and reproductive performance.

## MATERIALS AND METHODS

The present study was conducted at “Udgirkar Dairy Farm”, Dongargaon, Tal. Akola, Dist. Akola (M.S.) The experiment was conducted during the hottest period of the summer season for the duration of six weeks, 28<sup>th</sup> April to 4<sup>th</sup> June, 2006. For the study, 15 lactating Murrah buffaloes of similar age group, body weight and stage of lactation (mid-lactation) were selected. The buffaloes were housed under a conventional housing system. Data recorded on the days on which wallowing or splashing of water was practiced were treated as treatment group while data recorded on day on which no wallowing or splashing of water were practiced were treated as ‘control’.

### 1) Wallowing

All the riverine buffaloes have a natural predilection for water, and they enjoy wallowing in water ponds. A “Kaccha” water tank (40’x40’x10’) was constructed by the side of the farm. The selected animals were allowed a wallowing period of one hour during day time. In this tank selected animal were left for the wallowing for one hour in a day (13.00-14.00 h).

### 2) Splashing of water

Selected buffaloes were subjected to splashing of water on their body surfaces four times a day; water was applied with a flexible PVC pipe on the whole body surface of the animal. The treatment was given four times a day (at 9.00, 12.00, 15.00 and 18.00 h)

## RESULTS AND DISCUSSION

### BODY TEMPERATURE

#### 1. Effect of wallowing and splashing on body temperature

The average body temperature in the Murrah buffaloes was recorded as  $99.72 \pm 0.12^{\circ}\text{F}$ . The Murrah buffaloes maintained under wallowing and splashing showed a statistically non-significant effect on body temperature. The average body temperatures recorded of wallowing, splashing and control groups were  $99.71 \pm 0.12^{\circ}\text{F}$ ,  $99.71 \pm 0.12^{\circ}\text{F}$  and  $99.76 \pm 0.13^{\circ}\text{F}$ , respectively.

The present findings are not in agreement with the results obtained by Huang *et al.* (1987), Patel *et al.* (1990), Verma *et al.* (1990), Chen *et al.* (1993), Vijay Kumar *et al.* (1998); Pippal *et al.* (2006) as they reported significant effects on body temperature due wallowing and splashing of water on buffaloes.

#### 2. Effect of weeks on body temperature

There was highly significant effect ( $P < 0.01$ ) of weeks on the body temperature of the Murrah buffaloes. The lowest body temperature,  $99.34^{\circ}\text{F}$ , was observed in the last week of the experiment, i.e., the month of June, and the highest body temperature,  $100.15^{\circ}\text{F}$ , was recorded in the second week of the experiment, i.e., the month of May. This suggested that the environment influenced the thermoregulation mechanism of the Murrah buffaloes significantly. The present findings are in agreement with those reported by Roussel *et al.* (1970b) who studied zone cooling in cows.

#### 3. Effect of time on body temperature

The time had also highly significant ( $P < 0.01$ ) effect on the body temperature of the

Murrah buffaloes. The lowest body temperature, 98.93°F, was recorded at 6 am. The temperature then gradually but significantly increased to 99.62°F at 10 am and then it achieved the significantly highest level of 100.54°F at 2 pm, and at 6 pm it decreased significantly to lower level of 99.80°F. There was rise in body temperature to the extent of 1.63°F in the afternoon hours over the morning hours indicating discomfort to the animals. This suggested the necessity of cooling practice during the afternoon hours for body comfort of buffaloes. The present findings are in agreement with Bempong and Gupta (1989); Thiagarajan and Thomas (1992), who reported higher body temperature during afternoon hours than morning hours in cows. Verma and Hussain (1991) also reported that the morning values for body temperature were lower than the evening values in Murrah buffaloes.

## Respiration rate

### 1. Effect of wallowing and splashing on respiration rate

Wallowing and splashing had a highly significant ( $P < 0.01$ ) effect on respiration rate in Murrah buffaloes. The overall average respiration rate during the experimental period was  $28.98 \pm 1.39$  counts/minute. The average respiration rate in the Murrah buffaloes subjected to wallowing and splashing was found to be  $28.78 \pm 1.30$  and  $28.49 \pm 1.44$  counts/minute, respectively, which was significantly lower than the respiration rate observed in control group, i.e.,  $29.68 \pm 1.48$  counts/minute. This indicated that wallowing and splashing have beneficial effects on body comfort during the summer months.

Similar results were recorded by Sastry *et al.* (1973) in Murrah heifers. Patel and Dave (1990) also reported significant effect of splashing of water on respiration rate in crossbred heifers. Verma *et*

*al.* (1990) reported a significantly lower respiration rate with water application in Murrah buffaloes, Chen *et al.* (1993) also observed a significantly lower respiration rate by evaporative cooling of dairy cows, as did Vijay Kumar *et al.* (1998) in Murrah buffaloes. Tarazon Herrera *et al.* (1999); Pippal *et al.* (2006) also stated that wallowing and splashings were effective in lowering respiration in buffaloes.

### 2. Effect of week on respiration rate

The week had a highly significant effect ( $P < 0.01$ ) on respiration rate in the Murrah buffaloes. The lowest respiration rate (23.70 counts/minute) was observed in the first week of the experiment, i.e., the month of April, and the highest respiration rate (33.23 counts/minute) was observed during the third week of the experimental period, i.e., the month of May. This suggested that the respiratory mechanism of buffaloes was influenced by the climatic condition during the summer months.

Effect of Interaction (week and group) on respiration rate was also found significant ( $P < 0.05$ ) in Murrah buffaloes (Figure 1).

The present findings are in agreement with those reported by Roussel *et al.* (1970b), who studied zone cooling of cows.

### 3. Effect of time on respiration rate

There was a highly significant ( $P < 0.01$ ) effect of time on respiration rate in Murrah buffaloes. The respiration rate varied significantly due to time. The lowest respiration rate ( $21.45 \pm 0.67$  count/minute) was observed at 6 am the rate then significantly increased to ( $28.87 \pm 1.49$  counts/minute) at 10 am and it achieved the significantly highest level ( $33.55 \pm 1.97$  counts/minute) at 2 pm and gradually decreased to ( $29.07 \pm 1.75$  counts/minute) at 6 pm. The change in respiration rate from am to pm indicated that respiration rate of Murrah buffaloes increased with rise of ambient

temperature in all the three groups.

The present findings are in agreement with those of the previous workers, Bempong and Gupta (1989), who reported a higher respiration rate at 3 pm in cows, Verma and Hussain (1991), who reported a higher respiration rate in the evening than in the morning in Murrah buffaloes, and Vijay Kumar *et al.* (1998), who observed the highest respiration rate at 2 pm in Murrah buffaloes.

### Temperament score

#### 1. Relative frequency of temperamental behaviour

It is evident from the table that 80 percent of the animals in the control group exhibited slightly restless behaviour followed by docile (6.67 percent) and restless (13.33 percent) behavior. In the wallowing group, 20 percent, 66.67 percent and 13.33 percent of the animals exhibited docile, slightly restless and restless behavior, respectively. In the splashing group, 33.33, 60 and 6.67 percent animals exhibited docile, slightly restless and restless behaviour, respectively.

It is observed from the table that the highest proportion (80 percent) of the animals that exhibited a slightly restless temperament were in the control group. While when the same group of animals was shifted for wallowing and splashing practice, large proportions 20 percent and 33.33 percent, respectively of the animal exhibited docile behavior. This suggested that the animals were comfortable under splashing and wallowing practice.

#### 2. Effect of wallowing and splashing on temperament score

There was non-significant effect of wallowing and splashing on temperament score in the Murrah buffaloes. The overall mean temperament score was found to be  $2.11 \pm 0.06$  in the Murrah buffaloes during the experimental period. The average temperament score of the Murrah buffaloes maintained under wallowing and splashing of water was  $2.13 \pm 0.08$  and  $2.01 \pm 0.05$ , which was lower than the temperament score of the

Table 1. Temperament-wise frequencies and percentage values for Murrah buffaloes under wallowing and splashing.

Treatment	Number of animals	Temperament and temperament score				
		1	2	3	4	5
Wallowing	15	3 (20.00)	10 (66.67)	2 (13.33)	0.00 (0.00)	0.00 (0.00)
Splashing	15	5 (33.33)	9 (60.00)	1 (6.67)	0.00 (0.00)	0.00 (0.00)
Control	15	1 (6.67)	12 (80.00)	2 (13.33)	0.00 (0.00)	0.00 (0.00)
Average	15	3 (20.00)	10.33 (68.89)	1.67 (11.11)	0.00 (0.00)	0.00 (0.00)

(Figures in parentheses represent percentage values)

Table 2. Week and timewise mean for body temperature (°F) in Murrah buffaloes under different treatments group.

Week	Wallowing					Splashing					Control					Pooled				
	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.
1	99.03	99.59	100.71	99.92	99.81	98.93	99.63	100.65	99.98	99.80	98.87	99.50	100.35	99.40	99.53	98.94	99.58	100.57	99.77	99.71 <sup>a</sup>
2	98.76	100.00	101.19	100.55	100.13	98.92	100.14	100.77	100.53	100.09	99.15	100.43	101.08	100.27	100.23	98.94	100.19	101.01	100.45	100.15
3	98.68	99.76	100.97	99.99	99.85	99.07	99.89	100.80	99.83	99.90	98.81	99.93	100.92	100.07	99.93	98.85	99.86	100.90	99.96	99.89
4	99.03	99.54	100.44	99.76	99.69	99.00	99.22	100.44	99.81	99.62	99.33	99.93	100.41	99.92	99.90	99.12	99.56	100.43	99.83	99.74 <sup>a</sup>
5	98.99	99.24	100.27	99.53	99.51	98.99	99.29	100.35	99.57	99.55	98.87	99.17	100.44	99.39	99.47	98.95	99.23	100.35	99.50	99.51
6	98.71	99.19	99.87	99.27	99.26	98.67	99.32	99.89	99.25	99.28	98.83	99.46	100.23	99.42	99.49	98.74	99.32	100.00	99.31	99.34
Avg.	98.87 ± 0.07	99.55 ± 0.13	100.58 ± 0.20	99.84 ± 0.18		98.93 ± 0.06	99.58 ± 0.15	100.48 ± 0.14	99.83 ± 0.18		98.98 ± 0.09	99.74 ± 0.18	100.57 ± 0.14	99.74 ± 0.16		98.93 ± 0.05	99.62 ± 0.15	100.54 ± 0.15	99.80 ± 0.16	
Avg. for group					99.71 ± 0.12					99.71 ± 0.12					99.76 ± 0.13					99.72 ± 0.12

Means bearing same superscript do not differ significantly

Table 3. Week and timewise mean for respiration rate (counts/minute) in Murrah buffaloes under different treatment groups.

Week	Wallowing					Splashing					Control					Pooled					
	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 pm	Avg.	6 am	10 am	2 pm	6 p.m.	Avg.	
1	24.40	27.80	31.60	27.33	27.78	23.80	26.73	30.60	28.00	27.28	21.93	25.80	32.60	27.67	27.00	23.38	26.78	31.60	27.67	27.36 <sup>a</sup>	
2	24.40	30.27	37.40	32.00	31.02	24.33	29.40	38.67	33.27	31.42	25.53	32.07	41.00	35.20	33.45	24.76	30.58	39.02	33.49	31.96	
3	25.53	35.40	36.47	35.53	33.23	26.33	35.73	36.13	32.87	32.77	25.33	33.80	39.80	35.87	33.70	25.73	34.98	37.47	34.76	33.23	
4	25.67	27.87	34.13	27.33	28.75	26.33	27.27	32.60	27.53	28.43	25.33	28.47	34.53	29.00	29.33	25.78	27.87	33.76	27.96	28.84 <sup>b</sup>	
5	24.53	27.93	33.07	26.40	27.98	25.27	27.73	33.07	27.13	28.30	26.40	30.33	35.93	27.93	30.15	25.40	28.67	34.02	27.16	28.81 <sup>b</sup>	
6	22.47	24.60	25.13	23.47	23.92	21.40	23.13	24.13	22.27	22.73	21.07	25.27	27.00	24.47	24.45	21.64	24.33	25.42	23.40	23.70 <sup>a</sup>	
Avg.	24.50 ± 0.47	28.98 ± 1.49	32.97 ± 1.80	28.68 ± 1.78		24.58 ± 0.76	28.33 ± 1.71	32.53 ± 2.05	28.51 ± 1.68		24.27 ± 0.90	29.29 ± 1.40	35.14 ± 2.09	30.02 ± 1.86		24.45 ± 0.67	28.87 ± 1.49	33.55 ± 1.97	29.07 ± 1.75		
Avg. for group					28.78 <sup>a</sup> ± 1.30					28.49 <sup>a</sup> ± 1.44					29.68 <sup>a</sup> ± 1.48					28.98 ± 1.39	

Means bearing same superscript do not differ significantly

Murrah buffaloes maintained without any cooling ( $2.17 \pm 0.07$ ).

The average temperament score observed in the present study was similar to that reported by Rahman *et al.* (1988); Chahande (1992). However, the present findings are not in consonance with Dash *et al.* (1976), Basu *et al.* (1978); Nayak Mishra (1984); Gupta *et al.* (1988); Reddy and Thripathi (1987); Sastry *et al.* (1988) they found lower temperament score values.

### 3. Effect of week on temperament score

Week had a significant ( $P < 0.05$ ) effect on temperament score in the Murrah buffaloes. The highest temperament score (2.28) was recorded during the third week of May, i.e., the fourth week of the experimental period, and the lowest temperament score (1.87) was recorded in the first week of the experimental period, i.e., in the last month of April. This indicated that temperament score in the Murrah buffaloes varied significantly due to change in climatic variables during summer.

### 4. Effect of time of milking on temperament score

Time of milking had a non-significant

effect on temperament score of the Murrah buffaloes. The highest temperament score  $2.14 \pm 0.05$  was observed during the evening milking at 17:00 h and the lowest  $2.07 \pm 0.07$  at the morning milking at 5:00 h. Such variation in temperament might be due to wide variation in micro-climate during the early morning and afternoon hours during the summer months; however, this variation in temperament score during morning and evening milking was statistically non-significant. Similar findings are also reported Ambulkar and Ali (2002), who found that time of milking did not affect temperament score.

## CONCLUSION

Wallowing and splashing practice had a non-significant effect on body temperature of the Murrah buffaloes, but it had played important role in body comfort of animal which is evident significantly by lower respiration rate of buffaloes under wallowing and splashing practice by 2.17 counts/minute, 2.61 counts/minute, respectively at peak hot period (at 2 pm) Eighty percent of



Figure 1.

the buffaloes control group (without any cooling practice) exhibited slightly restless temperament but only 66.67 and 60.00 percent of the same buffaloes when subjected to wallowing and splashing practice exhibited slightly restless temperament, and there was remarkable change in temperament as 20.00 and 33.33 percent of animals in the wallowing and splashing groups exhibited docile temperament as against 6.67 percent in the control. Therefore, it is concluded that the wallowing and splashing practice has a beneficial effect in reducing temperament score.

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## COMPARATIVE ANALYSIS OF FOLLICULAR AND LUTEAL DYNAMICS IN OESTROUS CYCLES OF BUFFALOES AND CROSSBRED CATTLE

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## ABSTRACT

The ovarian follicular and luteal developmental patterns of Jersey crossbred cattle (n = 6) and Murrah graded buffaloes (n = 6) were compared to test the hypothesis that the reduced reproductive efficiency in buffaloes when compared with crossbred cattle might be due to variations in follicular and luteal dynamics. The follicular and luteal developmental patterns were studied in two consecutive cycles (n = 12) in all the animals. Ultrasonographic monitoring of ovarian follicular development throughout the oestrous cycle in crossbred cattle revealed that three (25 percent) and nine (75 percent) cycles had two waves and three waves, respectively. In buffaloes, two (16.7 percent) and ten (83.3 percent) cycles had two waves and three waves, respectively. In buffaloes, the dominant follicle (DF) of anovulatory waves reached the maximum size earlier and remained in the static phase for a significantly (P < 0.05) greater number of days (2.0-2.2 days) than in crossbred cows (0.67-1.67 days), which indicated early loss of LH receptors in the dominant follicle (DF) of buffaloes. The mean maximum diameter of the final wave DF (ovulatory) was significantly (P < 0.05) smaller ( $10.9 \pm 0.7$  mm) and its growth

rate was comparatively slower ( $1.73 \pm 0.10$  mm / day) in buffaloes when compared with crossbred cattle ( $13.33 \pm 0.72$ mm and  $1.95 \pm 0.30$  mm / day respectively). The smaller diameter and slow growth rate of the ovulatory follicle might be the cause for suboestrus and smaller CL in buffaloes. Thus follicular and luteal dynamics of buffaloes differ significantly in various vital parameters when compared with crossbred cattle, and this might contribute to their lower reproductive efficiency.

**Keywords:** ultrasonography, follicular waves, corpus luteum, crossbred cows, graded buffaloes

## INTRODUCTION

The world buffalo population can be estimated to be roughly 170 million head, of which approximately 97 percent is in Asian countries (Presicce, 2007). Buffaloes are the backbone of the dairy industry in India contributing above 55 percent of total milk production. However, because of their poor breeding capabilities (late maturity, poor oestrus expression, prolonged calving intervals and seasonal reproductive patterns) there has been minimal genetic selection for fertility in buffaloes

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when compared with cattle. In order to optimize buffalo reproduction, the physiological controls of recruitment, selection, growth, dominance and atresia of ovarian follicles need to be better understood. Since manual palpation of genitalia per rectum is not completely accurate in buffaloes because of the anatomical disposition and smaller structures of the ovaries (Saini *et al.*, 2007), the accuracy of real-time ultrasonography is a reliable method for arriving at the follicular and luteal developmental pattern in buffaloes. Extensive ultrasonographic studies have shown clear patterns of follicular dynamics in cattle and thus provided the basis for improving fertility and synchronizing oestrus with more precision in this species. These established reproductive management techniques in cattle can be successfully applied to buffalo because of similarities in the anatomy, physiology and endocrinology of reproduction between the two genera. Even though there have been reports comparing the gross reproductive parameters of buffalo and cattle (Drost, 2007), specific comparative analysis of follicular and luteal development between these two species are lacking. Hence, this study was undertaken to test the hypothesis that the reduced reproductive efficiency in water buffaloes (*Bubalus bubalis*) might be due to variations in follicular and luteal dynamics when compared with crossbred cattle (*Bos taurus* x *Bos indicus*).

## MATERIALS AND METHODS

Healthy Jersey crossbred pluriparous cows (n = 6) and Murrah graded pluriparous buffaloes (n = 6), aged 5-6 years, maintained at the Centralised Embryo Biotechnology Unit, Department of Animal Biotechnology,

Madras Veterinary College, Madhavaram, Chennai were selected for the study. All the experimental cows and buffaloes were maintained under ideal and identical stall fed conditions throughout the study. They were fed with adequate concentrates, green fodder, and paddy straw and had access to water *ad libidum*. They were monitored regularly for oestrus symptoms, and cyclicity of the animals was confirmed by frequent gynaeco-clinical examination.

The follicular and luteal developmental pattern was studied ultrasonographically in two consecutive cycles (n = 12) in all the animals. The ovaries of each cow / buffalo were examined ultrasonically every other day throughout an oestrous cycle starting from observed oestrus (Day 0) to the subsequent oestrus (Sianangama and Rajamahendran, 1996) using a real time B-mode ultrasound scanner (SONOVET 600) equipped with 7.5 MHz transrectal transducer. Data collection involved recording length and width of all detectable follicles  $\geq 4$  mm in each ovary and corpus luteum (CL) with the inbuilt scale provided with the ultrasound instrument. The diameters of each follicle and CL were determined by taking the mean of the length and width of the respective structures (Zeitoun *et al.*, 1996).

### *Study of follicular dynamics*

Each ovary was scanned and imaged in more than one plane to assure that all measurable follicles of  $\geq 4$  mm in diameter were detected. The follicle, which exceeded the diameter of all other recruited follicles of a wave by  $\geq 2$ mm and reached the maximum diameter, was determined as the dominant follicle (DF). The day of wave emergence was determined as the day the DF was first detected or retrospectively identified at a diameter of 4 mm

(Bo *et al.*, 1993). If the follicle was not detected until it was  $\geq 5$  mm, a growth rate of 1.5 mm / 24 h was used to retrospectively determine the first examination when the follicle would have been 4.0 mm (Bergefelt *et al.*, 2003). The growth, static and regression phases of DF during various waves were arrived at as described by Savio *et al.* (1988). A sketch of the ovaries was made recording the location and diameter of the individual identified follicles of  $\geq 4$  mm. The single data set was used to profile the day-to-day diameters of individual follicles by the identity method, as described by Ginther (1993) and pattern of follicular waves were arrived at.

#### ***Study of luteal dynamics***

After oestrus, ovulation was confirmed when the ovulatory follicle was no longer seen in the subsequent examination and was retrospectively confirmed with the visible recognition of luteal tissue in the same location (Kim and Kim, 2007). As in follicular study, the CL was also mapped during each examination and diameter was arrived at. The developmental pattern of the CL, the day at which it attained the maximum diameter and the regression pattern were recorded (Taponen *et al.*, 2000). Corpus luteum area (CLA) during the mid cycle (Day 10) was calculated using a formula: CL length $\times$ 0.5 $\times$ CL width $\times$ 0.5 $\times$ 3.14 (Kastelic *et al.*, 1990).

#### ***Statistical analysis***

Data on follicular and luteal characteristics in normal oestrous cycles of cows and buffaloes were analysed by student's *t*-test and by analysis of variance (ANOVA) with completely randomised design (Snedecor and Cochran, 1994). SPSS.10.0® software was used for analysis of data.

## **RESULTS**

#### ***Number of follicular waves***

Ultrasonographic monitoring of normal follicular wave pattern in crossbred cattle revealed that, nine (75.0 percent) and three (25.0 percent) oestrous cycles had three-and two-follicular wave patterns, respectively. In buffaloes, ten (83.3 percent) and two (16.7 percent) oestrous cycles had three- and two-waves, respectively. The incidence of three-wave cycles was significantly higher ( $P < 0.01$ ) than two-wave cycles in both cattle and buffaloes. Since the incidence of two-wave cycles was statistically insignificant, only three-wave cycles were taken for comparative analysis between crossbred cattle and buffaloes.

The follicular wave pattern and characteristics of DFs of various waves during the oestrous cycle of Jersey crossbred cows and Murrah graded buffaloes are presented in Table 1.

#### ***Mean inter-oestrus interval***

The oestrous cycle length was found to be greater in crossbred cows ( $22.67 \pm 1.20$  days) than in buffaloes ( $21.80 \pm 0.54$  days), but there was no significant difference between them.

#### ***Follicular wave emergence***

The day of emergence of the first follicular wave was significantly ( $P < 0.05$ ) earlier (Day  $0.89 \pm 0.31$ ) and the emergence of second wave was significantly ( $P < 0.05$ ) later (Day  $9.44 \pm 0.34$ ) in crossbred cows when compared with buffaloes (Day  $1.80 \pm 0.26$  and  $8.60 \pm 0.20$  respectively). However, there was no significant difference in the day of emergence of the third wave (Day  $15.67 \pm 0.29$  and  $15.40 \pm 0.72$  respectively) between them.

**Characteristics of dominant follicles****Dominant follicles of anovulatory waves**

In buffaloes, the DF of the first-wave reached the maximum size significantly ( $P < 0.01$ ) earlier (Day  $5.80 \pm 0.50$ ) at a significantly ( $P < 0.01$ ) faster growth rate ( $2.10 \pm 0.14$  mm / day) and remained in the static phase for a significantly ( $P < 0.05$ ) greater number of days ( $2.20 \pm 0.70$ ) than in crossbred cattle (Day  $7.33 \pm 0.58$ ,  $1.58 \pm 0.09$  mm / day and  $1.67 \pm 0.66$  days respectively). Similarly, the second-wave DF reached the maximum size non-significantly earlier in buffaloes than in the crossbred cows and remained static for a significantly ( $P < 0.01$ ) longer duration ( $2.00 \pm 0.41$  vs  $0.44 \pm 0.29$  days respectively).

**Dominant follicle of ovulatory wave**

In the present study, the mean maximum diameter of third-wave DF (ovulatory follicle) was significantly ( $P < 0.01$ ) smaller in buffaloes ( $10.9 \pm 0.7$ ) when compared with the crossbred cattle ( $13.33 \pm 0.72$ ). The growth rate of ovulatory follicles was non-significantly slower in buffaloes.

**Characteristics of corpus luteum**

In the case of buffaloes, the CL reached the maximum diameter ( $17.04 \pm 0.36$  mm) on Day  $7.17 \pm 0.58$ , after which the size significantly ( $P > 0.01$ ) reduced to  $12.13 \pm 0.26$  mm on Day  $11.92 \pm 0.31$ , then gradually increased to  $15.29 \pm 0.38$  mm on Day  $16.92 \pm 0.45$  and regressed constantly thereafter. In crossbred cows, the CL grew to a mean maximum diameter of  $21.58 \pm 0.36$  mm on the mean Day of  $9.33 \pm 0.51$  and remained fluctuating around this diameter till Days 14 or 15 of the cycle. Initiation of constant luteal regression occurred from the mean Day of  $16.25 \pm 0.76$ .

The CL diameter and the CLA during the mid cycle (Day 10) in buffaloes were

$14.42 \pm 1.0$  mm and  $164.60 \pm 22.6$  mm<sup>2</sup>, respectively. The respective values in crossbred cattle were  $20.83 \pm 0.41$  mm and  $330.22 \pm 16.0$  mm<sup>2</sup>. Crossbred cows had a significantly ( $P > 0.01$ ) larger CL than buffaloes.

**DISCUSSION****Number of follicular waves**

In the present study, the incidence of three-wave cycles was significantly higher ( $P < 0.01$ ) than two-wave cycles in both cattle and buffaloes. Sirois and Fortune (1988) supported the three-wave hypothesis and stated that 80 percent of the oestrous cycles in cattle had more than two follicular waves. However, Singh *et al.* (1996) observed one, two and three follicular waves in 4.3, 65.2 and 30.5 percent of oestrous cycles, respectively, and reported that the average number of follicular waves in crossbred cows was  $2.3 \pm 0.1$  per cycle. The studies by Taneja *et al.* (1996) in buffaloes confirmed the development of the ovarian follicles occurring in one or two-waves per oestrous cycle. Subsequently, three-wave oestrous cycles were reported in Murrah buffaloes (Baruselli *et al.*, 1997; Warriach and Ahmad, 2007), but with higher incidence of two-wave cycles. However, in the present study, there was a significantly ( $P < 0.01$ ) higher incidence of three-wave cycles than two-wave cycles in concurrence with the observation of Barkawi *et al.* (2009) in buffaloes. The variations in number of follicular waves were attributed to many factors like, status of nutrition, lactation, environment etc. at the time of study (Lucy *et al.*, 1992). The days of wave emergence in cattle and buffaloes were similar to the reports of Ginther *et al.* (1989); Baruselli *et al.* (1997), respectively. Even though the oestrous cycle length

was found to be greater in crossbred cows ( $22.67 \pm 1.20$  days) than in buffaloes ( $21.80 \pm 0.54$  days), there was no significant difference between them. The findings coincided with the earlier reports of Malhi *et al.* (2005) in cattle and Drost (2007) in water buffaloes.

### ***Characteristics of dominant follicles***

It was reported that the first wave DFs have more consistent characteristics than the subsequent waves (Ginther *et al.*, 2003) providing ample flexibility for regularizing the oestrous cycle. On perusal of the data in the present study, it was obvious that the characteristics of DF of first follicular wave were significantly varying in several parameters between buffaloes and crossbred cattle.

In buffaloes, the DF of anovulatory waves, i.e., the first and the second waves reached the maximum size earlier and remained in the static phase for significantly greater number of days than in the crossbred cows. Rastegarnia *et al.* (2004) stated that there will be a decrease in the number of LH receptors in the follicles at the static and regression phases and attributed this feature for lower ovulatory response for gonadotrophin releasing hormone (GnRH) treatment. Thus it was evident that in buffaloes the DFs were losing their LH receptors earlier than crossbred cattle, and in turn, their capacity to respond to endogenous or exogenous luteinizing hormone. These factors should be taken into account when designing protocols for oestrous / and follicular wave synchronization studies in buffaloes.

The third-wave DF (ovulatory follicle) in buffaloes developed at a slow pace and reached a maximum diameter ( $10.9 \pm 0.7$ mm) which was significantly smaller than its counterpart in crossbred cattle ( $13.33 \pm 0.72$ mm). The individual observations were in accordance with the reports

of Taneja *et al.* (1996); Drost (2007). Suboestrus or silent oestrus constitutes the single largest problem affecting the reproductive efficiency in water buffaloes thereby increasing the inter-calving period. Awasthi *et al.* (2007) correlated the slower growth rate and smaller size of the ovulatory follicle with silent oestrus condition in buffaloes and suggested that the DF in animals with silent oestrus grew slowly due to lower availability of LH to developing follicle which affected the bioavailability or synthesis of various growth factors and/or their binding proteins needed for terminal growth of the follicle. As the expression of oestrus behavior was dependent on amount of oestrogen produced by granulosa cells, it might be attributed to impaired oestradiol production which in turn was dependant on androgen production from theca cells under the influence of LH. However, further research is needed to identify the factor(s) affecting the growth rate of the ovulatory follicle resulting in silent oestrus in buffaloes.

### ***Characteristics of corpus luteum***

Overall observation during an oestrous cycle revealed that the crossbred cows had a significantly ( $P > 0.01$ ) larger CL than buffaloes. Sartori *et al.* (2002) reported a significant positive correlation between size of the ovulatory follicle and size of the CL. It seems likely that increased ovulatory follicular size would lead to increased numbers of granulosa cells which differentiate into large luteal cells following the LH surge and subsequent increased size of the CL (Smith *et al.*, 1994). So the variation in size of the CL between these two species could be at least partially the result of the variation in the size of ovulatory follicles of previous cycles.

In crossbred cows, the CL grew to a peak diameter of  $21.58 \pm 0.36$  mm on the mean Day of

Table 1. Characteristics of dominant follicles of three-wave oestrous cycles in Murrah graded buffaloes and Jersey crossbred cattle.

WAVE NO.	Characteristics of dominant follicle	Three-wave oestrous cycles		Significance
		Buffaloes (n = 10)	Cows (n = 9)	
		Mean $\pm$ SE	Mean $\pm$ SE	
FIRST	Day of wave Emergence	1.80 $\pm$ 0.26	0.89 $\pm$ 0.31	*
	Day of maximum diameter	5.80 $\pm$ 0.50	7.33 $\pm$ 0.34	**
	Maximum diameter (mm)	10.0 $\pm$ 0.30	11.22 $\pm$ 0.42	*
	Growth Phase (days)	5.00 $\pm$ 0.40	7.33 $\pm$ 0.58	**
	Growth Rate (mm/day)	2.10 $\pm$ 0.14	1.58 $\pm$ 0.09	**
	Static Phase (days)	2.20 $\pm$ 0.70	1.67 $\pm$ 0.66	*
	Regression Phase(days)	7.00 $\pm$ 0.70	8.0 $\pm$ 0.71	NS
	Regression Rate (mm/day)	1.50 $\pm$ 0.23	1.47 $\pm$ 0.14	NS
SECOND	Day of wave Emergence	8.60 $\pm$ 0.20	9.44 $\pm$ 0.34	*
	Day of maximum diameter	14.00 $\pm$ 0.62	15.78 $\pm$ 0.70	NS
	Maximum diameter (mm)	10.40 $\pm$ 0.70	10.11 $\pm$ 0.40	NS
	Growth Phase (days)	7.20 $\pm$ 0.70	7.44 $\pm$ 0.50	NS
	Growth Rate (mm/day)	1.50 $\pm$ 0.10	1.42 $\pm$ 0.10	NS
	Static Phase (days)	2.00 $\pm$ 0.41	0.44 $\pm$ 0.29	**
	Regression Phase(days)	5.40 $\pm$ 0.52	4.89 $\pm$ 0.48	NS
	Regression Rate (mm/day)	1.96 $\pm$ 0.10	2.24 $\pm$ 0.25	NS
THIRD (ovulatory)	Day of wave Emergence	15.40 $\pm$ 0.72	15.67 $\pm$ 0.29	NS
	Day of maximum diameter	21.80 $\pm$ 0.54	22.67 $\pm$ 1.20	NS
	Maximum diameter(mm)	10.90 $\pm$ 0.71	13.33 $\pm$ 0.72	**
	Growth Phase(days)	6.20 $\pm$ 0.40	6.83 $\pm$ 1.86	NS
	Growth Rate (mm/day)	1.73 $\pm$ 0.10	1.95 $\pm$ 0.30	NS
Oestrous cycle length		21.80 $\pm$ 0.54	22.67 $\pm$ 1.20	NS

\*\* (P < 0.01)      \* (P < 0.05)      NS-Not significant (P > 0.05)

9.33 ± 0.51. Taponen *et al.* (2000) also recorded a similar mean maximum diameter of 22.7 ± 1.1 mm in Finnish Ayrshire breed cows and heifers, but the size was attained at a later stage of cycle (Days 11 or 12). Similar to our study they also observed a fluctuation around this diameter till Days 14 or 15 of the cycle. Initiation of luteal regression occurred from the mean Day of 16.25 ± 0.76 which corroborated with the observations of Sianangama and Rajamahendran (1996). In the case of buffaloes, the CL reached the maximum diameter (17.04 ± 0.36 mm) on Day 7.17 ± 0.58 in concurrence with the findings of Barkawi *et al.* (2009). Unlike in cattle, the CL size reduced significantly during the mid luteal phase and regained its growth attaining a second peak before entering the phase of constant regression. Thus there was a significant difference in the luteal developmental pattern between these two species. The CL diameter and the CLA during the mid cycle (Day 10) in buffaloes were 14.42 ± 1.0 mm and 164.60 ± 22.6 mm<sup>2</sup>, respectively. The respective values in crossbred cattle were 20.83 ± 0.41mm and 330.22 ± 16.0 mm<sup>2</sup>. Veronesi *et al.* (2002) stated that the degree of agreement between

plasma progesterone concentrations and diameter of CL was highly significant. Thus the smaller CL would have contributed for low progesterone secretion thereof during the mid luteal phase, a period which was critical for embryonic sustenance, in buffaloes when compared with crossbred cows.

From the foregoing observations, it was concluded that ovarian follicular dynamics of Murrah graded buffaloes differed from crossbred cattle with the significantly increased static phase of DFs of anovulatory waves, the smaller size and the slower growth rate of ovulatory follicle. The resultant smaller corpus luteum too experienced a drastic fluctuation in developmental pattern during the mid luteal phase. These cumulative factors could be contributing for the lowered reproductive efficiency in buffaloes.

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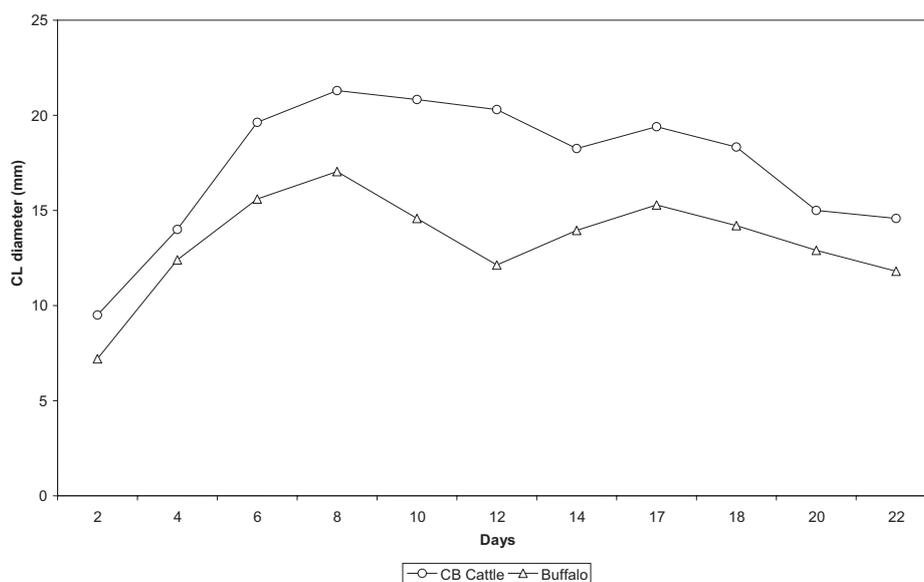


Figure 1. Luteal dynamics in rossbred cattle and buffaloes.

the project 'Transcriptional level of developmentally important genes in buffalo preimplantation embryos' for carrying out this research work.

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## FROZEN SEMEN PRODUCTION PERFORMANCE OF MURRAH BUFFALO BULLS

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**ABSTRACT**

A study was conducted to evaluate semen output characteristics of 3,933 ejaculates of 36 Murrah buffalo bulls maintained at the Artificial Breeding Complex, NDRI, Karnal, India. The objective of this study was to estimate expected frozen semen dose production. Age of bulls at collection during the study period (1996 to 2006) ranged from 2.31 to 7.36 years, with a mean of  $4.46 \pm 0.22$  years. The average ejaculate volume (VOL, ml), mass activity (MA), percent motility (IM), sperm concentration per ml (SPC, millions) and total sperm output per ejaculate (SPCE, millions) were  $2.58 \pm 0.09$  (1.79 - 3.61 ml);  $2.88 \pm 0.02$ ; (2.62 - 3.19);  $66.63 \pm 0.44\%$  (61.58 - 71.93%);  $998 \pm 10.90$  (853.49 - 1125.23) and  $2561.05 \pm 77.80$  (1783.59 - 3545.20, respectively). The average dilution rate was found to be 1:  $12.49 \pm 0.13$ , ranging from 1:10.67 to 14.07. The expected number of ejaculates that could be frozen per year per bull was 53.27 (21 - 74) and correspondingly expected frozen doses produced per year per bull were 6,879.49 (2,826.31 - 12,550.00).

**Keywords:** Murrah, semen quality, expected no. of ejaculate frozen, dose produced

**INTRODUCTION**

With globalization, the dairy industry has witnessed an increased demand for semen from superior sires. India has the largest breeding infrastructure in the world (64 frozen semen bull stations and more than 54,000 AI centers). Presently, total semen production is around 30 million frozen semen straws. It is envisaged that 60% of the breedable bovine population will be covered by A.I. (XI<sup>th</sup> five year plan; which would require around 66 million straws) and the remaining 40% through natural service with bulls of high genetic merit. In India, the result of AI is much below the desired level. As such, A.I. as a tool for livestock development is hardly applicable to 15% to 20% of bovine population. Artificial insemination using frozen semen is now the most widespread tool employed nationwide for improving the genetic potential of livestock. Attempts are being

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intensified to increase the coverage of AI so as to exploit the full potential of the technology. The use of the best bulls is often restricted by the limited number of doses of semen produced as there are several inherent and functional constraints in realizing the breeding goals through AI. Apart from the fact that buffalo bulls are known for poor libido, there are also anatomical and physical limitations to production of quality germplasm with good number of viable sperms throughout the year. The relatively smaller testicular size, lower daily sperm production rate and epididymal sperm reserve in buffalo bulls compared to cattle are some of the natural inbuilt constraints of this species (Suryaprakasam and Narsimha, 1993; Sudheer and Xavier, 2000; Singh *et al.*, 2003). Besides these constraints, seasonal influences (Mondal *et al.*, 2000) and prophylactic measures (Kammar and Gangadhar, 1998; Murugavel *et al.*, 2000; Mathur *et al.*, 2003) also adversely affect semen production performance directly or indirectly. There is much wastage of superior germplasm due to poor semen quality, poor freezability; and poor libido (Suryaprakasam and Narsimha, 1993; Sudheer and Xavier, 2000) resulting in immense economic losses as well as reduction in genetic gain. To satisfy the increasing demand for semen from superior sires, the AI industry has to optimize the number of spermatozoa per dose of semen in order to produce maximum number of straws with optimum conception rate. The objective of the present study was to gather basic information on semen characteristics and expected frozen semen production in Murrah buffalo bulls, which will facilitate in planning semen stations considering all the managemental factors affecting semen production in the tropical regions.

## MATERIALS AND METHODS

Data on ejaculates of Murrah buffalo bulls were collected from the Artificial Breeding Complex, National Dairy Research Institute, Karnal, Haryana, India. In the AI unit, young bulls were evaluated for sperm quality and production capability. Tests for sexual performance begin at the onset of puberty. Subsequently, bulls are tested for semen donates and judged on sexual behavior, size of the testes and sperm production. Bulls were kept in individual pens under a loose housing system on a concrete floor with the orientation of its long axis in the east-west direction. The bulls were fed concentrate ration 2.5 kg per bull. Institute farm - grown seasonal green fodder such as maize, cowpea, berseam, jowar etc., depending on their availability, along with mixture of maize and oat silage during lean periods was available *ad lib* to the animals. The data were compiled on a total of 3,933 ejaculates of 36 Murrah buffalo bulls maintained during the period from 1996 to 2006 from the Artificial Breeding Complex, NDRI, Karnal, India. The ejaculates were collected by AV technique once a week with two ejaculates with a gap of 20 to 30 minutes. Information collected on each ejaculate included date of collection, ejaculate number for the day, volume of the ejaculate, mass activity, concentration of sperm per ml and percentage of motile sperm. Bulls not donating semen were excluded from the data set. The bulls which gave freezable quality semen for at least six month were considered in the present study. Out of 72 Murrah buffalo bulls, data on 36 MU buffalo bulls were used to calculate the expected frozen dose produced. Total sperm production (million), no. of ejaculate frozen/bull/year and frozen dose produced per bull per year were derived from the available information. In this experiment, the

expected frozen dose producing ability per bull per year was calculated by utilizing semen production data using standard statistical tools (Snedecor and Cochran, 1967).

## RESULTS AND DISCUSSION

The AI industry aims at maximizing production of semen doses from bulls of high genetic merit. Therefore, the expected frozen dose producing ability per bull per year was calculated on the basis of semen data of MU bulls. This information will help in planning semen station establishment to meet the growing demand for AI considering all the managerial factors affecting semen production in tropical climates. The results of the performance of Murrah buffalo bulls regarding seminal attributes and expected frozen semen dose production are depicted in Table 1.

Age of the bulls at collection during the study period (1996 to 2006) ranging from 2.31 to 7.36 years, with a mean of  $4.46 \pm 0.22$  years. The average semen volume of 36 Murrah bulls was  $2.58 \pm 0.09$  ml (ranging from 1.79 to 3.61 ml) which can be compared to findings of other workers (Prajapati, 1995; Mondal, 1998). However, several workers reported higher (Singh *et al.*, 1992; Misra *et al.*, 1994; Rao and Sreemannarayana, 1996; Shukla and Mishra, 2005; Ravimurugan *et al.* 2008) ejaculate volume. The average mass activity i.e.,  $2.88 \pm 0.02$  (ranging from 2.62 to 3.19) was similar to earlier findings (Bhakat, 1994; Mondal, 1998); however, others have reported higher mass activity (Ram, 1988; Dharmi, 1992; Shukla and Mishra, 2005). The average initial motility ( $66.63 \pm 0.44\%$ , ranging from 61.58 to 71.93%) in the present study was comparable to the findings of Kumar *et al.* (1993) and Ravimurugan *et al.* (2008). However,

some reports showed higher percentages of initial motility (Bhosrekar *et al.*, 1994; Misra *et al.*, 1994; Sahu and Pandit, 1997; Pandey, 2001; Shukla and Mishra, 2005). The average sperm concentration ( $998.91 \pm 10.90$  millions/ml, ranging from 853.49 to  $1125.23 \times 10^6$ /ml) observed in the present study was in agreement with the findings of Ram (1988) whereas higher (Misra *et al.*, 1994; Pratap *et al.*, 1999; Prajapati *et al.*, 2000; Shukla and Mishra, 2005) values as well as lower (Ravimurugan *et al.*, 2008) were recorded by other researchers. These higher estimates in comparison to our study may be due to the fact that their studies might have been based on selected bulls with very high semen producing ability. The average total sperm output of Murrah buffalo bulls was  $2,561.05 \pm 77.80 \times 10^6$  (ranging from  $1783.59$  to  $3545.20 \times 10^6$ ). The average dilution rate was found to be  $12.49 \pm 0.13$ , with a range of 10.67 to 14.07. The expected number of ejaculates that could be frozen per year per bull was 53.27 (ranging from 21 to 74) and correspondingly, the expected frozen doses produced per year per bull could be 6,879.49 (ranged 2826.31 to 12550). Zafar *et al.* (1988) reported yearly production to be 8,412 semen doses per bull in Nili-Ravi buffalo bulls, which was higher than the estimate for Murrah bulls in the present study. The present values were higher than those reported by Roy (2006) in Murrah bulls (5,147.48 doses/year/bull).

From these findings, it can be concluded that the expected number of ejaculates that could be frozen per bull per year was 53.27 (ranging from 21 to 74) correspondingly, the expected frozen doses produced per bull per year could be 6,879.49 (ranging from 2826.31 to 12550.00) in Murrah bulls.

The production of semen from these bulls can be further increased by certain managerial interventions, i.e. control of housing and

Table 1. Average semen volume, mass activity, motility, sperm concentration and dilution rate in Murrah buffalo bulls. (No. of bulls = 36).

Parameters	Mean	S.E.	Range
Average age (Yr.)	4.46	0.22	2.31 - 7.36
Average volume (ml)	2.58	0.09	1.79 - 3.61
Average mass activity (0 - 5 scale)	2.88	0.02	2.62 - 3.19
Average Initial motility (%)	66.63	0.44	61.58 - 71.93
Average concentration (million/ml)	998.91	10.90	853.49 - 1125.23
Average total sperm production million/ ejaculate)	2561.05	77.80	1783.59 - 3545.20
Average dilution rate	12.49	0.13	10.67 - 14.07
Calculated no. of ejaculate frozen/year/bull	53.27	2.25	21 - 74
Calculated frozen dose produced per year per bull	6879.49	393.80	2826.31 - 12550.00

environmental variation can be controlled through providing comfortable housing conditions throughout the years. Feeding management with supplementation of minerals and vitamins from calfhood as evident on our farm, and vaccination rescheduling and development of; new vaccines with less anaphylactic stress.

The variations in semen quality parameters recorded in the present investigation, which were well supported by earlier reports, may be due to individual variations (Saxena and Tripathi, 1978), ejaculate frequency (Nath, 1988), differences in age (Bhat *et al.*, 2002), genetic make up of the bulls (Tomar *et al.*, 1966), season of study (Tuli, 1984) and agro climatic conditions.

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