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IBIC, KASETSART UNIVERSITY,
P.O. BOX 1084, BANGKOK 10903, THAILAND
E-mail : libibic@ku.ac.th
Tel    : 66-2-9428616 ext. 344
Fax    : 66-2-9406688
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RECONSTRUCTION OF LACERATED WOUND ON UPPER LIP IN A GRADED MURRAH HEIFER – A CASE REPORT

G. Kamalakar*, R. Mahesh and V. Devi Prasad

ABSTRACT

A 4 year old Graded Murrah Buffalo heifer was presented with extensive lacerated wound on upper lip and nostril on right side that occurred due to an accident. It showed excessive salivation, restlessness, presence of blood clots. The animal was examined thoroughly and prepared for surgery. Reconstructive surgery of upper lip and cheek was carried out under mental nerve block and linear block using Lignocaine hydrochloride. Good surgical technique and effective post operative management made the case successful recovery.

Keywords: graded Murrah buffalo, lacerated wound, lip, cheek, reconstructive surgery

INTRODUCTION

A wound is defined as discontinuity or separation of skin, mucous membrane, or any tissue surface. Lacerated wounds occur in animals more commonly due to accidents, while crossing fencing wire, injuries by sharp objects like glass pieces. A lacerated wound presents torn and irregular edges (Venugopalan, 2009). These wounds may occur at different places of body like face, inguinal region, abdomen, thorax, legs, etc. Because of the nosiposture ruminants are very often subjected to open wounds on near facial region due to automobile accidents, barbed fencing wires, snake bites, etc. Anterior facial region needs special attention in the surgical repair owing to the presence of important structures like buccal nerves, dental pads, incisors, etc.

CASE HISTORY AND CLINICAL OBSERVATIONS

A 4 year old Graded Murrah Buffalo heifer was presented to the clinic with a history of an extensive lacerated wound on face involving right side of upper lip and cheek due to an accident occurred yesterday. The animal showed excessive salivation and was unable to take feed and water properly due to the injury. On thorough examination the lacerated wound involved right side of lip, cheek and commissures tearing buccinator and levator naso labialis muscles. The injury was of full thickness, with serrated margins and measured 14 cm. Clotted blood was observed on wound margins with little contamination (F1).

TREATMENT AND DISCUSSION

The animal was casted in left lateral recumbency and prepared for aseptic surgery. It
was given mental nerve block and linear infiltration with 2% Lignocaine hydrochloride along the wound margins on upper and lower sides. The injury was irrigated with 1:1000 Potassium permanganate lotion thoroughly to remove debris and clots. To make suturing perfect the skin and subcutis was separated by under trimming and controlling the bleeding points. First the muscle layers were apposed using chromic catgut in continuous manner through the centre of the wound. Strepto penicillin powder was sprinkled on entire wound and skin edges were sutured in horizontal mattress pattern using 1/0 braided silk. Paste made of Zinc oxide and tincture benzoin was applied on suture line. Injection Amoxicillin+ Cloxacillin 3 g, injection Ketoprofen 2 mg/kg BW, injection Tribivet was administered intra muscularly. Owner was advised to give more of liquid diet. Wound dressing was done every day with paste of zinc oxide and tincture iodine. Sutures were removed on 10th post operative day (F2).

Lacerated wounds near nostrils are common in working bullocks due to irritation by bull nose rope and frequency of nose piercing injuries was 64% (Alam et al., 2010). Here the injury caused by a wheel adjustment guide pointer of a car. Suturing in these cases was very difficult because, surgical procedure require mouth to be opened till the suturing was complete as the thickness of the wound involved inner surface of the upper lip and commissures.

A series of opposition sutures were placed using chromic catgut no. 0, joining buccinator, levator naso labialis muscles including fascia restoring the normal anatomical configuration of the anterior half of the rostral facial region. Then subcutaneous sutures were placed using no. 0 chromic catgut. Cutaneous wound was sutured using no. 1 black braided silk in horizontal mattress pattern. The animal was kept on Intravenous alimentation for 3 days in order to restrict the movement of the jaws which otherwise would have impaired wound healing. The wound healing was observed to have been protracted which was thought to be because of the involvement of micro organisms inhabitants of the oral cavity.

Atropine sulphate was given subcutaneously 0.04 mg/kg BW in order to create dry mouth condition thus augmenting wound healing. The wound was found to heal by cicatrisation and the wound contraction was minimal. The animal was said to have normal prehension and masticatory habits indicating a complete and uneventful recovery.

The horizontal movement of jaws during mastication and rumination together with the presence of normal ruminal fauna and flora interfere with normal wound healing. The ruminant saliva is rich in bicarbonate content thereby further delaying wound healing. Normally ruminants sink their rostral part of the face into the gutters for drinking soiling the sutured part. Further if the animals were let outside they find their way into the pond for wallowing. These factors further pose a challenge before a surgeon for wound healing. Hence, the intravenous alimentation used in this case may be attributed for relatively quick healing.

Chromic catgut was used as it was readily available and cheaper. To prevent infection injection Amoxicillin and to alleviate pain injection Ketoprofen was administered. For better cutaneous wound healing different authors used various agents like fibrin glue (Michel and Harmond, 1990), honey (Bergman et al., 1983), sea buck thorn ointment (Gupta, 2002) and obtained better granulation tissue formation, reepithelialisation and other favourable histo-pathological factors. In this case paste made of Zinc oxide and tincture
Figure 1. Photograph showing lacerated wound of lip and cheek. Observe blood clots and contamination.

Figure 2. Photograph taken at the time of suture removal. Observe clear apposition.
benzoin was used on first day as an anti coagulant and from next day onwards combination of zinc oxide and tincture iodine was used because of their better wound healing and antiseptic properties respectively. Topical application of zinc oxide enhances reepithelialisation of partial thickness wounds in pigs. The cell division in wounds is connected with increased demand for zinc due to its function in enzymes required for cellular replication and zinc found to be slightly mitogenic to epithelial cells (Agren et al., 1991).

REFERENCES


SURGICAL TECHNIQUE FOR THE MANAGEMENT OF OBSTRUCTIVE UROLITHIASIS IN A BUFFALO CALF: A CASE REPORT

Md. Moin Ansari

ABSTRACT

In the present study a technique of tube cystostomy using Foley’s catheter in terms of tolerance by the animal and overall outcome of the patient suffering from obstructive urolithiasis is reported and discussed. Foley’s catheter was blocked permanently when free flow of urine was observed through urethra. Post-operatively, the calf remained in good health. Foley’s catheter was removed by pulling after deflating its balloon on 12 days after the free flow of urine had established. The wound healed uneventful and small opening left after removal of Foley’s catheter was dressed antiseptically until healing. Tube cystostomy and oral administration of tablets ammonium chloride along with cystone were resulted in speedy and uneventful recovery.

Keywords: Foley’s catheter, tube cystostomy, obstructive urolithiasis, buffalo

INTRODUCTION

Urolithiasis is defined as the formation of uroliths as a consequence of multiple congenital and/ or acquired pathophysiological process that result in increased concentration of less soluble crystalloids in the urine (Osborne and Kruger, 1984). A single urolith/calculus is usually responsible for obstruction in cattle, but sheep are normally affected by multiple calculi blocking the urethra for several centimeters leading to rupture of urethra or urinary bladder. Fatality rate in urolithiasis due to rupture of the urethra or urinary bladder is very high and so is the economic impact of this disorder (Gasthuys et al., 1993; Radostitis et al., 2000). Tube cystostomy is a less expensive procedure, which can be performed easily, require less time and preserve breeding ability. However, problem of ascending infection, recurrent obstruction and displacement of the tube by the animal may occur (William and White, 1991). The utility of the tube cystostomy with medical dissolution of urethral/ cystic calculi has been reported (Singh, 2005; Ansari and Moulvi, 2009). The present paper describes obstructive urolithiasis and its surgical management in a male buffalo calf and puts on record.

CASE HISTORY AND OBSERVATIONS

A male buffalo calf of three and half months age presented with the complaint that there
was only few drops of urine passed or none at all for the last 72 h despite making painful attempts and showing signs of uneasiness. The calf was in poor condition and off-feed. Physical examination revealed there was marked pitting edema on ventral abdominal area and penis could not be extruded due to marked subcutaneous edema (Figure 1). Percutaneous massage of penis did not provide any relief except the removal of some minute particle through external orifice. Examination of the preputial orifice has disclosed chalky white flakes precipitated of the preputial hairs and resemble calculi in shape. On the basis of clinic-physical examinations the animal was diagnosed as suffering from obstructive urolithiasis. Keeping in view the fact of the complete blockage of the urinal passage may result in rupture of urethra or the bladder, it was decided to performed tube cystostomy using Foley’s catheter to correct the disorder.

**SURGICAL TECHNIQUE**

Paramedian anterior to the brim of the pubis was shaved and prepared for aseptic surgery. The animal was restrained in right dorsolateral recumbency. The animal was operated under lumbosacral epidural analgesia, induced with 3.5 ml of 2% lignocaine hydrochloride (Xylocaine, Astra-IDL, Bangluru). This is usually accomplished using the “hanging drop” or the “lack of resistance” technique during injection (Figure 2). Additional local infiltration analgesia at the site operation was done as and when required. An incision was made at the caudal-ventral abdomen, lateral and parallel to the penis. Abdominal muscles were separated by dissection. A Foley’s catheter (no.14) (Uro-cath, Romsons Medicons, Agra) was passed through a subcutaneous tunnel of about 8 inch in length parallel to the penis up to the level of preputial orifice and brought up to the site of the incision. A K-wire was anchored in the eye of Foley’s catheter and it was inserted into the bladder through its ventral aspect with a sudden thrust without incising the urinary bladder (Figure 3). After insertion of the catheter its balloon was inflated by infusing 10 ml of sterile physiological normal saline to prevent it from dislodgement from the bladder and the K-wire was pulled out slowly. The laparotomy incision was sutured in standard procedure and the catheter was fixed to the abdominal wall with simple interrupted sutures along the length of the tube (Figure 4). The post-operative care included daily dressing of the skin wound with 0.5% povidone iodine solution till healing. Antibiotic cover with injection Ampicillin plus Cloxacillin at the dose rate of 5 mg/kg (AC Vet, Intas Pharma, Ahmedabad) used twice in a day intramuscularly for 7 days, injection Meloxicam at the dose rate of 0.2 mg/kg (Melonex, Intas Pharma, Ahmedabad) body weight intramuscularly once daily for 5 days, injection vitamin A (Intavita, Intas Pharma, Ahmedabad) 2 ml intramuscularly on six day interval for three time. Tablet ammonium chloride 500 mg/kg body weight and tablet cystone 3 tablets in thrice in a day orally for 10 days were advocated. Foley’s catheter was blocked permanently when free flow of urine was observed through urethra. Post-operatively, the calf remained in good health. Foley’s catheter was removed by pulling after deflating its balloon 12 days after the free flow of urine had established. The wound healed uneventful and small opening left after removal of Foley’s catheter was dressed antiseptically until healing.
Figure 1. A buffalo calf suffering from urolithiasis.

Figure 2. Hanging drop technique during lumbosacral epidural analgesia.
Figure 3. Placement of Foley’s catheter into the bladder With the help of K-wire.

Figure 4. Fixing of the external part of the Catheter with abdominal wall after completion of tube cystostomy.
RESULTS AND DISCUSSION

The case was attended instantly without delay as the complete blockage of urinary passage may result in rupture of urethra or the bladder. The Foley’s tube cystostomy was performed in this as advocated by William and White (1991) in dog and cat. Foley’s catheter was much flexible and its inflated balloon or cuff covered the entire circumference of the catheter, which prevented leakage of urine. This is in agreement with the findings of Singh (2005). Foley’s catheter was well tolerated by the buffalo calf. The surgical maneuvering of the bladder through para-median was found easier when the calf was kept in dorsolateral recumbency as reported by Ansari (2005). Difficulty in placing the catheter and suturing of bladder as observed in other approaches (Prasad et al., 1978) could be overcome since the bladder lies very superficially and can be approached conveniently. The abdominal wound healed without any complication in 12 days and calf urinated through the urethra without any difficulty. Additional combination therapy can be considered more fruitful in combating uraemic toxaemia primarily due to bladder rupture. Ammonium chloride was used for acidification of the urine to induce dissolution of the calculi. The efficacy of ammonium chloride in the management of urolithiasis has been described by others also Jones et al. (2009). Cystone tablet by virtue of its marked diuretic action would have contributed to the diuresis. No occurrence of urolithiasis or other condition leading to retention of the urine was observed. Author is of the opinion that post-operative oral administration of ammonium chloride and cystone tablets might have helped to avoid recurrence. Similar observation has earlier been reported by Ansari (2005) in a cow calf.

REFERENCES

OCULAR SQUAMOUS CELL CARCINOMA IN A BUFFALO: A CASE REPORT

Deepak Kumar Tiwari¹, Sandeep Saharan¹, Satbir Sharma², R.N. Choudhary², Neelesh Sindhu², Vikas Jaglan¹ and Sandeep Potaliya¹

ABSTRACT

A four and half year old female murrah buffalo was referred to the Teaching Veterinary Clinical Complex (TVCC) with the history of growth on the nictitating membrane of the left eye near the medial canthus since one month. Animal showed little discomfort due to the growth but have normal vision. Neoplastic outgrowth was excised after ligating its base with 3-0 chromic catgut and lavaged with normal saline solution. The animal was recovered uneventfully within 15 days.

Keywords: buffalo, eye, squamous cell carcinoma

INTRODUCTION

Squamous cell carcinoma is a tumour of epidermal cells in which the cells show differentiation to keratinocytes. Squamous cell carcinoma is the most commonly occurring neoplasm afflicting the bovine eye (Fazili et al., 2001; Kohlirn and Mashadi, 2008; Sivaseelan et al., 2008). The most common areas affected are limbus (junction of the cornea and the sclera), third eyelid, and on the upper and lower eyelid margins primarily at muco-cutaneous junctions (Goldschmidt and Hendrick, 2002). The malignant tendency of this disease makes early recognition critical. The etiology of the disease is multifactorial. However, prolonged exposure to sunlight (ultraviolet light) also seems to be a driving force for the disease (Anderson and Badzioch, 1991).

This report communicates a case of ocular squamous cell carcinoma in a female buffalo, which was successfully treated by surgical intervention.

CASE HISTORY AND CLINICAL EXAMINATION

A four and half year old female Murrah buffalo was referred to the Teaching Veterinary Clinical Complex, LUVAS, Hisar with the history of growth on the left eye near the medial canthus (Figure 1). There was watery discharge from the affected eye since one month. The animal was treated with parental administration of antiobiotic and topical eye drop since last 15 days but no significant improvement was noticed.

Clinical examination revealed a hard growth on nictitating membrane near the medial canthus of the left eye. The animal had normal vision with mild opacity of cornea. The rectal

¹Department of Veterinary Surgery and Radiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India
²Teaching Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India
temperature, heart rate, pulse rate and respiratory rate were within the normal physiological limits. Blood and serum biochemical values were also within the normal reference range. The surgical excision was decided and the site was prepared for asepsis.

RESULTS AND DISCUSSION

Animal was sedated with Injection Xylazine at the rate of 0.05 mg/kg body weight intravenously and regional anaesthesia was achieved by performing Peterson nerve block using 10 ml 2% lignocaine hydrochloride. Neoplastic outgrowth was excised after ligating its base with 3-0 chromic catgut (Figure 2). The eye was lavaged with normal saline solution.

Post operatively parental antibiotics Enroflloxacin at the rate of 5 mg/kg body weight and analgesic Meloxicam at the rate of 0.5 mg/kg body weight were administered intra muscularly, daily for five consecutive days. Eye ointment was applied topically in the affected eye (thrice per day) for 10 days. The animal was recovered uneventfully within 15 days and no complication has been reported since one month after operation.

On histopathological examination, proliferating epithelial cells with concentrating layer of keratin forming cell nest was found. The tumourous growth was diagnosed as a squamous cell carcinoma as similar finding reported by Patel.

Figure 1. Growth near the medial canthus of the left eye.
Figure 2. Excision of neoplastic growth.

CONCLUSION

Early recognition and evaluation of squamous cell carcinoma is necessary and easily be removed successfully without much complication.

REFERENCES

MANAGEMENT OF IRREGULAR SHARP MOLARS IN A BUFFALO – A CASE REPORT

P. Ramesh¹*, P. Ravi Kumar¹, M. Raghunath² and P. Vidya Sagar¹

ABSTRACT

A four year old Graded Murrah Buffalo was presented with a history of bilateral distension of cheeks, persistent salivation and occasional quidding since one year. Physical examination revealed asymmetrical hard bulge of the both cheeks which was pitting on pressure. Upon oral examination noticed abnormal accumulation of feed material on either side of the cheeks, ulcerative lesions in oral mucosa and irregular sharp molars. The impacted material from both the sides of buccal cavity was removed and irrigated with potassium permanganate solution. The irregular sharp edges of the teeth were rasped by tooth rasp manually to level the teeth, resulting in complete recovery.

Keywords: Murrah buffalo, sharp molars, tooth rasp, teeth

INTRODUCTION

Affections of irregular and sharp molar teeth occurs as quidding, bulging of the cheeks due to impaction of the food material and salivation. Present report puts on record a case of overgrown sharp molar teeth and its management in a graded Murrah buffalo.

CASE HISTORY AND CLINICAL OBSERVATIONS

A four year old, graded Murrah buffalo was presented to the Teaching Veterinary Clinical Complex with a history of bilateral distension of cheeks since one year with persistent salivation and occasional quidding. The animal was anorectic with decreased water intake since a week. All the vital parameters were within normal range with mild dehydration. Physical examination revealed hard bulge of the both cheeks, which was asymmetrical and pitting on pressure.

Needle aspiration revealed presence of greenish colour fluid resembling rumen fluid of neutral pH. Examination of oral cavity revealed abnormal accumulation of feed material on either sides of the cheeks (Figure 1), with ulcerative lesions in the oral mucosa and irregular sharp molars. All laboratory findings were within

¹Teaching Veterinary Clinical Complex, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India, *E-mail: rameshvety777@gmail.com
²Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, India
Figure 1. Photograph showing abnormal accumulation of feed material in cheeks.

Figure 2. Photograph showing tooth rasping of overgrown molars.

Figure 3. Photograph showing recovery after treatment.
normal range. Based on the findings, the case was diagnosed as feed accumulation in the cheeks due to irregular sharp molars.

**TREATMENT**

By physical restraint, the mouth was opened and the impacted food material was removed with a long forceps from both the sides of buccal cavity and irrigation of oral cavity was done with potassium permanganate solution. The irregular sharp edges of the teeth were rasped by firm and controlled strokes of the tooth rasp until a uniform level of the molar teeth was obtained (Figure 2). Dehydration was corrected with injection Dextrose Normal Saline 25 ml/kg body weight intravenously. The animal was supplemented with rumenotorics to improve the feed intake and was advised semi solid diet like gruel and semi cooked cereals until complete recovery. The oral mucosa was painted with boroglycerine paste.

**RESULT AND DISCUSSION**

After 10 days of the treatment, the animal started taking feed normally and water and made an uneventful recovery (Figure 3). Irregular and sharp molars are the common condition which hampers the mastication and cause simple indigestion in animals. Usually last molars are more predisposed, outer edges of upper molars and inner edges of lower molars become sharp, such animals usually throw feed material and salivation as observed in the present case. (Dollar, 1958).

The condition can be diagnosed by detailed oral examination and tooth rasping can effectively reduce the sharp edges.

**REFERENCES**


ABSTRACT

A rare case of complete persistence of imperforate hymen in murrah buffalo was diagnosed and treated successfully. Animal got permanent relieve from unwanted symptoms like continuous straining during urination and defecation. Though the animal cycled regularly but failed to conceive with follow-up for one year.

Keywords: imperforate hymen, Murrah buffalo, pyometra, pyocervix, pyovagina

INTRODUCTION

The hymen is formed from the epithelial lining of the paramesonephric ducts and the urogenital sinus at the vestibulovaginal junction (Roberts, 1971). Canalization of the hymen is usually complete at birth and leads to communication between the lumen of the caudal vagina and vestibule (Roberts, 1971). Congenital imperforate hymen is rare in cattle and similar condition in a buffalo heifer reported by Gupta and Sharma (1973). The complete blockage results in accumulation of uterine and cervical secretions and formation of mucometra, mucocervix and mucovagina (Parkinson, 2001). The present report puts on a record a case of imperforate hymen with secondary pyometra, pyocervix and pyovagina in a buffalo heifer.

CASE HISTORY AND CLINICAL OBSERVATIONS

A five year old Murrah buffalo heifer was attended by local practitioner with complaint of intense straining during urination and defecation. The case was diagnosed as a urinary obstruction and around three litters non-smelling mucous like fluid was evacuated by inserting a medium size canula connected to a rubber tube into buldge fluctuating portion through rectum. However, animal expressed temporary relief only for few days. After 48 days, animal developed same symptoms in an intense form (Figure 1) and the animal was referred to Referral Veterinary Polyclinic, IVRI, Izatnagar. Detailed anamnesis revealed that the buffalo was bred two times naturally with last mating three months back, during which the male had apparent difficulty in positioning himself. Further, each mating was followed by intense form of straining lasting few days. Since last mating, female developed permanent sign of prolong straining during and after defecation. Further, animal had an abnormal flow of urine and was also associated with straining.

Per rectal examination revealed a...
voluminous fluid filled fluctuating mass descending into abdomen and it was astonishing to find a tough membrane obstructing hand to palpate cervix per vaginally (Figure 2). These findings lead to diagnose the case as imperforate hymen.

**TREATMENT AND DISCUSSION**

The animal was subjected to epidural anaesthesia with 2% lignocaine hydrochloride and then restrained in lateral recumbency. The hymen membrane was punctured with a trocar guarded by finger and the hole was dilated to its maximum by digital pressure. Around 4 litres of intense foul smelling pus like fluid gush through vagina (Figure 3) and an apparent decompression of abdomen was noticed (Figure 4). Immediate per rectal examination failed to locate cervix and uterine horn. However, per vaginal examination reveals tough corrugation all over the wall of uterine horn. Uterus was flushed with normal saline mixed with

**Figure 1. Tenesmus during defecation.**

**Figure 2. Visualisation of hymenal membrane cranial to urethral orifice.**

**Figure 3. Intense flow of from genitalia.**

**Figure 4. Apparent decompression of abdomen after evacuation of pus.**
potassium permagnate (1:1000) and lignocain jelly (Lignocain Hydrochloride 4%) along with pulv. Antibiotic 10 gm containing Neosporin Polymyxin b sulfate, Bacitracin zinc and Neomycin sulphate applied locally. Animal was discharged with the prescription of parentrel anti-inflammatory Meloxicam 0.05 gm, antibiotic 2.50 gm containing Streptomycin Sulphate, Penicillin G Sodium, Procain penicillin G and intrauterine medication with 45 ml 5% Povodone Iodine for five days. After 45 days, follow-up was made to examine the animal. Cervix and both uterine horn were palpated per-rectally and no reunion of membrane were detected on per-vaginal examination. Animal cycled regularly but could not able to conceive with follow-up for one year. The animal was culled and failed to follow further.

The case, imperforate hymen with secondary pyometra, pyocervix and pyovagina was diagnosed on the basis of history clinical observation and per-vaginal palpation. The case was earlier mis-diagnosed as urinary obstruction and insertion of canula into buldge portion of vagina leading to breach in aseptic environment that might lead to subsequent infection with pus farming bacteria leading to accumulation of pus. In present case the wall of vagina and uterus become very thin and difficult to locate the horns but per-vaginal examination revealed that the inner surface of vagina and uterus was very rough and corrugated. This occurs because the normal outflow of the uterine secretions is prevented by complete persistency of imperforate hymen leading to accumulation of fluid that increases with the age and the cyclic ovarian activity of the female (Troiano and McCarthy, 2004). The duration and volume of fluid accumulation could have affected the endometrium via pressure atrophy leading to embryonic loss if fertilization occurred or some permanent blocked might have occurred that preventing fertilizations. And this may be the reason for the reproductive failure in the present case. Scanning through literatures revealed no sufficient information to suggest that hymen persistency is hereditary. Thus, opens the window for systematic research on prognostic reproductive life of an affected animal and its heritability which would help the clinicians as well as owners to take appropriate decision in time.

REFERENCES


MONOCEPHALIC THORACOPAGUS TETRABRACHIUS TETRAPUS MONSTER IN MURRAH BUFFALO- A CASE REPORT

Vikas Sachan¹, Brijesh Kumar²*, Vipin Sonkar³ and Atul Saxena¹

ABSTRACT

A conjoint monster was delivered by caesarean section in a pluriperous murrah buffalo. Partially duplicated, two female fetuses joined at the thoracic region (Thoracopagus) and having well developed eight limbs, i.e. four forelimbs (Tetabrachius) and four hind limbs (Tetrapus) and both pelvis are separate (Dicaudatus). There was clear four nostrils (Tetrarhino) and the post-mortem examination revealed that internal organs were paired.

Keywords: conjoined twin, monster, Thoracopagus, Tetabrachius, Tetrapus, Dicaudatus, Tetrarhino

INTRODUCTION

Monstrosity is a disturbance of the development that involves various organs and systems which can cause great distortion of the individual (Vegad, 2007). The incidence of fetal monsters, though rare, was reported by Khasatiya et al., 2009; Jerome et al., 2010; Ravikumar et al., 2012 in cows, Dhami et al., 2000; Prasad et al., 2006; Sharma et al., 2010 in buffaloes. Conjoined twins arise from a single ovum and are monozygotic in nature (Arthur, 1956) and are the frequent cause of dystocia in cattle and buffalo. Conjoined twins are also known as diplopagus monsters or Siamese twins. Structural or numerical duplication during the embryonic stage give rise to fetuses whose body structures are partially but not completely duplicated (Roberts, 1971). They are the result of incomplete division of a fertilized ovum and show great variation from partial duplication to almost complete separation of two individuals, joined in just a few places. Dystocia is a common sequel of monstrosity and most of the cases resolved by caesarean section. In the present study a case of monocephalic thoracopagus tetrarhino tetrabrachius tetrapus dicaudatus monster was relieved by caesarean section.

CASE HISTORY AND CLINICAL OBSERVATIONS

A seven year old murrah buffalo presented to Teaching Veterinary Clinical complex, Veterinary University (DUVASU) Mathura in recumbent condition with history of full term gestation and straining since last two days, water bag ruptured 12 h before and also case was handled by local practitioner to relieve the dystocia but failed. The

¹Department of Veterinary Obstetrics and Gynaecology, Deen Dayal Upadhayay Veterinary and Animal Sciences University (DUVASU), Mathura, Uttar Pradesh, India
²ICAR RC for NEH Region Sikkim Centre Gangtok, Sikkim, India, *E-mail: drbrijeshvet02@gmail.com
³Department of Animal Husbandry, Government of Uttar Pradesh, Uttar Pradesh, India
clinical parameter such as heart rate 48/minute, respiration rate 30/minute, rectal temperature 101.5°F and animal was lethargy, dull, depressed and straining sign was completely ceased. Detail Gynaeco-clinical examination revealed that birth canal was completely impacted with fetal head and legs and two amputated legs were also palpated. Further detail examination revealed that head relatively big and no clear demarcation of thorax (Figure 1) and palpation of many legs at untoward places confirmed the fetal monstrosity and might be prime cause for dystocia.

TREATMENT AND DISCUSSION

Attempts were made to relieve dystocia through obstetrical maneuver but futile then it was decided to go for cesarean section to relieve the dystocia. The buffalo was stabilize with fluid therapy comprises of inj. Dextrose Normal Saline and Normal Saline 4 litter each, Ca-borogluconate 450 ml and antibiotic, antihistaminic and anti-inflammatory were administered. Lower left flank laprohystrectomy was made and a full term dead female monster was extracted out. The animal

Figure 1. Conjoint Monster with separate pelvis.
Figure 2. Four nostrils depression and complete absence of epithelium on pole.
Figure 3. A pair of hearts, fused liver and two pairs of kidneys.
was stood after 6 hrs of cesarean section and took the water and walks little and after 24 h animal showing sign of clear improvement and returning to normal and discharge with written prescription advising the owner to continue the same treatment five more day along with local dressing of surgical site and intrauterine medication. After 12th day of operation suture was removed and animal looks totally recovered.

The monster was a conjoined female twin with fusion at the thoracic region containing two pairs of fore limbs and posterior regions of both twins were well developed and having separate pelvis with external genitalia and rectum and pair of hind limbs in each but in one pelvis both limbs was in broken condition probably because of previous handling (Figure 1). The heads were fused, lacked distinct eyes, having two ears, four clear nostrils (tetrarhino) with two complete jaws. There was depression and complete absence of epithelium on pole region (Figure 2). On post-mortem examination, the conjoined twin monster was found to be attached to the thoracic region and encloses a pair of hearts. Other visceral organs like fused liver and two pairs of kidneys, (Figure 3). There was well developed urogenital system and small and large intestine were present with separate rectums for both fetuses.

Conjoined twins may be caused by number of factors such as genetic, environmental, and infectious agents. Assisted reproductive techniques such as In vitro fertilization (IVF) and Intra cytoplasmic sperm injection (ICSI) may be a factor (Romero et al., 1988). The embryonic disk starts to differentiate on the 13th day of conception. If the split occurs after day 13, then the twins will share body parts in addition to sharing their chorion and amnion (Finberg, 1994). This type of foetus is due to congenital embryonic duplication of germinal layer arising from single ovum (Kumar and Reddy, 2008) that gives rise to monozygotic foetus with partial duplication of body structures. Simon et al., (2009) stated that conjoined twins were always genetically identical and shared the same sex. Dystocia due to conjoined twin monsters, though uncommon, have been reported earlier in buffalo (Urankar et al., 1994; Dhami et al., 2000) and in cow (Honnappagol et al., 2005). The present case seemed to be a non-inherited teratogenic defect of development as there was no history of monstrosity in previous calving.

REFERENCES


ABSTRACT

The study was conducted on 18 clinical cases of ketosis presented in the Teaching Veterinary Clinical Complex, Faculty of Veterinary Science and Animal Husbandry (F.V.Sc & A.H), R.S. Pura and areas around R.S. Pura Tehsil. Clinical and haemato-biochemical parameters were studied in the affected animals. A minor decrease in mean body temperature with an increase in severity of ketosis was noted. The severe cases had diminished rate of ruminal motility and prominent clinical signs were sudden and unexpected drop in milk production, depraved appetite, wasting and depression. Biochemical parameters viz. plasma glucose, total plasma protein, LDL and HDL-cholesterol, calcium and magnesium decreased significantly.

Keywords: ketosis, buffalo, biochemical parameters

INTRODUCTION

Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation period characterized by increased levels of ketone bodies in the blood, urine, and milk. In buffalo, ketosis remains one of the major diseases that decrease the productivity (Ghanem and El-deeb, 2010). Ketosis can be clinical or subclinical depending on the subjectivity of the clinical signs. It is generally accepted that clinical ketosis occurs in ruminants when they are subjected to demands on their resources of glucose and glycogen that cannot be met by their digestive and metabolic activity. Clinical ketosis has visible clinical symptoms and typically occurs within the first six to eight weeks post-calving, resulting in anorexia, licking and blindness, hard dry feces, rapid loss of condition, and decreased milk production (Youssef et al., 2010). In addition, the milk fat yield of ketotic cows is increased due to the availability of beta-Hydroxybutyric acid (BHBA) and fatty acids. Clinical ketosis is easy to diagnose by its clinical
symptoms. The present study was conducted to study the clinico-biochemical parameters in buffaloes with clinical ketosis.

**MATERIALS AND METHODS**

The study was undertaken for 8 months duration. Buffaloes with history of anorexia, hard dry feces, rapid loss of condition, and decreased milk production drop in milk production presented at Teaching Veterinary Clinical Complex of the college, constituted the cases for this study. A total of 18 cases (Murrah, Jaffarabadi and few Non-descript) were found to be suffering from ketosis diagnosed by history, clinical signs and by urine and milk nitroprusside tests viz. Modified Rothra’s, Ross Modification of Rothra’s test and Multidiagnostic strip reaction (supplied by Siemens, India). Three buffaloes were used as control. Blood samples from the buffaloes were collected from jugular vein. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and was stored at -20°C. About 2 ml of blood was collected for glucose estimation. Blood glucose, total serum protein, albumin, Alanine transaminase( ALT), Aspartate Aminotransferase (AST), Blood urea nitrogen, Createnine, HDL-Cholesterol, LDL-Cholesterol, calcium, phosphorus, and magnesium were estimated using UV-Spectrophometer by employing standard kits. The statistical analysis was done as per the method described by Snedecor and Cochran (1994).

**RESULTS AND DISCUSSION**

The present study revealed varying degrees of frequency of occurrence of symptoms in ketosis with sudden decline in milk yield being present in 100 per cent of the cases (Table 1). This was followed by selective feeding (79.14%), wasting (49.91%), depression (36.67%), complete anorexia (29.37%), acetone smelling breath (19.35%), dry mucus coated feces (12.90%) and signs of central nervous system involvement (6%) of the cases of bubaline ketosis. The pattern of signs observed in this study was similar to clinical profile described by other workers (Roy and Ghorui, 2000; Radostits et al., 2007).

The present study elucidated that ketosis was predominantly accompanied by a drop of 3.44±0.1 litres milk/animal/day estimating 34.92 on per cent basis. Decline of 25-60 percent in milk production in bovine clinical ketosis has also been placed on record by, Swain and Tripathy (1987) and Mir and Malik (2003). The possible reason for the decreased milk production could be reduced capacity of the animal to supply the lactogenic precursors to mammary gland than the capacity of the gland to produce due to homeorhetic drive for production (Lean et al., 1992). Moreover, elevated blood ketones also result in decreased milk production (Andersson and Lundstrom, 1985).

In the present study depressed ruminal motility was recorded in ketotic buffaloes and this could be attributed to excessive generation of ketone bodies, as ketones bodies are reported to effect ruminal motility causing incomplete and depressed ruminal contraction (Lean et al., 1991). In severe cases mean rumen motility was as low as 1.77±0.11 against 2.17±0.19 per two minutes in mild cases.

In the present study a significant decrease (P<0.05) in Glucose (36.64 mg/dl), Total protein (5.52 g/dl), Albumin(2.03 g/dl) and A:G ratio (0.57) was observed when compared with healthy control (Table 2). The decrease of glucose level may occur in response to intake of low energy diet specially at
the early stage of lactation when high rate of glucose utilization in the mammary gland is required (Nazifi et al., 2008). Hypocalcemia can exert an additional depressive effect on endogenous glucose production, hence, aggravating hypoglycemia (Schlumbohm and Harmeyer, 2003). Decrease in plasma glucose level in ketosis has also been by Youssef et al., (2010) in lactating buffaloes. Since albumin is indicative of the liver’s synthetic function (West 1990), the reduction in total protein and albumin in our study is an indicator for hepatic injury. In the energy deficient ketotic animals labile pool of body protein also serves as an important source for energy synthesis of milk lactose and milk protein (Radostits et al., 2007). This protein catabolism for an increased rate of gluconeogenesis may be the reason for a reduction in total plasma protein levels. Similar results have also been recorded by Youssef et al., (2010).

Buffaloes suffering from ketosis showed a non significant increase in blood urinary nitrogen (22.27mg/dl) level and creatinine (2.18 mg/dl) (Table 2). The high levels of blood urea results from either increased breakdown of tissue or dietary protein or impaired excretion. Significantly higher average values of AST (144.81 U/L) and ALT (144.8 U/L) were observed in ketotic buffaloes when compared to healthy control (Table 2). Although AST is non-specific liver enzyme estimation of its activity in dairy cows is most often associated with fatty liver syndrome (Cebra et al., 1997). AST has been found to increase significantly in ketotic cows compared with healthy ones (Youssef et al., 2010). The infiltration of hepatic cells with fat increases cell membrane permeability with subsequent release of AST enzyme that serves as a good tool for metabolic liver diseases (Karasai and Schefar 1984). Consequently, in the present study, the elevated serum AST in ketotic buffalo compared with control ones could be due to negative energy balance. Similarly, ALT has been found to increase in liver and bile duct malfunctions (Steen et al., 1997). Consequently, in the present study, the high AST and ALT support the occurrence of hepatic damage in ketotic buffalo.

Significantly lower values of Cholesterol were observed in buffaloes 84.9 mg/dl, when compared with the control group (Table 2). The low cholesterol observed could be attributed to mild liver asteatosis which cause reduction in cholesterol formation in the liver (Grummer, 1995). However, Anantwar and Singh (1993) reported that there was an increase of cholesterol levels in ketotic animals. The decrease of serum cholesterol in ketotic buffaloes is similar to clinical conditions caused by liver injuries and fatty liver syndrome in cattle (Marcos et al., 1990). HDL and LDL-cholesterol level (64.90 mg/dl and 30.42 mg/dl, respectively) showed a significant decrease in ketotic buffalo in comparison to control groups (Table 2). These results coincide with those of Turk et al. (2008) and Youssef et al. (2010). These results may be attributed to moderate liver steatosis, which causes reduction in cholesterol level. In contrast to present study, Youssef et al. (2010) observed there was non-significant decrease in LDL-cholesterol levels in ketotic buffalo in comparison to the normal ones.

Significantly lower levels of plasma calcium, phosphorus and magnesium were observed in ketotic buffaloes 8.14 mg/dl, 4.77 mg/dl and 1.90 mg/dl respectively when compared with the control group (Table 2). The decrease of phosphorus and magnesium level coincided with the findings obtained by Ziogas et al. (2007) and Youssef et al. (2010). Insufficient phosphorus supply in the diet, prolonged anorexia, and increased urinary phosphorus excretion due to hyperparathyroidism could explain presence of hypophosphatemia in this
Table 1. Important clinical signs observed in clinically ketotic buffaloes (n=18).

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>Clinical Signs</th>
<th>Percent animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nervous signs</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Constipation</td>
<td>12.90</td>
</tr>
<tr>
<td>3.</td>
<td>Acetone smell in breath</td>
<td>19.35</td>
</tr>
<tr>
<td>4.</td>
<td>Complete anorexia</td>
<td>29.37</td>
</tr>
<tr>
<td>5.</td>
<td>Depression</td>
<td>36.67</td>
</tr>
<tr>
<td>6.</td>
<td>Wasting/ woody appearance</td>
<td>49.91</td>
</tr>
<tr>
<td>7.</td>
<td>Selective feeding (partial anorexia)</td>
<td>79.14</td>
</tr>
<tr>
<td>8.</td>
<td>Sudden drop in milk yield</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Plasma biochemical and mineral values in ketotic buffaloes.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Ketotic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>58.4±2.86</td>
<td>36.64±1.19*</td>
</tr>
<tr>
<td>T Protein (g/dl)</td>
<td>7.44±0.26</td>
<td>5.52±0.13*</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.13±0.15</td>
<td>2.03±0.05*</td>
</tr>
<tr>
<td>A:G ratio</td>
<td>0.71±0.01</td>
<td>0.57±0.007*</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>31±3.1</td>
<td>43.90±3.22*</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>118.2±4.07</td>
<td>144.8±2.19*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>179.11±7.18</td>
<td>84.9±3.16*</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dl)</td>
<td>48.4±4.60</td>
<td>30.42±1.05*</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dl)</td>
<td>86.5±2.97</td>
<td>64.90±1.62*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.71±0.05</td>
<td>2.18±0.05</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>18.3±1.24</td>
<td>22.27±1.10</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>10.8±0.41</td>
<td>8.14±0.09*</td>
</tr>
<tr>
<td>P (mg/dl)</td>
<td>5.80±0.19</td>
<td>4.77±0.07</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>2.20±0.05</td>
<td>1.90±0.03*</td>
</tr>
</tbody>
</table>

Means marked with asterisk (*) differ significantly (p<0.05) from the control group value in a column.
condition. However, the decrease of magnesium level could be attributed to ketonuria which results in decreased tubular resorption or to the need of magnesium that regulate metabolism for milk secretion. In reference to the fall in calcium levels it is suggested that there might be increased loss of base in urine to compensate ketosis induced acidosis. Besides, Cote et al. (1969) indicated that reduced feed intake may also lead to secondary hypocalcemia.

REFERENCES


Methods, 8th ed. Iowa State University Press, Ames, Iowa, USA.


ABSTRACT

Thirty healthy Murrah buffaloes of 5-9 years of age with second to fifth lactation were randomly chosen from well organized dairies located at Jabalpur to elucidate alteration in concentration of some enzymes viz. serum alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transferase (ALT), and aspartate amino transferase (AST), in serum of normal cyclic, repeat breeder and anoestrus Murrah buffaloes. Gynecological examinations were employed for the diagnosis of reproductive status of animals. The selected 30 Murrah buffaloes were divided into three groups and each group comprised of 10 animals for the generation of experimental data. Blood samples were collected from animals of different groups on the day of estrus from normal cyclic, repeat breeder and anoestrus buffaloes on the same day for gynaeco-clinical examination. The serum was prepared following routine procedure. The serum enzymatic activities was monitored immediately for serum alkaline and acid phosphatase, alanine amino transferase and aspartate amino transferase. The serum alkaline phosphatase and acid phosphatase values were 9.190 and 1.357; 19.778 and 2.667; 14.064 and 2.100 KA units /100 ml in anoestrus, repeat breeder and normal cyclic groups, respectively. The values of serum alkaline phosphatase and acid phosphatase values were significantly (P<0.01) different among the groups. It was recorded to be highest in repeat breeder followed by normal cyclic and lowest in anestrus buffaloes. The serum alanine aminotransferase and aspartate aminotransferase values were 26.402 and 69.124; 32.035 and 104.435; 34.007 and 85.093 U/L in anoestrus, repeat breeder and normal cyclic groups, respectively. The values of serum alanine aminotransferase and aspartate aminotransferase of repeat breeder was significantly (P<0.01) higher than normal cyclic and lowest in anoestrus group.

Keywords: Serum alkaline phosphatase, Serum acid phosphatase, Alanine amino transferase and Aspartate amino transferase

INTRODUCTION

In India, estimated populations of buffaloes are 105.3 million among which female buffaloes consists of 54.5 million (NDDB, 2012). These animals play an important role in Indian livestock economy. The success of dairy cattle and buffalo economy lies in proper and optimal reproductive rhythm of each individual cow and buffalo in the herd, within normal physiological range (Dhaliwal, 2005). Any deviation or prolongation in the breeding rhythm results in a progressive economic loss due to widening of dry period reduced calvings and

1College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.), India
2College of Veterinary Science and Animal Husbandry, Anjora, Durg (C.G.), India
lactations during the life span of the animal (Singh et al., 2006). Barren or infertile buffaloes means a direct loss in milk production, whereas reduced calf crops hamper the selection efficiency in long term dairy herd improvement (Baghel, 2006). About 25 percent dairy animals. However, repeatability and anoestrus conditions are recognized as the serious problem in increasing the calving interval, therefore by subjecting the farmer into heavy economic loss. In general incidence of anestus has been reported between 9.09-82.50 percent in buffalo (Thakor and Patel, 2013).

Blood profile might be potential in characterizing the problem and diagnosing a deficient condition (Eltohamy et al., 1989; Jain, et al., 2003). The present investigation was designed to study the biochemical changes during different reproductive states (Normal cyclic, repeat breeder and anoestrus) in Murrah buffaloes. The study was aimed to elucidate alteration in concentration of some enzymes in serum of normal cyclic, repeat breeder and anoestrus Murrah buffaloes.

MATERIALS AND METHODS

In the present investigation, healthy Murrah buffaloes (30) were randomly chosen from well organized dairies located at Jabalpur. These animals were in 5-9 years of age group and within second to fifth lactation. These animals were screened as per the approved technical program. Gynecological examinations were employed for the diagnosis of reproductive states of animals. The selected 30 Murrah buffaloes were divided into three groups and each group comprised of 10 animals for the generation of experimental data. Blood samples were collected from animals of different group on the day of estrus from normal cyclic buffaloes.

Samples from repeat breeder and anoestrus buffaloes were taken on the same day for gynaeco-clinical examination. The serum was prepared following routine procedure. Separated serum was centrifuged at 3000 rpm for 10 minutes. Serum was used immediately for monitoring assay of enzyme activities serum alkaline phophatase (ALP) and acid phophatase (ACP), alanine amino transferase (ALT) and aspartate amino transferase AST). Serum alkaline phosphate was measured as per Tietz, (1976). Acid phosphatase was estimated by the calorimetrically method of King and Jagatheesan, (1959), alanine aminotransferase and aspartate aminotransferase as per Henry, (1974). The data were analyzed statistically using analysis of variance technique (ANOVA) and the differences between means were compared using critical difference (Snedecor and Cochran, 1996).

RESULTS AND DISCUSSION

Concentration of serum enzymes in different reproductive states in Murrah buffaloes have been given in Table 1.

1. The serum alkaline phosphatase (ALP) activity was significantly (P<0.01) increased in repeat breeder (P<0.01) followed by that in normal cyclic and lowest in anestrous condition of buffaloes. The trend of higher ALP activity in repeat breeder was in agreement with the findings reported by Sharma et al., (1986). They found higher serum ALP activity in primary infertile Kankrej heifers than normal cyclic heifers. Mehta et al. (1989) also recorded similar results on comparison between repeat breeder and normal cyclic cows. Gandotra et al. (1993) reported higher level of ALP activity in repeat breeder cattle and
buffaloes as compared to normal cyclic cattle and buffaloes. Chandrakar (1999) found serum activity concentration to be significantly higher in repeat breeder than normal fertile cows. Yaqub et al. (2013) reported that Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentration non significantly fluctuated during the estrous cycle in Red Sokoto goats.

In this study ALP activity during anoestrus condition was significantly lower than the levels in normal cyclic Murrah buffaloes. It might be due to malnutrition. The result can be correlated with the findings of Kalnath et al. (2007) who reported higher Alkaline Phosphatase (ALP) activity during follicular development. However, Derashri et al. (1984) reported the ALP levels in anoestrus condition to be higher as compared to the animals in oestrus but the difference was non-significant. In the light of present results it could be hypothesised that decreased level of AKP activity in normal cyclic as compared to Repeat breeders might be due to enhanced folliculogenesis resulting in increased pace of conception while reverse is true in repeat breeder (Sharma et al., 1986).

2. Serum acid phosphatase (ACP) activity showed a similar trend as ALP which was significantly (P<0.01) highest in repeat breeder followed by reduced in normal cyclic and lowest in anestrous condition. The result correlates with the findings of Gandotra et al. (1993). They recorded significantly higher values of ACP activity in repeat breeder than normal cyclic buffaloes. The result was also similar to Ganguly (2013) who reported that increase in Acid phosphate concentration decreases with increase in follicular size. It can be inferred that higher concentration of acid phosphate concentration increases follicular activity resulting in repeat breeding. On the contrary, Sharma et al. (1986) reported that mean values of ACP were significantly (P<0.01) higher in normal cyclic Kankrej heifers. Increased activity of ACP might be helpful in hydrolysing the organic phosphomonoesters and thus provide energy in the form of phosphates in normal cyclic animals.

Table 1. Concentration of serum enzymes in different reproductive states in murrah buffaloes.

<table>
<thead>
<tr>
<th>Attributes (KA unit/dl)</th>
<th>Normal Cyclic</th>
<th>Repeat Breeder</th>
<th>Anoestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum alkaline phosphatase (ALP)</td>
<td>14.064 ± 0.58(^b)</td>
<td>19.778 ± 0.385(^c)</td>
<td>9.190 ± 0.48(^a)</td>
</tr>
<tr>
<td>Serum acid phosphatase (ACP)</td>
<td>2.100±0.234(^b)</td>
<td>2.667±0.187(^c)</td>
<td>1.357±0.133(^a)</td>
</tr>
<tr>
<td>Serum alanine aminotransferase (ALT) (U/L)</td>
<td>34.007±1.366(^c)</td>
<td>32.035±1.423(^b)</td>
<td>26.402±0.654(^a)</td>
</tr>
<tr>
<td>Serum aspartate aminotransferase (AST) (U/L)</td>
<td>85.093±3.070(^c)</td>
<td>104.435±2.416(^b)</td>
<td>69.124±2.608(^a)</td>
</tr>
</tbody>
</table>

Note: 1. Mean ±SE values with different superscripts in row are highly significantly different (P<0.01).
2. The enzyme unit (U) is a unit for the amount of a particular enzyme.
3. The enzyme activity unit (KA) is expressed I the term of KA/100 ml.
The result indicating significantly higher ACP activity in normal cyclic as compared to anoestrous buffaloes was in contrary to the findings of Derashri et al. (1984) who observed ACP activity level higher in anoestrus surti buffaloes than the normal oestrus buffaloes. The serum ACP activity level might be influenced by physiological conditions. Its activity levels may be used as an index in assessing the estrogen level in buffaloes.

3. The serum ALT activity level was significantly (P<0.01) increased in normal cyclic, followed by repeat breeder. The level of ALT was significantly (P<0.01) lower in anoestrus buffaloes as compared to other two groups of buffaloes. The findings of result were in agreement with the observation of Derashri et al. (1984). He reported the same trend of ALT activity in oestrus and normal cyclic condition and suggested that possible involvement of hormonal levels prevailing during normal oestrus/normal cyclic helps in regulation of ALT activity levels. Sharma et al. (1986) recorded significantly lower activity of ALT in infertile group of heifers than normal group. Pal et al. (1991) found level of ALT activity in the cyclic heifers and cows to be comparatively significantly higher than non cycling ones. In the present study the level of ALT was significantly lower in repeat breeder than normal cyclic buffaloes. These findings were again in agreement with Gandotra et al. (1993). They recorded that the ALT activity level was higher in normal cyclic cattle and buffaloes than the repeat breeder cattle and buffaloes. Higher level of serum ALT activity might be due to increase in metabolic activity mediated by physiological activity during oestrus cycle condition.

4. The serum AST activity level was significantly (P<0.01) increased in repeat breeder compared to normal cyclic and lowest in anestrous group. In the present result AST activity being significantly lower in normal cyclic as compared to repeat breeders buffaloes, supported the findings of Pal et al. (1991). They reported that the AST activity in cyclic heifers and cows were comparatively higher and statistically significant (P<0.01) than the non-cyclic heifers. This enzyme activity was indicative of increased physiological activity and pathological condition of the tissue. In the present study AST activity was higher in repeat breeder than normal cyclic buffaloes and differences between two groups were significantly different. This was in agreement with Gandotra et al. (1993). They reported that AST activity level in repeat breeder cows and buffaloes were significantly higher than the normal cyclic cows and buffaloes. The possible cause of increased AST activity level may be uterine tissue damage in repeat breeder cows & buffaloes. The present result was in contrary to Sharma et al. (1986) who found AST activity to be higher in primary infertile Kankrej heifers as compared to normal cyclic heifers, however, the difference was found to be non significant. Sarwar et al. (2002) found that AST and ALT were significantly higher in endometritis in Nili-Ravi buffalo which can be correlated with the result of repeat breeder. However, result is dissimilar to those of findings of Yaqub et al. (2013) who reported ALT and AST to non significantly fluctuate during estrus cycle in goat. This might be because goat is a seasonal breeder whereas buffalo is a regular breeder.

Estrus cycle may produce measurable stress during estrus phase resulting in increased physiological activity which increases the AST activity in blood. But in case of repeat breeder higher values might be due to tissue damage.
CONCLUSION

Serum alkaline phosphatase and acid phosphatase activities in anoestrus group were significantly lower than normal cyclic and repeat breeders, Alanine amino transferase and aspartate amino transferase activities in normal cyclic were also significantly higher than anoestrus buffaloes.

REFERENCES


CROSS-SECTIONAL SURVEY OF HELMINTHIASIS IN BUFFALOES AT TEHSIL JATOI AND TEHSIL MUZAFFAR GARH, SOUTHERN PUNJAB, PAKISTAN

Muhammad Asif Raza¹, Muhammad Mazhar Ayaz²*, Muhammad Mudasser Nazir², Muhammad Saleem Akhtar², Mubashir Aziz², Saeed Murtaza² and M. Ali Khosa²

ABSTRACT

In the four year study from 2009 to 2013, a total of 500 faecal samples from buffaloes from different locations of Tehsil Jatoi and Tehsil Muzaffar Garh, Southern Punjab were analyzed to confirm the presence of gastrointestinal parasitic infection. The recovered parasites were five nematodes viz Toxocara vitulorum (16.6%), Oesophagostomum radiatum (3.2%), Bunostomum phlebotomum (1.6%), Cooperia spp. (1.6%), Trichostrongylus spp. (0.8%). The two trematodes were Fasciola hepatica (8.4%), Paramphistomum cervi (15%). Age-wise prevalence was 79.5% and 47% in buffalo calf and adult buffalo, while sex-wise prevalence was 78.4% and 50.93% in male and female buffalo, respectively. Chi-square statistical design was applied to data to know the dependence of helminth’s prevalence on sex and age of animals.

Keywords: buffaloes, helminths, southern Punjab, Pakistan

INTRODUCTION

Helminths are recognized as a major constraint to livestock production throughout the world (Ibrahim et al., 1984 and Waler et al., 1987). Water buffaloes are considered common host for helminthiasis in tropical and sub-tropical area. Helminthiasis inflicts huge economic losses even deaths of infected animals. The economic losses are in a variety of ways like lowered the fertility, reduction in work capacity, involuntary culling of emaciated animals, reduction in food intake, lesser weight gains, lower milk production, huge treatment costs, and mortality in heavily parasitized animals (Lebbie and Irungu, 1994), the other can be reduced weight gains and the condemnation of infected organs at slaughter (Liu et al., 2009). Prevalence of Gastrointestinal Tract helminthes in ruminants has been reported up to 25 to 92% in different areas of Pakistan by various workers like Ali et al., 2000; Raza et al., 2007; Ijaz et al., 2008; Al-Shaibani et al., 2008; Kakar and Kakar-sulemankhel, 2008; Raza et al., 2010; Ayaz et al., 2013 and Iqbal et al. (2002) has pointed out various parasitic problems like Facioliasis, hydatidosis, coccidiosis, theileriosis and babesiosis as the major parasitic problems of ruminants in Pakistan in the order of priority. A number of factors that influence the prevalence of helminthes that includes age (Mckenna, 1981), sex (Asanji and Williams, 1987), breed (Miller, 1998; Mirza and Razzak, 1998), worm population (Ankers et al., 1997), weather condition (Asanji

¹Faculty of Veterinary Medicine, Higher College of Technology, Al-Ain Men’s College, United Arab Emirates
²Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan, *E-mail: mazharayaz@bzu.edu.pk
and Williams, 1987, Mohiuddin et al., 1984) and husbandry or managemental practices (Tan et al., 1996). Some helminths infecting buffaloes primarily has zoonotic importance such as schistosomiasis, cystic echinococcosis, and fasciolosis (Liu et al., 2009; Cringoli et al., 2007). Punjab province in Pakistan has largest population of buffalo so considering the health implications and the economic potential of water buffaloes, the issue of investigating parasitic infections of buffaloes is of relevance (Rinaldi et al., 2007; Veneziano et al., 2007) for the cross sectional study.

MATERIALS AND METHODS

The present cross sectional study was initiated from January 2009 to June 2013 to determine the point prevalence of Gastrointestinal tract helmintiasis in the buffalo under field conditions at Tehsil Jatoi and Tehsil Muzaffar Garh, Southern Punjab, Pakistan.

Study area

From January 2009 to June 2013, fecal sample of 500 buffaloes (Between age ranging six months to two years and from three years to six years and Sex-wise) were brought to the Laboratory of Veterinary Parasitology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan for identification of eggs/larvae or adult helminths.

Sample analyses

Fecal samples were examined for helminthes eggs/larvae/adult by using direct and indirect techniques (Ayaz, 2010) and for identification of certain nematodes, copro-culture/Baermann method (Ayaz, 2010) were performed to obtain larval stages. Both eggs and larvae from copro-culture were identified by using standard techniques as described by MAFF (1979) and Soulsby (1982). Briefly, one gram of fecal sample was mixed well in a drop of water and a relatively homogenous and transparent preparation was obtained and examined under microscope by placing a drop of suspension on slide with cover slip. At least three direct smears were examined from each sample. All the samples were also examined by using concentration techniques, i.e. floatation and sedimentation. For floatation technique, five grams of feces was mixed in 50 ml of water and strained through a sieve no. 60 micron mesh to remove the course material. The mixture was allowed to sediment for half an hour. The supernatant was discarded and sediment was mixed with saturated salt solution. The suspension was centrifuged at 1000 rpm for two minutes. The upper 0.1 ml of centrifuged suspension was transferred to a glass slide and examined under microscope at 10 X for the presence of helminthes eggs. More over a relatively new technique for “a single slide positive sample” was also developed as “micro-floatation technique” (Ayaz, 2010). For sedimentation technique to examine heavy eggs, five grams of faeces was mixed in 50 ml of water and strained through a sieve mesh no. 60 micron to remove the course material. The mixture
was allowed to sediment for half an hour. After centrifugation, the supernatant was decanted and washing was continued until supernatant became clear. A drop of 0.1 ml was taken from sediment with the help of Pasteur’s pipette on slide and was examined under microscope at 10 X for the presence of helminthes eggs.

**Feco-Copro-Culture**

Feco-Copro-Culture provides an environment suitable for hatching and development of helminths eggs. Samples found positive for nematode eggs were broken up finely, using either a large pestle and mortar or spatula and were placed in a glass jar or petri-dish for incubated at 26°C for 3-7 days. After incubation, samples were examined for the presence of larvae and were identified with the help of key by MAFF (1979).

**Statistical analyses**

Data on the prevalence of helminthiasis was analyzed using Chi-square statistical design and percentage on the basis of sex and age. Graphical representation of tabulated data was also done.

**RESULTS**

The present cross sectional study was commenced from January 2009 to June 2013 to determine the prevalence of GIT helminthes in buffaloes. Overall Age-wise and sex-wise prevalence in buffalo was 60% and 57.8%, respectively. The highest prevalence for nematodes (119/500; 23.2%) followed by trematodes (117/500; 23.4%) was recorded. A total seven species of helminths including five species of nematodes, i.e. *Toxocara vitulorum*, *Oesophagostomum radiatum*, *Bunostomum phlebotomum*, *Cooperia* spp., *Trichostrongylus* spp. and two trematodes species i.e. *Fasciola hepatica*, *Paramphistomum cervi* were recorded. Among various species of helminthes *Toxocara vitulorum* was the most prevalent species of helminthes which was followed by *Paramphistomum cervi*, *Fasciola hepatica*, *Oesophagostomum radiatum*, *Bunostomum phlebotomum*, *Cooperia* spp. and *Trichostrongylus* species respectively. The mixed helminthes infections (52/500; 12.4%) was often composed of 07 species including *Toxocara vitulorum*, *Oesophagostomum radiatum*, *Bunostomum phlebotomum*, *Cooperia* spp., *Trichostrongylus*

<table>
<thead>
<tr>
<th>GIT Helminths Species</th>
<th>Number of Examined Fecal Samples</th>
<th>Number of Positive Fecal Samples</th>
<th>% age Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxocara vitulorum</em></td>
<td>500</td>
<td>83</td>
<td>16.6</td>
</tr>
<tr>
<td><em>Paramphistomum cervi</em></td>
<td>500</td>
<td>75</td>
<td>15</td>
</tr>
<tr>
<td><em>Fasciola hepatica</em></td>
<td>500</td>
<td>42</td>
<td>8.4</td>
</tr>
<tr>
<td><em>Oesophagostomum radiatum</em></td>
<td>500</td>
<td>16</td>
<td>3.2</td>
</tr>
<tr>
<td><em>Bunostomum phlebotomum</em></td>
<td>500</td>
<td>8</td>
<td>1.6</td>
</tr>
<tr>
<td><em>Cooperia</em> spp.</td>
<td>500</td>
<td>8</td>
<td>1.6</td>
</tr>
<tr>
<td><em>Trichostrongylus</em> spp.</td>
<td>500</td>
<td>4</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Table 2. Age-wise prevalence of different species of helminths in buffaloes (n=500).

<table>
<thead>
<tr>
<th>Species of helminths</th>
<th>Calf</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxocara vitulorum</em></td>
<td>60/200; 30%</td>
<td>23/300; 7.67%</td>
</tr>
<tr>
<td><em>Paramphistomum cervi</em></td>
<td>40/200; 20%</td>
<td>35/300; 11.67%</td>
</tr>
<tr>
<td><em>Fasciola hepatica</em></td>
<td>16/200; 8%</td>
<td>28/300; 9.33%</td>
</tr>
<tr>
<td><em>Oesophagostomum radiatum</em></td>
<td>4/200; 2%</td>
<td>12/300; 4.00%</td>
</tr>
<tr>
<td><em>Bunostomum phlebotomum</em></td>
<td>4/200; 2%</td>
<td>4/300; 1.33%</td>
</tr>
<tr>
<td><em>Cooperia spp.</em></td>
<td>2/200; 1%</td>
<td>6/300; 2.00%</td>
</tr>
<tr>
<td><em>Trichostrongylus spp.</em></td>
<td>1/200; 0.5%</td>
<td>3/300; 1.00%</td>
</tr>
</tbody>
</table>

Table 3. Sex-wise prevalence of different species of helminths in buffaloes (n=500).

<table>
<thead>
<tr>
<th>Species of helminth</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxocara vitulorum</em></td>
<td>25/125; 20%</td>
<td>58/375; 15.47%</td>
</tr>
<tr>
<td><em>Paramphistomum cervi</em></td>
<td>30/125; 24%</td>
<td>45/375; 12%</td>
</tr>
<tr>
<td><em>Fasciola hepatica</em></td>
<td>13/125; 10.4%</td>
<td>29/375; 7.73%</td>
</tr>
<tr>
<td><em>Oesophagostomum radiatum</em></td>
<td>8/125; 6.4%</td>
<td>8/375; 2.13%</td>
</tr>
<tr>
<td><em>Bunostomum phlebotomum</em></td>
<td>5/125; 4%</td>
<td>3/375; 0.8%</td>
</tr>
<tr>
<td><em>Cooperia spp.</em></td>
<td>2/125; 1.6%</td>
<td>6/375; 1.6%</td>
</tr>
<tr>
<td><em>Trichostrongylus spp.</em></td>
<td>0/125; 0%</td>
<td>4/375; 1.07%</td>
</tr>
</tbody>
</table>

Table 4. Presence of various species of GIT helminths in buffalo.

<table>
<thead>
<tr>
<th>Species of helminths</th>
<th>Buffaloes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nematodes</strong></td>
<td></td>
</tr>
<tr>
<td><em>Toxocara vitulorum</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Oesophagostomum radiatum</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Bunostomum phlebotomum</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Cooperia spp.</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Trichostrongylus spp.</em></td>
<td>+</td>
</tr>
<tr>
<td><strong>Trematodes</strong></td>
<td></td>
</tr>
<tr>
<td><em>Fasciola hepatica</em></td>
<td>+</td>
</tr>
<tr>
<td><em>Paramphistomum cervi</em></td>
<td>+</td>
</tr>
<tr>
<td><strong>Cestodes</strong></td>
<td>-</td>
</tr>
<tr>
<td>Total Number of Helminths spp.</td>
<td>7</td>
</tr>
</tbody>
</table>
The prevalence of helminthes was higher in buffalo (Table 2) calves as compared to adult buffalo. A total of 12.4% buffalo (62/500) had mixed infection comprising 16% (32/200) in calves and 10% (30/300) in adult buffalo.

In Table 2 and 3 The prevalence of helminthes was higher in young animals compared with the older ones and the prevalence of helminthes was higher in males compared with the females.

In Table 4 a total of seven species of helminthes including five nematodes, including *Toxocara vitulorum*, *Oesophagostomum radiatum*, *Bunostomum phlebotomum*, *Cooperia* spp., and *Trichostrongylus* spp. were found while two species of trematodes, i.e. *Fasciola hepatica*, *Paramphistomum cervi* were recorded. The most prevalent nematode recovered in this study area from buffaloes was *T. vitulorum* which was reported to be the most frequent occurring nematode in cattle and buffaloes by other scientists like El-Maukddad (1979), Iqbal et al. (1984), Mourad et al. (1985), Anwar et al. (1996) and Motahar et al. (2000).

The infection was higher in young animals as compared to older ones that may be attributed to lesser immunity because of fewer/ maiden exposure to various species of helminthes. It might be interesting that prevalence was higher in males as compared with females. Normally, females are assumed to be more infected due to stress of pregnancy and parturition due to stall feeding in females around the termination of pregnancy and thus lesser exposure to pasture contamination. Most of the researchers have observed higher rates of nematode burden in female hosts as compared with males in *B. bubalis* and *B. bubalis*.

**DISCUSSION**

Helminthiasis being one of the major problems affecting the productivity of buffaloes remains on top of the list. This severity depends on the prevalence, intensity of infection, presence of intermediate host, fauna and flora, frequency of infection, fecunditity and mal-management practices. In this cross sectional survey, the Age-wise prevalence of helminthes was higher in young animals as compared to elder ones, and in sex-wise higher in males as compared with the females. In buffaloes, a total of seven species of helminthes including five nematodes, including *Toxocara vitulorum*, *Oesophagostomum radiatum*, *Bunostomum phlebotomum*, *Cooperia* spp., and *Trichostrongylus* spp. were found while two species of trematodes, i.e. *Fasciola hepatica*, *Paramphistomum cervi* were recorded. The most prevalent nematode recovered in this study area from buffaloes was *T. vitulorum* which was reported to be the most frequent occurring nematode in cattle and buffaloes by other scientists like El-Maukddad (1979), Iqbal et al. (1984), Mourad et al. (1985), Anwar et al. (1996) and Motahar et al. (2000).

The infection was higher in young animals as compared to older ones that may be attributed to lesser immunity because of fewer/ maiden exposure to various species of helminthes. It might be interesting that prevalence was higher in males as compared with females. Normally, females are assumed to be more infected due to stress of pregnancy and parturition due to stall feeding in females around the termination of pregnancy and thus lesser exposure to pasture contamination. Most of the researchers have observed higher rates of nematode burden in female hosts as compared with males in *B. bubalis* and *B. bubalis*.

<table>
<thead>
<tr>
<th><strong>Age-wise animals</strong></th>
<th><strong>Calf</strong></th>
<th><strong>Adult</strong></th>
<th><strong>Overall prevalence</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffaloes</td>
<td>159/200; 79.5%</td>
<td>141/300; 47.00%</td>
<td>300/500; 60%</td>
</tr>
<tr>
<td>Sex-wise animal</td>
<td><strong>Male</strong></td>
<td><strong>Female</strong></td>
<td><strong>Overall prevalence</strong></td>
</tr>
<tr>
<td>Buffaloes</td>
<td>98/125; 78.4%</td>
<td>191/375; 50.93%</td>
<td>289/500; 57.8</td>
</tr>
</tbody>
</table>

Table 5. Age-wise and sex-wise prevalence of GIT helminths in buffalo (n=500).
Figure 1. Prevalence of different species of helminths in buffaloes.

Figure 2. Prevalence of different species of helminths in buffaloes.
Figure 3. Age-wise prevalence of different species of helminths in buffaloes.

Figure 4. Sex-wise prevalence of different species of helminths.
the males like Asanji and Williams, 1987; Pal and Qayyum, 1992; Iqbal et al., 1984; Maqsood et al., 1996; Komoin et al., 1999; Valcarcel and Garcia Romero, 1999.

In contrast to the current results, Gulland and Fox (1992) reported that prevalence and intensity of infection (faecal egg counts) were higher in males than females, except during the lambing periods, and decreased with increase in age in both sexes. Effect of reproductive cycle has been reported to affect the worm burdens in animals, which has an important epidemiological significance as Lyons et al. (1987, 1992) reported a progressive increase in the egg per gram (EPG) and number of helminths in ewes during and after the parturition period. This phenomenon has been attributed to a variety of reasons like seasonal changes, host factors, activation of hypobiotic larvae, parturition stress, poor nutritional status, peri-parturient relaxation in immunity (PPRI) or spring rise phenomenon, hormonal changes around parturition, breed differences etc. etc. In many parts of the world, parturition of grazing animals is synchronized to occur with the favorable climate to pasture growth and also suitable for development and survival of free-living stages of most helminths (Wedderburn, 1970).

REFERENCES


Veneziano, V., M. Santaniello, S. Carbone, S. Pennacchio, M.E. Morgoglione, M.


HISTOLOGICAL STUDY ON STROMAL TISSUE IN MAMMARY GLAND AT LACTATING, INVOLUTION AND PREGNANT STAGE IN MURRAH BUFFALO

D. Chaurasia¹, R.S. Dalvi², S.B. Banubakode², N.C. Nandeshwar², R. Churchan², S.P. Ingole¹ and B. Sinha¹

ABSTRACT

The present histological study was conducted on mammary gland tissue of sixty Murrah buffaloes. The samples were categorized into three stages as lactating, nonlactating nonpregnant (involution stage) and nonlactating pregnant by ascertaining the stage of lactation, dry period and pregnancy period. Stroma was found to be comprised of interalveolar, interlobular and interlobar connective tissue. The amount of stromal tissue varied during different stages of lactation. In late pregnant and colostrum stage the interalveolar connective was minimum and alveoli were almost touching each other. The amount of stromal tissue increased from colostrum stage to ten months of lactation. It was maximum in nonlactating nonpregnant stage from one to two months (later stage of involution). Through stomal tissue blood and lymph vessels and nerve goes into the parenchymal tissue.

Keywords: mammary gland, stromal tissue, collagen fibers, Murrah buffalo

INTRODUCTION

Mammary gland stromal tissue undergoes dramatrical histological changes in the various stage of lactation under the hormonal influences. The ratio of glandular parenchyma to the stromal tissue is one of the important parameter for selection of cattle as a milch breed. That’s why histological study of the mammary gland is pre requisite. The name “Black Gold” has emerged as synonym for the one very popular breed of buffaloes i.e. Murrah, which serves as capital reserve or cash crops to rural folk by producing economic stability, livelihood security and social status (Balbhadra, 2013). During the past one year, a United States dairy firm had purchased Murrah buffaloes, each yielding over 25 kg milk a day, at a cost of Rs 2.5 lakh each from Haryana. This shows that rich countries will soon switch over to Murrah husbandry (Sing, 2013). There is paucity of detail literature of histological study of mammary gland stromal tissue in various stage of lactation in buffalo. So keeping in view the importance of Murrah buffalo in Indian economy, mammary

¹Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Chattishgarh Kamdhenu Viswavidalaya, Anjora, Durg, Chattishgarh, India, *E-mail: durgavet2010@gmail.com
²Department of Veterinary Anatomy and Histology, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India
gland as an important accessory reproductive organ as well as scanty information, present experiment was proposed on the mammary gland of Murrah buffalo to study histological changes in stromal tissue of mammary gland.

MATERIALS AND METHODS

The present histological study was conducted on mammary gland tissue of sixty Murrah buffaloes. The mammary gland samples of buffaloes were collected from dairy farms nearby Nagpur in Maharashtra and Durg, Rajnandgoan and Raipur District of Chhattisgarh after their natural death. The samples were ensured for not having any pathological lesions. The samples were categorized into three stages as lactating, nonlactating nonpregnant and nonlactating pregnant by the stage of lactation, dry period and pregnancy period. Lactating stage was further categorized in five groups as: colostrum stage/phase, three months of lactation, five months of lactation, seven months of lactation and ten months of lactation. Nonlactating nonpregnant stage was categorized in two groups as: Upto one month and from one to two month. Nonlactating pregnant stage was categorized into three stage as early pregnant stage, mid pregnant stage and late pregnant stage.

Mammary tissue of 3-5 mm thickness was fixed in 10% neutral buffered formalin fixative for histological and histochemical studies. After fixation tissue were dehydrated in alcohol, cleared in benzene and embedded in paraffin as per the method of Drury and Wallington (1980). Three to five micron thick sections were cut and stained in Haematoxylin and Eosin, Van Gieson’s, Gordon and Sweets, Orcein and Masson’s Trichrome stain for histological structure, collagen fibers, reticular fibers, elastic fibers and muscle fibers respectively as per the method of Bancroft and Cook (1994).

RESULTS AND DISCUSSION

During the present work, the stromal tissue was found to be comprised of interalveolar, interlobular and interlobar connective tissue (Figure 1). The amount of interalveolar connective tissue varied during different stages of lactation. The interalveolar connective tissue was dense and mainly composed of collagen fibers. Blood capillaries were predominantly seen in lactating stage in interalveolar connective tissue (Figure 2). In colostrum stage, the interalveolar connective tissue was very scanty and alveolus was almost touching to each other (Figure 2). From colostrum stage onwards, the amount of interalveolar connective tissue was increased with the advancement of lactation upto ten month (Figure 2 and 3). Comparatively, more amount of interalveolar connective tissue was observed in between resting alveoli than active alveoli (Figure 2 and 4). In nonlactating nonpregnant animals, more amount of interalveolar tissue was found (Figure 1). In the nonlactating pregnant stage, the interalveolar connective tissue was seen throughout the pregnancy (Figure 5). Intalobular duct, blood and lymph vessels and nerve were present in interalveolar connective tissue (Figure 6). These finding were in agreement with the Trautmann and Fiebtger (2002) and Riviere (2007) in domestic animals, Sordillo and Nickerson (1988) and Bragulla and Konig (2004) in cow, Sulochana et al. (1989) in sheep, Parmar et al. (1985) in goat and Nosier (1973) in camel. The blood capillaries in the interalveolar connective tissue observed in mammary gland during lactating stage could be
Figure 1. Photomicrograph of mammary gland of nonlactating nonpregnant upto one month stage showing interalveolar connective tissue (IAC), interlobular connective tissue (ILC) and interlobar connective tissue (IBC).

(Van Gieson’s X 100)

Figure 2. Photomicrograph of mammary gland of colostrum stage of lactation showing active alveoli (A), interalveolar connective tissue (IAC) and blood capillaries (BC).

(Haematoxylin and Eosin X 400)
Figure 3. Photomicrograph of mammary gland of ten months of lactation showing collagen fibers (CF) in interalveolar connective tissue (IAC), and interlobular connective tissue (ILC).

(Van Gieson’s X 100)

Figure 4. Photomicrograph of mammary gland of ten months of lactation showing resting alveoli (RA) and interalveolar connective tissue (IAC).

(Haematoxylin and Eosin X 400)
Figure 5. Photomicrograph of mammary gland of nonlactating late pregnant stage showing collagen fibers (CF) in interalveolar connective tissue (IAC).

(Van Gieson’s X 400)

Figure 6. Photomicrograph of mammary gland of colostrum stage of lactation showing interalveolar connective tissue (IAC), interlobular connective tissue (ILC) and interlobular duct (D).

(Van Gieson’s X 100)
Figure 7. Photomicrograph of mammary gland of five months of lactation showing fat cells (FC) in interlobular connective tissue (ILC).

(Van Gieson’s X 100)

Figure 8. Photomicrograph of mammary gland of nonlactating nonpregnant one to two month stage showing elastic fibers (EF) in interlobular connective tissue (ILC) and interlobar connective tissue (IBC).

(Orcein X 100)
attributed to the higher blood supply demanded by the mammary tissue for the synthesis of milk.

Mammary parenchyma was divided into lobules by the bundles of thick dense connective tissue fibers. These fibers bundles were interlobular connective tissue present in the form of septae. Interlobular artery and vein, lymph vessels, nerves and interlobular ducts were present in the interlobular connective tissue. At places, few fat cells were seen in the interlobular connective tissue. However, in some places, fat cells were found to predominate the other connective tissue elements (Figure 7). This was in accordance to the Chaurasia et al. (2012) they found fully developed stromal tissue formed chiefly of the massive fat pad in prenatal period. The most abundant fibers were collagen fibers (Figure 1 and 3). The amount of collagen fibers were increased apparently with advancement of lactation from colostrum stage to ten months of lactation. Five to eight times increased were noticed in the amount of interlobular connective tissue from colostrum stage of lactation to involution stage (Figure 1 and 6). Elastic fibers were observed in interlobular connective tissue in lactating and nonlactating stages in buffalo (Figure 8). The amount of elastic fibers was comparatively more in the nonlactating nonpregnant stage. However, present study did not show reticular fibers in the lactating and nonlactating stages in buffalo. In early pregnant stage lobulations were not distinct.

In lactating and late pregnant stage the lobes were not seen in tissue section because of large size of alveoli and lobule. The interlobar connective tissue was found between lobes (Figure 1 and 9). The interlobar connective tissue collagen fibers were more compact and dense than interalveolar and interlobular connective.
It was found that the amount of interlobar connective tissue was increased after weaning during involution stage. Approximately there was two to three fold increase in amount of interlobar connective from initial one months of involution to later period of involution (from one to two months). These findings were in agreement with the findings of Bloom and Fawcett (1975). They opined that after few days of milk cessation, the secretion that remains in alveoli and duct, get absorbed and increases in the activity of lysosomal enzyme leading to degeneration of epithelium. This desquamation of epithelium gradually leads to collapse of alveoli and get associated with increase in stromal tissue. The findings of the present study reflect on the active glandular dynamism in the terms of alteration in the glandular complex under the influence of pituitary and gonadal hormones in tune with the physiological demands and status of the animal health.

ACKNOWLEDGEMENTS

The author (Durga Chaurasia) thank the Former Vice Chancellor (Dr. Hazara) of Indira Gandhi Krishi Vishwavidyala, Raipur, Dean, Dr. S. Jogi for granting me study leave to pursue Ph. D. programme from Maharashtra Animal and Fishry Science University. I am thankful to my Husband Dr. R. K. Chaurasia for procurement of sample. We thank Dean (Nagpur Veterinary College, Nagpur, MAFSU) Dr. B. P. Danndge for providing me all facilities in Nagpur veterinary college to complete the research project.

REFERENCES


Philadelphia, USA.


ABSTRACT

The aim of the study was to investigate efficiency of test-day model (TDM) compared to lactation model (LM) for genetic evaluation of Murrah buffalo bulls. Use of TDM instead of LM is of more interest in genetic evaluation because of variability of lactation days in dairy animals. Data pertaining to first lactation monthly test-day milk yield (FLMTDMY) and first lactation 305-days or less milk yield (FL305DMY) of 1105 Murrah buffaloes during 1993 to 2010 were collected and adjusted against significant environmental influences. It was found that test-day6 milk yield (FLMTD6MY) had the highest genetic and phenotypic correlation with FL305DMY. An attempt is being made in the present investigation to compare the estimated breeding values (EBVs) of Murrah bulls through contemporary comparison method for FL305DMY and FLMTD6MY. The rank correlations between two traits were highly statistically significant indicating that FLMTD6MY equally effective to discriminate amongst sires.

Keywords: Contemporary comparison, lactation model, least-squares, Murrah buffalo, test-day model

INTRODUCTION

India is regarded as a treasure house of world’s best buffalo germplasm. Buffalo is not only a better source of milk but also provides meat and works as a draught animal. Indian buffalo contributes 17% of world milk production and 48% of Asian milk production (Food and Agriculture Organization, 2012). Among the various buffalo breeds available in India, the Murrah buffalo is the cynosure for dairy type. Murrah buffalo produces good quantity of milk and it is now well established that it represents a unique breed in terms of feed conversion ability with low grade feeds, ability to sustain under adverse climatic conditions, resistance to diseases and production of high value milk containing a higher fat per cent. Keeping the importance of buffalo in India, Network Project on Buffalo Improvement was initiated with the objective to envisage and undertake progeny testing for improvement of buffalo breeds at various farms in different parts of the country.

In India test bulls are evaluated based on their daughters first lactation 305-days or less milk yield without taking into account variation in lactation days though the variation in lactation length is reflected in persistency. Genetic evaluation of
dairy bulls for milk production based on individual monthly test-day yields rather than 305-days or less milk yield has a number of benefits (Jamrozik and Schaeffer, 1997). Because of variability of lactation days in dairy animals, the use of test-day models (TDM) instead of lactation model (LM) is of more interest in genetic evaluation. First lactation monthly test-day 6 milk yield (FLMTD6MY) had the highest genetic and phenotypic correlation with FL305DMY as obtained by Kumar et al. (2014). So FLMTD6MY of daughter was used in the present study. Information on test-day is lacking in Murrah buffaloes and hence, the present study was carried out.

MATERIALS AND METHODS

Source of Data

In the present study, information were collected from 7 sets of progeny testing under Network Project on Murrah buffalo Improvement. In 7 sets of progeny testing 95 (11, 12, 15, 14, 15, 16, and 12) Murrah bulls were evaluated. Lactation records of 1105 Murrah buffaloes during 1993 to 2010, were collected from the history-cum pedigree sheets and milk yield registers maintained at the National Dairy Research Institute (NDRI), Karnal; Central Institute for Research on Buffalo (CIRB), Hisar; Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana and Choudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar.

Information on Murrah buffalo

Sires were evaluated on the basis of first lactation 305-days or less milk yield (FL305DMY) and first lactation monthly test-day 6 milk yield i.e. 155th day milk yield (FLMTD6MY) in the present study. The records of the buffaloes with normal lactation were considered for this study. Data of buffaloes with a minimum of 500 kg of milk production in at least 100 days of lactation, calving and drying under normal physiological conditions were included in the analysis. The buffaloes showing abortion, dystocia and other reproductive disorders were not included in the study. To ensure the normal distribution of records, the outliers were removed and data within the range of Mean ± 3 standard deviation was only considered for the study. Hence after standardization and normalization, records of 832 Murrah buffaloes were retained for analysis.

Statistical analysis

The data were adjusted for significant non-genetic factors for buffaloes calved in different farms, years and seasons of calving using fixed linear models. Since the data was non-orthogonal, the least-squares technique suggested by Harvey (1990) was used to estimate the effect of non-genetic factors, and the means were compared using Duncan’s multiple range test (Kramer, 1957). The model considered was as follows:

\[ Y_{ijkl} = \mu + P_i + S_j + F_k + e_{ijkl} \]

where, \( Y_{ijkl} \) is the lth observation in Kth farm, jth season and ith year of calving; \( \mu \) the overall mean; \( P_i \) the fixed effect of ith year of calving; \( S_j \) the fixed effect of jth season of calving; \( F_k \) the fixed effect of the kth farm; and \( e_{ijkl} \) the random error~NID (0, \( \sigma^2 e \)).

After adjusting data for significant fixed effects, EBVs of Murrah buffalo bulls were estimated for FL305DMY and FLMTD6MY. In 7 sets of progeny testing 95 (11, 12, 15, 14, 15, 16 and 12) Murrah bulls were evaluated by contemporary
comparison (CC) method (Sundaresan *et al.* 1965) as follow:

\[
\begin{align*}
I & : \text{is the sire index} \\
H & : \text{is the herd average} \\
N & : \text{is the number of daughters of the sire} \\
D & : \text{is the average performance of trait of daughters’ of the sire} \\
CD & : \text{is the average performance of trait of contemporary daughters}
\end{align*}
\]

where,

The Spearman’s rank correlation method (Steel and Torrie, 1960) was used to judge the effectiveness of test-day and lactation models of sire evaluation. To compare two models, Spearman’s rank correlations were estimated using ranks of bulls based on EBVs for FLMTD6MY and FL305DMY. Two models were compared for the 7 sets separately and significance of rank correlations were tested.

**RESULTS AND DISCUSSION**

The data were adjusted for significant non-genetic factors. In the present study FLMTDMY and FL305DMY were significantly affected by farm. EBVs of sires based on FL305DMY and FLMTD6MY were estimated by CC methods and then sires were ranked subsequently (Table 1 and Table 2). Comparison of two models of sire evaluation was done by comparing the spearman’s rank correlations between ranks of sires based on EBVs for FL305DMY and FLMTD6MY. The rank correlations between corresponding ranks (Table 3) based on FL305DMY and FLMTD6MY ranged from 0.566 (in set 2) to 0.882 (in set 1). The rank correlations between two traits for sire evaluation were highly statistically significant in 5 sets of progeny testing indicating that both traits of sire evaluation were equally effective to discriminate amongst sires.

Use of test-day record is easier and advance than lactation record as in this case only one or two particular day (test-day) record is required instead of taking all day records up to 305-days of lactation. Test-day milk yields offered a better modeling opportunity and more accurate in genetic evaluation. Also, test-day milk yields in farm should be taken into consideration for selection of the buffalo for milk yield. The test-day models have been suggested as the method of choice for the analysis of milk yield traits in order to maximize the use of all available information. This method becomes even more important in smaller herd size and without well-established milk recording schemes. In fact, the test-day model appears to be a better alternate of 305-day lactation model because early selection on the basis of test-days could reduce generation interval. It could economize the genetic evaluation of dairy animals and improve accuracy of evaluation. Estimation of breeding value based on test-day milk yield would offer a solution to handle complex situation like lack of necessary infrastructure for daily milk recording and hence cost of recording could be reduced substantially.

**REFERENCES**


Harvey, W.R. 1990. Mixed model least squares’ and maximum likelihood computer program, PC-2 version, Ohio, USA.
Table 1. Rank of sires on the basis of estimated breeding values for first lactation 305-days or less milk yield.

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Table 2. Rank of sires on the basis of estimated breeding values for first lactation monthly test day-6 milk yield.

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Table 3. Spearman’s rank correlations for the ranks between first lactation 305-days or less milk yield and first lactation monthly test day-6 milk yield.

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* Significant at (P < 0.05) ** Significant at (P < 0.01).


EVALUATION OF FRESH SEMEN QUALITY AND PREDICTING THE NUMBER OF FROZEN SEMEN DOSES IN JAFFRABADI BUFFALO BULL

S.N. Ghodasara*, P.U. Gajbhiye, A.R. Ahlawat and K.S. Murthy

ABSTRACT

Scientific information on frozen semen characteristics of Jaffrabadi buffaloes (*Bubalis bubalis*) is scanty and centre to this research is to evaluate frozen semen characteristics in Jaffrabadi buffalo bulls. The study included six buffalo bulls with average age 95.83±5.47 months and average body weight of 834.16±47.63 kg maintained at Cattle Breeding Farm, Junagadh Agricultural University, Junagadh. A total of 206 ejaculates were collected during the period of study. Ejaculates collected from the six Jaffrabadi buffalo bulls were clean, dense to very dense (D = 67.2%, DD = 32.8%) and milky white (72.5%) to creamy (27.5%) in colour. During this observatory study average (Mean±SE) semen parameter like, ejaculatory volume, mass activity, sperm concentration, initial progressive sperm motility and total sperm number per ejaculate were 5.11±0.17 ml, +3.43±0.04, 838.30±25.74×10⁶/ml, 79.41±0.60% and 4053.99±150.56×10⁶ spermatozoa, respectively. Average dilution rate in 0.5 ml medium straw (40 million sperm/straw) was found to be ideal at 10.48±0.32 ml. In a year expected number of ejaculates that could be frozen from the 6 bulls was 34.34±6.43 and correspondingly, the expected number of frozen doses produced from bulls could be 3546.46±540.30 numbers.

Keywords: Jaffrabadi bulls, ejaculates, frozen doses, semen parameter

INTRODUCTION

Jaffrabadi buffalo is one of the heaviest buffalo breeds of world, inhabitant of Gir forest area in Saurashtra region of Gujarat, India. These buffaloes are known on their higher milk fat per cent (>8%) and larger fat globular size and hence the milk is preferred for Ghee and Khoa making (Thomas and Sastry, 2005). High quality Jaffrabadi frozen semen producing centers in the region are few and studies on semen characters and sexual behavior are scanty. There are many agencies/organizations of government and non government organizations working in the field of breed improvement in the Jaffrabadi buffaloes, but information need to be assessed on the reproductive performance, behavior and semen characteristics of Jaffrabadi bull. Changes in the environmental condition influence sperm output, accessory sex gland secretion and epididymal function, all of which are reflected in the ejaculate as volume, sperm numbers or sperm motility, morphology, viability etc. (Koonjaenak et al., 2007). The knowledge of sexual behavior and semen evaluation are valuable tools to estimate the reproductive efficiency of a breeding bull (Brohi, 1993).
MATERIALS AND METHODS

Six Jaffrabadi buffalo bulls of Cattle Breeding Farm, Junagadh Agricultural University, Junagadh aged 95.83±5.47 months (mean±SE, ranges 75 to 108 months) with live weight of 834.16±47.63 kg (mean±SE, ranges 730-1000 kg) with typical breed characters (Figure 1 and 2) formed the experimental material. Buffalo 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 green fodder such as maize, sorgum, sunflower and lucern according to the season and availability along with ad lib mature pasture grass hay. Concentrate component of ration comprised of mixture (50:50) of commercial concentrate pellet (Amul power dan) and cotton seed cake at the rate of 4.5 kg per bull/per day along with mineral mixture powder at the rate of 40 g/bull/day. The bulls were drenched with 10 eggs and 500 ml edible oil (cotton seed oil) 3 times a month.

A clinical history of each bull was taken at the start of this study, including previous illnesses, mating behavior and libido. The data were compiled on a total of 206 ejaculates of 6 Jaffrabadi buffalo bull during the period from July 2011 to June 2012.

Bulls were properly cleaned in perpetual area with plenty of clean water and semen was collected using male dummy without giving any false mounting. Samples were collected early in the morning once a week, using artificial vagina (AV) maintaining inner temperature 40-42°C. The temperature of semen processing room was maintained at 20-25°C during the whole study hours. Immediately after collection, each sample was transferred to laboratory and placed in a water bath at 35°C. Sterilization of all items were maintained before the day of collection and kept in an incubator at 45°C.

Ejaculates were collected by AV technique by trained person (Figure 3). Two ejaculates with a gap of 20 to 30 minutes were collected (Figure 4). Immediately after collection ejaculate was evaluated for clarity/cleanliness (1 = clean, 2 = dirty or contaminated), colour (1 = watery, 2 =
Figure 3. Semen collection in Jaffrabadi buffalo bull.

Figure 4. Thick creamy white semen.

Figure 5. Live and dead count of spermatozoa using eosin and nigrosin staining.
milky, 3 = creamy), density (0 = thin, D = dense, DD = very dense) and Volume (ml, graduated collection tube). Mass activity of spermatozoa was recorded by placing a drop of semen on a warm slide at 100 × magnification under a microscope with attached stage warmer (temperature set at 37°C), camera and LCD screen (0 = no mass activity, +1 = slow waves, +2 = quick waves, +3 = very quick waves, +4 = Waves, churning of whirls and eddies) (Nath, 1988). Motility, as a percentage of individually motile spermatozoa, was estimated by examining a drop of diluted fresh semen (with buffer solution) under a microscope at 200×. Motility percentage was scored on the basis of the percentage of spermatozoa with normal forward progressive movement, while those showing circling movements or those oscillating at one place were regarded as immotile (Ahmad, 1994). Sperm concentration was assessed with Bovine Accucell photometer, (IMV) by diluting 1:100 time neat semen in to Sodium chloride solution 0.9% W/V. Live and Dead spermatozoa count has been carried out using Eosin and Nigrosin staining technique (Figure 5) (Campbell, 1956).

RESULT AND DISCUSSION

Age of the bulls at the beginning of collection during the study period ranging from 75 months to 108 months, with a mean of 95.83±5.47 months. A total of 217 ejaculates were collected during the period of study. The distribution of collections per bull is shown in Table 1. Eleven ejaculates were considered very thin (watery) or dirty and were therefore discarded from semen processing and freezing. Semen characteristics of the remaining 206 ejaculates are summarized in Table 1.

Colour of semen studied was actually the thickness of the semen together with pigment. The ejaculates collected from the six Jaffrabadi buffalo bulls were clean, dense to very dense (D = 67.2%, DD = 32.8%) and milky white (72.5%) to creamy (27.5%) in colour (Figure 4). Javed et al. (2000) reported milky-white coloured semen in Nili-Ravi and in Swamp, buffalo bulls. Ejaculate volumes from Jaffrabadi bulls ranged from 2-12 ml (5.11±0.17 ml), similarly, nearly same ejaculatory volume were reported by Tomar et al. (1966); Shukla and Mishra (2005) in Murrah bulls and Javed et al. (2000) in Nili-Ravi bulls. However, scientists (Bhakat et al., 2011; Pant et al., 2003; Koonjaenak et al., 2007; Rehman et al., 2012) reported lower ejaculated volume as compared to Jaffrabadi in Murrah, swamp and Kundhi buffalo breeds, respectively. Differ in the semen volume in various breeds of buffaloes might be due to differences in genetics, reproductive health status of bulls, age of bulls, frequency of collection, pooled volume, nutrition, season and management (Nazir, 1988; Soderquist, 1992). Variations can also be due to skill of semen collector/attendant and temperature of AV.

Mass activity of experimental Jaffrabadi bulls (range from +2.5 to +4 with a mean of +3.43±0.04) is similar to earlier reports of mass activity reported by various researcher (Ram, 1988; Dhami, 1992; Shukla and Mishra, 2005) in Murrah bulls and Javed et al. (2000) in Nili-Ravi bulls. However, scientists (Bhakat et al., 2011; Pant et al., 2003; Koonjaenak et al., 2007; Rehman et al., 2012) reported lower ejaculated volume as compared to Jaffrabadi in Murrah, swamp and Kundhi buffalo breeds, respectively.
Table 1. Mean (±SE) of semen parameter/characteristics, dilution rate/ml of semen and Number of doses frozen/year/bull from Jaffrabadi bulls.

<table>
<thead>
<tr>
<th>Name or Number of Bulls</th>
<th>Laxman</th>
<th>Bhagro</th>
<th>Moti</th>
<th>Nagraj</th>
<th>Sundar</th>
<th>Raja</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of bulls (Months)</td>
<td>102</td>
<td>96</td>
<td>75</td>
<td>108</td>
<td>89</td>
<td>105</td>
<td>95.83±5.47</td>
</tr>
<tr>
<td>No. of ejaculates</td>
<td>54</td>
<td>46</td>
<td>40</td>
<td>23</td>
<td>25</td>
<td>18</td>
<td>34.34±6.43</td>
</tr>
<tr>
<td>Volume of semen/ ejaculate</td>
<td>4.59±0.27</td>
<td>6.89±0.51</td>
<td>4.94±0.24</td>
<td>4.82±0.27</td>
<td>4.20±0.39</td>
<td>4.17±0.36</td>
<td>5.11±0.17</td>
</tr>
<tr>
<td>Cleanliness of semen (Score: 1-2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Colour (Score: 1-3)</td>
<td>2-3</td>
<td>2-3</td>
<td>2-3</td>
<td>2-3</td>
<td>2-3</td>
<td>2-3</td>
<td>-</td>
</tr>
<tr>
<td>Density (Score: 0-DD)</td>
<td>D-DD</td>
<td>D-DD</td>
<td>D-DD</td>
<td>D-DD</td>
<td>D-DD</td>
<td>D-DD</td>
<td>-</td>
</tr>
<tr>
<td>Mass activity</td>
<td>3.39±0.08</td>
<td>3.30±0.09</td>
<td>3.53±0.08</td>
<td>3.48±0.14</td>
<td>3.48±0.10</td>
<td>3.50±0.12</td>
<td>3.43±0.04</td>
</tr>
<tr>
<td>Initial progressive sperm motility (%)</td>
<td>79.43±0.98</td>
<td>75.65±1.92</td>
<td>81.00±1.15</td>
<td>80.43±1.38</td>
<td>83.00±1.10</td>
<td>79.17±1.52</td>
<td>79.41±0.60</td>
</tr>
<tr>
<td>Sperm concentration (10^6/ml)</td>
<td>732.00±29.16</td>
<td>561.37±41.44</td>
<td>960.38±45.02</td>
<td>916.17±78.22</td>
<td>1212.76±96.86</td>
<td>974.00±85.33</td>
<td>838.30±25.74</td>
</tr>
<tr>
<td>Total sperm number (10^6)</td>
<td>3348.18±227.45</td>
<td>3314.45±314.13</td>
<td>4817.27±345.80</td>
<td>4394.16±486.14</td>
<td>5025.76±535.66</td>
<td>4069.78±477.79</td>
<td>4053.99±150.56</td>
</tr>
<tr>
<td>% of Live sperm</td>
<td>85.17±0.76</td>
<td>84.76±1.02</td>
<td>85.70±0.99</td>
<td>87.09±1.09</td>
<td>84.52±1.32</td>
<td>85.94±1.44</td>
<td>85.38±0.42</td>
</tr>
<tr>
<td>Average dilution rate/ ml of semen</td>
<td>9.15±0.36</td>
<td>7.02±0.52</td>
<td>12±0.56</td>
<td>11.45±0.98</td>
<td>15.16±1.21</td>
<td>12.18±1.07</td>
<td>10.48±0.32</td>
</tr>
<tr>
<td>Post Thaw progressive motility</td>
<td>57.13±0.86</td>
<td>57.39±0.94</td>
<td>59.26±1.08</td>
<td>59.57±1.11</td>
<td>60.60±0.99</td>
<td>61.94±0.94</td>
<td>58.71±0.42</td>
</tr>
<tr>
<td>Calculated no. of ejaculate frozen(dose)/ year/bull</td>
<td>4535.8</td>
<td>4449.83</td>
<td>4742.4</td>
<td>2538.69</td>
<td>3183.6</td>
<td>1828.46</td>
<td>3546.46±540.30</td>
</tr>
</tbody>
</table>
During the study initial progressive sperm motility ranged from 65 to 95%, with mean of 79.41±0.60 and is in agreement with the findings of Koonjaenak et al. (2007) in swamp buffalo. Sahu and Pandit (1997) and Shukla and Mishra (2005) recorded higher initial progressive motility in Murrah bulls. Lower percentage of initial motility than the present findings in Murrah bulls was also reported by Bhakat et al. (2011) and Kumar et al. (1993). The post thaw motility ranged from 45% to 65% with average mean of 58.71±0.42 during the entire study period. Percentage of live and dead spermatozoa of all the Jaffrabadi bulls was ranged from 75 to 96 percent live spermatozoa with average of 85.38±0.42 percent live spermatozoa.

Total sperm number per ejaculate ranged from 2400 to 10136 million sperm with mean of 4053.99±150.56×10⁶ spermatozoa. Mean dilution rate was found to be 10.48±0.32, with a range of 4.5 to 25.83 ml. Expected number of ejaculates that could be frozen from the 6 bulls was 34.34±6.43 (ranging from 18 to 54) and correspondingly, expected frozen doses produced from bulls could be 3546.46±540.30 (ranged 1828.46 to 4742.4). Out of six bulls, ejaculates were collected from three bulls for entire year that produced 4576±67.13 frozen doses/year. Two bulls were used for semen collection for only 8 months and one bull was used for 5 months. Each semen doses were of 0.5 ml with 40 million sperm concentration per straw. Bhakat et al. (2011) revealed average total sperm output of Murrah buffalo bull was 2,561.05±77.80×10⁶. Average dilution rate was found to be 12.49±0.13. Expected number of ejaculates that could be frozen per year per bull was 53.27 and correspondingly, the expected frozen doses produced per year per bull could be 6,879.49. Zafar et al. (1988) reported yearly production to be 8,412 semen doses per bull in Nili-Ravi buffalo bulls and Roy (2006) produced 5,147.48 doses/year/bull in Murrah bulls which was higher than the estimate for Jaffrabadi bulls in the present study.

In vitro semen evaluation parameters used in the present study are used to determine fresh semen motility in post-thaw samples. Some research workers established a correlation between motility and field fertility; others did not (Christensen et al., 1999; Tardif et al., 1999). Variations in semen quality parameters recorded in the present investigation, 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 makeup of the bulls (Tomar et al., 1966), season of study (Tuli, 1984) and agro climatic conditions.

Present study reveled that semen characters of Jaffrabadi bulls are comparable to Murrah and there characteristics can be made use of to meet the high demand for semen from selected Bulls of high genetic merit. Harvesting maximum semen doses per bull was the other approach to increasing the number of inseminations possible per bull. Information on expected semen doses per bull will help in planning the functioning of A. I. center at field level as per the capacity of the semen station running at Cattle Breeding Farm.

**REFERENCE**


Nazir, M. 1988. semen evaluation and sperm morphology - monography on reproductive pattern of Riverine buffaloes and recommendations to improve their reproductive performance at small farmer level. PARC, Islamabad.


ABSTRACT

Nutritional deficiency including mineral deficiency may decrease reproductive efficiency in buffaloes. Therefore, aim of the present study was to assess effect of minerals Calcium (Ca) Magnesium (Mg) and inorganic phosphorus (Pi) on cyclicity of Nili Ravi Buffaloes. The present experiment was performed at Livestock Experiment Station, Bhunikey. The female buffaloes (n=90) were divided into 3 groups; cyclic (n=30), non-cyclic (n=30) and repeat breeders (n=30). Mineral profile was measured through serum of the buffaloes under study. Calcium and Magnesium were measured through calorimetric method while inorganic phosphorus was measured through UV method. The level of calcium was significantly (P<0.05) higher in normal cyclic buffaloes than that of the non-cyclic and repeat breeders. The level of magnesium was non-significant in normal cyclic and non-cyclic buffaloes, while it was significantly (P<0.05) higher in repeat breeders as compared to cyclic and non-cyclic buffaloes. Phosphorus was not in balance in non-cyclic and repeat breeders. It is concluded from the present study that Ca: P should be 2:1.

Keywords: repeat breeder, non-cyclic, calcium, inorganic phosphorus, magnesium

INTRODUCTION

Reproductive efficiency is the primary factor affecting productivity of a dairy buffalo and is greatly influenced by late attainment of puberty, seasonal breeding, long calving intervals, increased number of services per conception, increased days open, uterine infections and various obstetrical problems (Samad et al., 1987). Anoestrus and repeat breeding are the biggest factor decreasing reproductive efficiency. Among the various factors that cause anoestrus and repeat breeding, major one is under-nutrition (Francos et al., 1977; Bhaskaran and Patil, 1982). The minerals play vital role in development of reproductive potential and maintenance of functional integrity of the reproductive system in domestic animals (Leathem, 1966). The calcium (Ca), Phosphorus (P) and Magnesium (Mg) are important minerals in this respect. The exact mechanism by which mineral deficiency reduces fertility is not clear (Luca et al., 1977). Calcium, inorganic phosphorus and magnesium may lead to reproductive failure (Hidiroglo, 1979). The ovarian activities are most prone to minerals imbalances and their deficiency suppresses ovarian activity (Haq et al., 1999). Therefore, this study was conducted to assess the effect of minerals (Calcium, Phosphorus, and Magnesium) on anoestrus and repeat breeding in

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1Buffalo Research Institute, Pattoki, District Kasur, Pakistan, *E-mail: raoqaisarshahzad@gmail.com
2University of Veterinary and Animal Sciences, Lahore, Pakistan
3Department of Animal Health, University of Agriculture, Peshawar, Pakistan
MATERIALS AND METHODS

Nili-Ravi buffaloes (n=90) of 3-10 years of age having similar BCS (Body Condition Score) were selected from Livestock Experiment Station, Bhunikey.

Buffaloes were grouped into three categories; Cyclic (n=30), Non Cyclic (n=30) and Repeat Breeder (n=30). Buffaloes having smooth and inactive ovaries in 10 days interval of rectal palpation were called as non cyclic. Buffaloes having no abnormality on palpation and not being pregnant after artificial inseminations in three consecutive estrus period were called as repeat breeder.

Blood samples were collected from the jugular vein of the animal aseptically. The samples were kept at room temperature for 24 h for serum separation. The serum were collected and stored at -20°C for further studies.

Analytical procedure
i) Diagnostic kits: Diagnostic kits bearing cat. No. Ca 590, Mag 570 and PH. 1016 (Randox International Lab. Ltd, UK) were used and standard procedures were applied for estimating concentrations of serum calcium, magnesium and inorganic phosphorus.

ii) Analyses of samples: Colorimetric method was used for the estimation of serum calcium (Sarkar, 1967) and magnesium and UV method was applied for inorganic phosphorus concentration (Teiz, 1983). All these analyses were performed through spectronic-21.

iii) Computation of concentration: Concentration of calcium, magnesium and inorganic phosphorus will be computed as following.

**Calcium**

Concentration (mg/dl) = Absorbance of sample x 9.82
Absorbance of standard

**Magnesium**

Concentration (mg/dl) = Absorbance of sample x 2.31
Absorbance of standard

**Inorganic phosphorus**

Concentration (mg/dl) = Absorbance of sample x 5.18
Absorbance of standard

Statistical Analysis

The data thus collected was analyzed by Analysis of Variance (ANOVA) by using SPSS version 13 (Steel and Torrie, 1982).

RESULTS AND DISCUSSIONS

Normal cyclic buffaloes had significantly (P<0.05) higher calcium than non cyclic and repeat breeder buffaloes. The results of the present study are in line with Pasha et al. (2012) who reported similar (9-11 mg/dl) calcium levels in the serum of buffaloes in Punjab. Results are not in coincidence with Husnain et al. (1981), who reported the calcium level in serum of milking buffaloes were slightly lower (6.70- 8.00 mg/dl). These results are not in line with the study of Hedaoao et al. (2008) who reported that there is no difference of calcium between normal cycling and anoestrus buffaloes.

Normal cyclic buffaloes had similar levels
of magnesium to that of non cyclic, while repeat breeder buffaloes had higher levels of magnesium as compared to both cyclic and non cyclic buffaloes. Results of the present study are in line with Hedaoo et al. (2008) who reported that magnesium has no effect on cyclicity of the buffaloes. Magnesium values in buffaloes blood were slightly higher than Pasha et al. (2012) who reported 2.68 mg/dl in Punjab, Iqbal. (1990), who reported 2.57-2.58 mg/dl magnesium in blood serum of cattle and Hussain (1991) who reported 1.75-280 mg/dl in blood serum of cattle. Results are similar to Oba and Ramos (1988), who reported 3.84±1 mg/dl magnesium in serum of cattle.

Normal cyclic buffaloes had low levels of inorganic phosphorus than that of non cyclic and repeat breeder buffaloes. Calcium and phosphorus should be 2:1 in mammals. In the present in anoestrus and repeat breeder buffaloes, this ratio was high. Results in the present study were in line with Hignett. (1959) who reported that phosphorus higher than 2:1 may result in infertility.

It’s concluded from the above trial that calcium and phosphorus imbalance may result in infertility while magnesium may have no effect on fertility. It’s further suggested that further studies comprising blood mineral profile, fodder mineral profile and soil mineral profile should be measured to sort out exact problem of the nutritional deficiency.

### REFERENCES


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### Table 1. Mean ± S.E values of calcium, magnesium, and inorganic phosphorus among different buffalo groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calcium (mg/dl)</th>
<th>Magnesium (mg/dl)</th>
<th>Inorganic Phosphorus (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Non cyclic</td>
<td>10.55±0.43⁠ᵃ</td>
<td>4.76±0.22⁠ᵃ</td>
<td>5.39±0.28⁠ᵃ</td>
</tr>
<tr>
<td>Repeat breeder</td>
<td>10.01±0.42⁠ᵃ</td>
<td>5.36 ±0.38⁠ᵇ</td>
<td>5.55±0.32⁠ᵇ</td>
</tr>
<tr>
<td>Cyclic</td>
<td>11.77±0.37⁠ᵇ</td>
<td>4.98±0.38⁠ᵇ</td>
<td>4.64±0.33⁠ᵇ</td>
</tr>
</tbody>
</table>

Mean values with in the same column bearing different superscripts differ significantly (P<0.05) among groups.

Biochem., 20: 155-166.
ABSTRACT

The present study reports prevalence of dermatophilosis due to *Dermatophilus congolensis* among buffaloes in Kerala. Five buffaloes presented with skin lesions primarily on the lower limbs, udder and tail were subjected to detailed investigations to identify the etiological factors. Skin swabs and scabs were collected from the lesions under sterile conditions and were subjected to direct microscopical and cultural examinations. Direct microscopical examination of Giemsa and Gram's stained smears of scabs revealed typical tram track appearance of *Dermatophilus congolensis*. Culture of skin scabs in sheep blood agar yielded typical greyish beta haemolytic adherent colonies. The isolates were further confirmed by morphological appearance and biochemical reactions. Direct microscopical examination of skin scrapings yielded negative results for fungal elements and mites. This forms the preliminary report of dermatophilosis among buffaloes in Kerala.

Keywords: *Dermatophilus congolensis*, prevalence, buffaloes, Kerala

INTRODUCTION

Dermatophilosis is an exudative, pustular dermatitis that affects domestic, aquatic and wild animals and man, caused by *Dermatophilus congolensis*. It is an economically important disease which causes considerable loss in terms of skin damage, reduced meat and milk production, culling or death of affected animals and costs of control and treatment (Zaria, 1993). It has been reported by the Food and Agricultural organization (FAO) to be one of the four major bacterial diseases which affect cattle and other animals in the tropical and subtropical regions (Hashemi Tabar *et al*., 2004). Diagnosis of the condition is by demonstration of typical tram track appearance of the organism in stained skin scabs and confirmation by isolation and identification of organisms. This disease is of worldwide occurrence but more prevalent in tropical and subtropical countries. The disease is a chronic dermatitis and could occur in any part of the body and occasionally become generalised. Accurate diagnosis and early treatment are found to be useful for better clinical recovery from the condition. There are few reports of Dermatophilosis among buffaloes from India (Pal, 1995; Sharma *et al*., 1992). The present study forms the first report of dermatophilosis from buffaloes in Kerala.
MATERIALS AND METHODS

Five Murrah buffaloes presented with dermatological problems during 2011 were included in the study. Detailed clinical examination of these animals was carried out and type of lesions was recorded. Skin scabs and scrapings and impression smears from lesions were collected under sterile conditions for laboratory examination. Small pieces of skin were taken from the underside of the scabs and softened in few drops of distilled water on a clean microscopic slide; a smear was made and stained with Giemsa and Gram’s stains (Quinn et al., 1994). The impression smears taken from the lesions were also stained with Giemsa’s stain and Gram’s stain and examined under the oil immersion objective of microscope. The skin scrapings were also subjected to direct microscopical examination using 10 percent potassium hydroxide to rule out fungal elements and mites.

Isolation of D. congolensis was carried out using Haalstra’s technique (Haalstra, 1965). Skin scabs were minced with a sterile scalpel blade and placed in glass bottles. One millilitre of sterile water was added to each specimen. The bottles were allowed to stand open for three and a half hours at room temperature. Then the opened bottle was transferred to candle jar, with a candle was burned within the jar to obtain 10 to 20 percent carbon dioxide tension. Under CO₂ tension the motile zoospores if present, were chemotactically attracted to the surface of the distilled water. After 15 minutes, the bottle was carefully removed and a loopful taken from the water surface was seeded on blood agar plates and incubated at 37°C in 20 percent carbon dioxide for 24 to 48 h. The plates were examined for colonies of D. congolensis (Quinn et al., 1994).

The isolates were stained by Gram’s method and the preliminary tests were done based on it. The morphological, cultural, biochemical and sugar fermentation tests of the isolates were determined as per the methods described by Cowan (1974).

RESULTS AND DISCUSSIONS

Detailed clinical examination of animals revealed characteristic exudative dermatitis lesions with formation of scabs, crusts and fissures with matted hair at their bases, suggestive of dermatophilosis (Figure 1). Similar types of lesions were described by most of the workers irrespective of the species of the animals affected (Koney, 1996; Gitao et al., 1998; Wabacha et al., 2007). All the animals had lesions on the lower limbs, three had lesions on the udder (Figure 2) and two had lesions on the tail (Figure 3). One of the animals had severe generalised lesions involving all these areas (Figure 4).

Microscopical examination of Giemsa or Gram’s stained smears of the scab material from the lesions revealed characteristic Gram positive septate branching filaments which were longitudinally as well as transversely divided to form spherical or ovoid cocci in multiple rows, with typical ‘tram-track appearance’ suggestive of D. congolensis in all samples (Figure 5). This distinctive morphology of the organism was demonstrated by most of the workers as the most practical diagnostic method for dermatophilosis (Abu-Samra, 1978; Quinn et al., 1994). The organisms were observed in different forms depending on the stage of development varying from long branching filaments, filaments packed with zoospores and mature free zoospores released from the filaments. Kaminski and Suter (1976) and Hyslop (1980) described pleomorphic
Figure 1. Lesions with thick scabs and fissures on limb.

Figure 2. Lesions on the udder.

Figure 3. Lesions on the tail.

Figure 4. Severe generalised dermatophilus dermatitis.

Figure 5. Branching filaments of *D. congolensis* in scabs (Gram’s stain x1000).
nature of \textit{D. congolensis} in stained smears of scabs and stated that the organism might be seen in any form of the various stages of its lifecycle.

Culture of the scab materials from all five animals yielded typical beta haemolytic colonies of \textit{D. congolensis} in sheep blood agar in presence of 10 percent carbon dioxide (Figure 6). There were variations in the shape, colour and texture of the colonies. Similar observations were also made by Gordon (1964) and EL-Nageh (1971).

Microscopical appearance of organisms in Gram stained smears from colonies were also highly variable with Gram positive branching filaments in different stages of segmentation, packets of coccoid forms, germinating spores or combinations of the above forms depending on the age of the culture and strain of the isolate (Figure 7). The wet mount preparation of all the isolates revealed motile zoospores. All the isolates were haemolytic producing clear zones of haemolysis in seven percent sheep blood agar within a period of 24 to 72 h of incubation. The isolates were positive for catalase, oxidase and urease tests and were able to digest gelatin and Loefflers coagulated serum, indicating proteolytic activity. The isolates showed hydrolysis of starch and casein. All the isolates showed negative results with nitrate test and indole test. Similar biochemical characteristics were also reported for \textit{D. congolensis} by several workers (Pal, 1995; Mannan \textit{et al.}, 2009).

All the five isolates produced acid from glucose, fructose and sucrose within 24 h of incubation. But variable results were obtained with maltose, mannitol and lactose. The isolates were unable to produce acid from sorbitol and xylose. This is in agreement with previous findings (Mannan \textit{et al.}, 2009; Shaibu \textit{et al.}, 2011). None of the isolates produced gas from the sugars. No fungal elements or mites could be detected on microscopical examination of skin scrapings using 10 percent potassium hydroxide.

The results of the present study confirmed occurrence of Dermatophilosis among buffaloes in Kerala. The presence of predisposing factors such as prolonged wetting, high humidity, high temperature and various ectoparasites might have predisposed to the occurrence of the condition. An early and prompt diagnosis and treatment of the condition has to be undertaken to reduce the economic loss to farmers.

\textbf{REFERENCES}

Abu-Samra, M.T. 1978. Morphological, cultural and biochemical characteristics of


ABSTRACT

This study was carried out during breeding season on 125 repeat breeding buffaloes to evaluate the therapeutic efficacy of various hormonal and non-hormonal drugs in improving their reproductive efficiency. Repeat breeding buffaloes (112) were treated parenterally with 5 different drugs, keeping 13 animals as untreated control, and results were compared with 22 normal cyclic buffaloes. The conception rates in treatment cycle and overall of 3 cycles post-treatment were compared between different groups. For 25 and 32 repeat breeding buffaloes treated with 0.02 mg Gonadotropin Releasing Hormone (GnRH; Receptal 5 ml i/m), just after artificial insemination (AI) and 500 mg of hydroxy-progesterone caproate (Duraprogen 2 ml i/m) on day 4th or 5th post-AI, the conception rates (CRs) in the treatment cycle were 60.00 and 43.75% and overall CRs within 3 cycles were 76.00 and 62.50% (P<0.05), respectively, with a mean treatment to fertile oestrus interval of 6.58±3.27 and 8.25±3.28 days. For 26, 23 and 6 repeating buffaloes treated with Enroloxacin (Inj. Bayrocin single shot 30 ml) i/m at the time of AI, Ceftriaxone (Inj. Vetacef 2 g) intrauterine (i/ut) at 12-24 h post-AI, and Povidone plus Metranidazole (Ranvidone 20-40 ml) i/ut for 2-4 days (AI in next cycle), the first service conception rates were 23.08, 34.78 and 33.33%, respectively, and overall CRs 53.85, 65.22 and 83.33% (P<0.01), with the fertile oestrus intervals of 20.86±5.53, 12.20±4.49 and 17.20±8.17 days, respectively. The results of ceftriaxone were better as compared to enrofloxacin. The overall CRs for the GnRH, progesterone and antibacterial therapies were 76.00, 62.85 and 53.85 to 83.33% (P<0.01), respectively (normal cyclic group 81.82%, repeat breeding control 38.46%), with significantly (P<0.05) shorter fertile oestrus interval in GnRH and progesterone treated groups as compared to antibiotics treated one. Thus, all these regimes, and GnRH in particular, are recommended to the practitioners for their use in the field to ameliorate the problem of repeat breeding in buffaloes.

Keywords: repeat breeding, buffaloes, hormonal/non-hormonal therapy, conception rate
INTRODUCTION

The term repeat breeder or cyclic non-breeder describes the animal that has failed to conceive after 3 or 4 services of a fertile bull/artificial inseminations (AI). Repeat breeding is a major constraint in dairy farming. It is an important cause of low reproductive efficiency in buffaloes. The incidence of repeat breeding varies from 15-32% and seems to be lower in animals kept individually on small-holdings than in large herds. Endocrine imbalance, nutrition, faulty breeding management, early embryonic mortality and infectious agents leading to clinical and sub-clinical endometritis are amongst the major causes of repeat breeding in dairy animals (Zemjanis, 1980). Fertilization failure is rare in females but zygote does not survive and therefore subsequent oestrus follows normally. Luteal dysfunction leading to inadequate progesterone production post-breeding could be a cause of embryonic death. Gonadotropin Releasing Hormone (GnRH) / Human Chorionic Gonadotropin (hCG) and/or progesterone analogues have been successful to sustain early pregnancy and improve conception rate in repeat breeding bovines (Sreenan and Diskin, 1983; Dhami et al., 2009; Patel et al., 2014). Similarly post-insemination antibiotics therapy intrauterine is beneficial in enhancing conception rate in repeat breeders of unknown etiology and particularly with low grade genital infection (Mahto et al., 2006; Dhami et al., 2009). However, in most of the reports only one protocol has been tested at a time, and the literature on comparative efficacy of hormonal and antibacterial approach in the same condition is scarce. Hence the present study was planned to evaluate the comparative therapeutic efficacy of GnRH, Progesterone and Antibiotics/Antiseptics at a time in repeat breeding buffaloes under field conditions.

MATERIALS AND METHODS

This study was conducted under field conditions in villages of Anand district in Gujarat (India). The buffaloes managed by the farmers individually at their door-step and brought to the AI Centers of the concerned village co-operative societies for AI, pregnancy diagnosis and sexual health control camps were initially screened through gynaeco-clinical examinations. In all 125 repeat breeding buffaloes that had taken more than 3 infertile services, even with good quality frozen-thawed semen, beyond 6 months to 1 year postpartum and confirmed by rectal palpation twice 10 days apart, were selected and subjected to different therapeutic regimes (112), keeping 13 as untreated control. Moreover, 22 buffaloes exhibiting spontaneous estrus within 90 days postpartum and inseminated without any treatment served as normal cyclic controls. Buffaloes in estrus were inseminated by the concerned lay inseminator of the society.

All the animals identified were dewormed using Albendazole 3000 mg (Helmiguard 3000, Vetcare India Ltd.) and were also treated for ectoparasites, if any, by using Flumethrin (Flupor, Vetnex-RFCL India Ltd). Owners of the ear-marked animals were supplied with mineral mixtures (Amul brand) for supplementing to their animals 50-55 g per day for 15 days. The following were the treatment protocols used (Table 1).

Animals of all seven groups once inseminated were followed for 1 to 3 cycles post-treatment, and overall as well as cycle-wise conception rates and fertile oestrus intervals were compared between groups by Chi-square test,
and completely randomized design, respectively (Snedecor and Cochran, 1986). The results are presented in Table 2 and also illustrated by Figure 1.

RESULTS AND DISCUSSION

Effect of gonadotropin releasing hormone (GnRH)

Twenty five repeat breeding buffaloes were treated with 20 µg GnRH intramuscularly (i/m), just after AI. The conception rates obtained were 60 and 76% in the treatment cycle and overall of 3 cycles post-treatment, respectively, with a mean treatment to fertile oestrus interval of 6.58±3.27 days. Maximum buffaloes conceived in the treatment cycle itself indicating beneficial effect of GnRH in inducing fertile ovulation and CL growth (Table 2, Figure 1). The present findings of 76 and 60% conception rate using GnRH as against 38.46 and 14.29% in untreated control group coincided well with the reports of Ghulam et al. (2002); Vijayarajan et al. (2007); Sharma and Dhani

Table 1. Different approaches used in the treatment of repeat breeding (Gr. 1 to 6) in buffaloes under field conditions.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment Groups</th>
<th>No. of Buffaloes</th>
<th>Status of Repeat Breeding / Normal Cyclic Animals</th>
<th>Treatment Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GnRH</td>
<td>25</td>
<td>Long oestrus, free from visible genital infection</td>
<td>Buserelin acetate-GnRH 20 µg i/m simultaneous to AI (Receptal, 5 ml)</td>
</tr>
<tr>
<td>2</td>
<td>Progesterone</td>
<td>32</td>
<td>Normal oestrus, apparently free from visible genital infection</td>
<td>Hydroxyprogesterone 500 mg i/m 4th or 5th day post-AI (Duraprogen, 2 ml)</td>
</tr>
<tr>
<td>3</td>
<td>Enrofolxacin</td>
<td>26</td>
<td>Normal oestrus, discharge free from visible genital infection</td>
<td>Enrofloxacin 3 g i/m (Bayrocin 1 shot, 30 ml), AI simultaneously or in next cycle</td>
</tr>
<tr>
<td>4</td>
<td>Ceftriaxone</td>
<td>23</td>
<td>Normal oestrus, discharge free from visible genital infection</td>
<td>Ceftriaxone 2 g i/uterine in 20 ml DW (Vetaceph 2 g) 12-24 h post-AI</td>
</tr>
<tr>
<td>5</td>
<td>Ranvidone</td>
<td>06</td>
<td>Repeat breeding with clear unhealthy discharge</td>
<td>Povidone + Metronidazole 20-30 ml i/uterine for 2-3 days, AI in next cycle</td>
</tr>
<tr>
<td>6</td>
<td>Untreated Control</td>
<td>13</td>
<td>Normal oestrus, discharge free from visible genital infection</td>
<td>No any treatment, only AI and follow up</td>
</tr>
<tr>
<td>7</td>
<td>Normal Cyclic Control</td>
<td>22</td>
<td>Normal first oestrus within 90 days postpartum, free from visible genital infection</td>
<td>No any treatment, only AI and follow up</td>
</tr>
</tbody>
</table>
(2008); Dhami et al. (2009); Savalia et al. (2013) in repeat breeding buffaloes and Patel et al. (2014) in crossbred cows. Stevenson et al. (1990) found overall conception rate of 32.1 vs 41.6% (P<0.01) following AI alone and AI+100 µg GnRH i/m among hundreds of repeat breeding cows. Morgan and Lean (1993) recorded 12.5% increase in overall conception rate with the use of GnRH (250 µg) in normal cows and up to 22.5% in repeat breeding cows, while in other studies the conception rates of 60 vs 40% were found for GnRH treated (0.02 mg i/m) vs untreated repeat breeding cows (Ata and Tekin, 2001; Shelar et al., 2002). Further, Mandal et al. (2004) found first service conception rates of 50.0 vs 37.5% and overall conception rates 87.5 vs 75.0% in GnRH (2.5 ml Receptal) treated vs untreated repeat breeders. The beneficial results with GnRH injection at AI could be due to induction of timely ovulation with improved CL function.

**Effect of progesterone supplementation**

For 32 repeat breeding buffaloes treated with 500 mg of hydroxy-progesterone caproate on day 4th or 5th post-AI, the conception rate obtained was 43.75% in the treatment cycle itself, and 62.50% overall, with a mean treatment to fertile oestrus interval of 8.25±3.28 days (Table 2, Figure 1). These findings of first service and overall conception rates obtained with progesterone supplementation, as against 14.29 and 38.46% in untreated control group, are closely comparable with the earlier reports of Kavani and Kodagali (1984); Awasthi et al. (2002); Kumar et al. (2003); Dhami et al. (2009) in cows and buffaloes. Sharma et al. (2004) and Patel et al. (2005) found conception rates of 66.7 vs 50.0% and 50.0 vs 33.3% for 4th day post-AI progesterone treated vs untreated repeat breeding buffaloes and HF cows, respectively. Sharma and Dhami (2008) recorded 20 and 40% rise in conception rate over control group with 250 and 500 mg progesterone supplementation, respectively, on 4th day post-AI in repeat breeding buffaloes suggesting beneficial role of higher dose of therapy. According to Das et al. (1992), the most critical period for the embryo survival was the late blastocyst. The failure of blastocyst to implant might be due to the progestational changes in the endometrium at the appropriate time. Kastelic (1994) stated that most embryonic losses occur during early pregnancy, and the cause is usually unknown. Embryonic loss before 125 days is usually preceded by, and may be caused by, luteal regression. Hence, hormonal treatment to increase plasma progesterone concentrations may improve pregnancy rates, particularly in repeat breeding cows.

**Efficacy of antibiotics/Antimicrobials**

Twenty six repeating buffaloes were treated with long acting enrofloxac in intramuscularly at the time of insemination. The conception rates in treatment cycle, and overall of 3 cycles were 23.08 and 53.85%, with the fertile oestrus interval of 20.86±5.53 days. Among 23 repeating buffaloes treated with intrauterine infusion of 2 g Ceftriaxone 12 to 24 h post-AI, 34.78% buffaloes conceived in treatment cycle with an overall conception rate of 65.22% after a mean treatment to fertile oestrus interval of 12.20±4.49 days. Among 6 repeat breeding buffaloes with clear endometritis treated with intrauterine infusion of Ranvidone 20-30 ml, 33.33% buffaloes conceived in post-treatment 1 cycle, with an overall conception rate of 83.33% after a mean treatment to fertile oestrus interval of 17.20±8.17 days. The results of ceftriaxone were better as compared to enrofloxac in treated group in terms of first service (34.78 vs 23.08%) and overall conception rates (65.22 vs 53.85%) and even for
fertile oestrus interval (12.20±4.49 vs 20.86±5.53 days). Further, the overall conception rate (83.33%) with Ranvidone was significantly higher than the antibiotics treated groups without significant difference in the time interval, but the number of animal included in this group was comparatively less (Table 2, Figure 1).

Present findings of varying conception rate in treatment cycle and overall with different modes of antibiotics therapy corroborated well or partly with the previous report of Sharma and Dhami (2008); Dhami et al. (2009), who obtained significantly higher overall conception rates within 3 cycle among repeat breeding buffaloes treated with cephalaxin 4 g and ceftriaxone 2 g intrauterine 24 h post-AI as compared to untreated control group, while Kumar et al. (2004) obtained 80% conception in repeat breeding cows treated with enrofloxacin intramuscularly as against only 20% in untreated control group and 45% in normal breeding group. Rane et al. (2003) obtained 71.67% conception rate within 33 days among 60 repeat breeding buffaloes treated with i/ut enrofloxacin at 1500 mg (15 ml) for 2 days at previous oestrus as per the sensitivity of isolates of cervical mucus. Mahto et al. (2006) achieved 50% CRs each with pre- and post-AI ceftriaxone treatment in repeat breeding cows as compared to

Table 2. Fertility response following various hormonal and antibacterial treatments in repeat breeding buffaloes under field condition.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of Animals Treated</th>
<th>Conception Rate within 3 Cycles</th>
<th>Treatment to Fertile OI (Days)</th>
<th>Cycle-wise Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>GnRH (Receptal)</td>
<td>25</td>
<td>19</td>
<td>76.00b</td>
<td>6.58±3.27b</td>
</tr>
<tr>
<td>Progesterone (Duraprojen)</td>
<td>32</td>
<td>20</td>
<td>62.50c</td>
<td>8.25±3.28b</td>
</tr>
<tr>
<td>Antibiotic (Bayrocin)</td>
<td>26</td>
<td>14</td>
<td>53.85c</td>
<td>20.86±5.53a</td>
</tr>
<tr>
<td>Antibiotic (Vetacef)</td>
<td>23</td>
<td>15</td>
<td>65.22c</td>
<td>12.20±4.49a</td>
</tr>
<tr>
<td>Antiseptic* (Ranvidone)</td>
<td>6</td>
<td>5</td>
<td>83.33a</td>
<td>17.20±8.17a</td>
</tr>
<tr>
<td>Overall Treated</td>
<td>112</td>
<td>73</td>
<td>65.18c</td>
<td>11.66±2.00b</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>13</td>
<td>5</td>
<td>38.46d</td>
<td>13.40±5.47a</td>
</tr>
<tr>
<td>Normal Cyclic Control</td>
<td>22</td>
<td>18</td>
<td>81.82a</td>
<td>7.00±2.40b</td>
</tr>
</tbody>
</table>

OI = oestrus induction.
$ AIs were done in the next cycle following 2-4 days treatment with Ranvidone intrauterine.
Values bearing common superscript within the column do not differ significantly (P>0.05).
only 16% in untreated group. The usage of many other antibiotics and antiseptics have been reported with variable beneficial effects among repeat breeding and endometritic buffaloes and cows by earlier workers, viz. Dhabale et al. (1997) as 50.0 vs 16.7% conception with gentamicin and Singh et al. (2001) as 69.23% with cephalaxin and 55.56 to 64.28% with other antibiotics. Present results and many of the above researchers, thus, suggest that mild genital infection prevails in repeat breeders and it can be cleared with use of effective antibiotics, either locally in uterus or parenterally thereby improving conception rate, at par with the normal fertile animals, and reducing the calving interval to a desired goal.

**Comparison of treatment response in repeat breeders Vs. Normal cyclic group**

Among 112 repeat breeding buffaloes treated with hormonal and antibacterial drugs, 40.18% buffaloes conceived in treatment cycle, and another 18.75% and 6.25% conceived in post-treatment I and II cycles, respectively, with an overall conception rate of 65.18% after a mean interval from treatment to fertile oestrus as 11.60±2.00 days. Among 22 normal cyclic and 13 repeat breeding control buffaloes that were inseminated without any treatment on spontaneous oestrus, 54.55% and 15.38% buffaloes, respectively, conceived in I cycle. The overall conception rates for the two groups were 81.82% and 38.46%, with mean fertile oestrus intervals of 7.00±2.40 and 13.40±5.47 days, respectively, from the day of first AI. The differences between groups were significant for all the traits (Table 2).

As regards relative efficacy of different treatment protocols of repeat breeding problem, the conception rate in the first (treatment) cycle itself was the highest for GnRH treated group (60%), at par with normal cyclic group (54.55%), followed by progesterone treated (43.75%) and antibiotics/antiseptic treated groups (23.08 to 34.78%). The

![Figure 1](image_url)
overall conception rates for the respective three protocols were 76.00, 62.85 and 53.85 to 83.33% (normal cyclic group 81.82%). The results with hormone therapy, particularly GnRH, were better and comparable with the normal cyclic group, followed by antibiotics treated group, and all these results were significantly better or superior than those of untreated control group. Moreover, cost-wise and looking to the period of response, use of GnRH was the most economic in repeat breeding buffaloes as compared to progesterone or antibiotics in the present study.

Studies on the comparative efficacies of different treatment protocols of repeat breeding cows or buffaloes on farm (Patel et al., 2005) or even field conditions (Sharma and Dhami, 2008; Dhami et al., 2009; Patel et al., 2014) are meagre. Patel et al. (2005) recorded conception rates of 66.66, 83.33 and 50.00% within two cycles following GnRH (0.02 mg), hCG (1500 IU) and Progesterone (500 mg) i/m treatments post-AI in repeat breeding HF cows as against 33.33% in untreated control. Sharma and Dhami (2008) also obtained significantly higher overall conception rates with i/m use of GnRH (90%), at par with normal cyclic group (88.2%) and hydroxy-progesterone caproate (80%), and i/ut cephalexin (80%) and ceftriaxone (70%) in repeat breeding buffaloes than in untreated control group (40.00%). The present relatively better findings with GnRH and progesterone suggested that ovulatory problem, endocrine imbalance and luteal insufficiency leading to fertilization failure and/or embryonic mortality may be major causes of repeat breeding in buffaloes under the study area. Antimicrobials were also to some extent beneficial, but the relatively low results could be due to resistance developed by the genital microflora against them, since Ranvidone—an antiseptic gave the best results though in a limited number of animals.

CONCLUSIONS

Our findings clearly support that the use of GnRH at the time of insemination and of progesterone therapy 4th or 5th day post-insemination definitely improves conception rate by 10-20% depending upon the cause in repeat breeding buffaloes, and hence can be advocated to deal with this problem under field conditions. Present results with antimicrobials suggest that mild genital infection prevails in repeat breeders and it can be cleared with use of effective antibiotics, either infused locally in uterus or parenterally, thereby improving conception rates in repeat breeders and achieving the optimum calving interval.

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REFERENCES


The study was carried out to test the efficacy of Gonadotrophin Releasing Hormone (GnRH) protocols for induction of estrus and fertility in buffaloes. Impact of the GnRH preparations used by the field veterinarians to treat a total of 499 buffaloes with history of anestrus and infertility belonging to different agro-climatic zones of Haryana was analyzed during the period of study. The data of treated buffaloes so obtained was divided into two major groups depending upon the treatment protocol used, viz., GnRH group (n=300) and Controlled Internal Drug Release (CIDR) + GnRH group (n=399). GnRH group animals were subdivided into GnRH alone, GnRH-PG (Prostaglandin F2α) and GnRH-PG-GnRH (Ovsynch) protocols. CIDR + GnRH treated animals were categorized into CIDR + GnRH, CIDR + GnRH-PG and CIDR + GnRH-PG-GnRH (Ovsynch) protocols. The overall estrus induction rate was recorded to be 100% in animals treated with Ovsynch, followed by CIDR + GnRH (98.20±1.80%), then CIDR + GnRH-PG (96.87±3.12%) and CIDR + GnRH-PG-GnRH protocols. However, the overall conception rate was observed to be significantly higher in animals of CIDR + GnRH group. It can be concluded that GnRH in combination with Progesterone based CIDR protocol subsequently improves the estrus induction and pregnancy rates in buffaloes under field conditions.

**Keywords:** anestrus, buffalo, conception rate, estrus, gonadotrophin releasing hormone, GnRH

**INTRODUCTION**

Buffalo is very sensitive to environmental temperature and radiation due to black thick skin and very few sweat glands. Although buffalo is polyestrous animal, however there is distinct seasonal variation in display of estrus, conception rate and calving interval (Singh et al., 2000). During summer, there is reduction in feed intake along with alteration of the profile of reproductive hormones. Lower circulating concentrations of FSH (Razdan et al., 1982), LH (Rao and Pandey, 1982) and progesterone have been detected during summer along with higher prolactin levels (Kaker et al., 1982). This results in weak estrus symptoms during summer months. Also longer inter-calving interval in buffalo due to prolonged postpartum anestrus (Barile, 2005), is mainly attributed to lower circulating concentration of hypophyseal and gonadal hormones (Madan et al., 1983) and suboptimal functioning of hypothalamo-hypophyseal and gonadal axis (Rao and Shreemannarayan, 1982).
Early re-establishment of cyclic ovarian activity after calving is essential because more the estrus cycles a female has before 30 days postpartum, the fewer services per conception are required (Metwelly, 2001). The productive life of a buffalo can be maximized if it is bred within 100-150 days after parturition to produce a calf and start a new lactation every year (Abdalla, 2003). The treatments given in the first month postpartum in order to initiate normal estrus cycles, also improve reproductive performance (Zain et al., 2001). Thus many managemental strategies and hormonal regimens have been administered to stimulate ovulation and resumption of normal cyclicity of anestrus in buffalo during peak breeding and low breeding periods (Singh and Singh, 1986; Aminudeen, 1991; Malik, 2005). These hormones act directly on the reproductive organs or indirectly on the pituitary gland to stimulate the release of naturally occurring hormones, which in turn act on the reproductive organs.

In view of above, the present study was planned in order to test the efficacy of gonadotropin hormone (GnRH) in combination with other hormones for induction of estrus and fertility in anestrus buffaloes.

**MATERIALS AND METHODS**

The basic purpose of the study was to evaluate the efficacy of the hormonal preparations being used for treatment of anestrus, induction of estrus and fertility in buffaloes under field conditions. The study constituted the survey of different villages of the selected ten districts of Haryana state (namely Hisar, Sirsa, Fatehabad, Kaithal, Karnal, Panipat, Sonipat, Jind, Rohtak and Bhiwani) known for higher buffalo population (Livestock Census, 2007) to obtain the information related to different hormonal protocols used for induction of estrus and fertility in buffaloes. At least a ten per cent of total hormonal preparations used by field veterinarians were selected randomly and impact analysis of different hormonal preparations was done.

The hormones were supplied in the field by the Department of Animal Husbandry and Dairying, Haryana. In order to quantify the effect of different hormonal protocols used, data collected was broadly classified into Group I (n=300) and Group II (n=199) with three subgroups in each, based on combination of hormones used.

**Group I: GnRH Group**

1. **GnRH Alone** (n=100)

   Injection Receptal® 2.5 ml was administered intramuscularly (I/m) and animals were then observed for heat. This was used in animals with follicular cyst on the ovaries.

2. **GnRH- PG Protocol** (n=153)

   The first injection of Receptal® 2.5ml was administered I/m on day 1 of treatment. Injection Clostenol® 2 ml was administered I/m 7 days after the Receptal® injection. Animals were observed for estrus and inseminated 12 h after onset of estrus.


   The first injection of Receptal® 2.5 ml I/m was administered on the first day of treatment followed by injection Clostenol® 2 ml I/m after 7 days. A second injection of Receptal® 2.5 ml was administered I/m 48 h after Clostenol® injection. All the animals were inseminated either at the time of second injection of Receptal® (0 h) or 12 h later.
**Group II: CIDR + GnRH Group**

1. **CIDR- GnRH protocol (n=58)**
   CIDR device was inserted on the first day of treatment and was removed on day 7. Heat was observed for 2-6 days and insemination was done 12 hours after observing heat. Injection Receptal® 2.5 ml was injected I/m at the time of AI.

2. **CIDR- GnRH – PG protocol (n=111)**
   On the first day of treatment, CIDR was inserted and injection Receptal® 2.5 ml was injected I/m. On day 7, the CIDR was removed and injection Clostenol® 2 ml was administered I/m. The animals were inseminated 12 h after heat observation.

3. **CIDR- GnRH - PG- GnRH / CIDR Ovsynch FT AI protocol (n=130)**
   On the first day of treatment, the CIDR was inserted intravaginally and injection Receptal® 2.5 ml was administered I/m. On day 7, CIDR was removed and injection Clostenol® 2 ml was given I/m. Injection Receptal® 2.5 ml was administered I/m 48 h after Clostenol injection and insemination by clock was followed between 0 and 12 h after GnRH injection.

Estrus induction rate, conception rate/ pregnancy rate at 1st service (induced heat), conception rate/ pregnancy rate at 2nd service (spontaneous heat) and overall pregnancy rate were recorded in order to evaluate the response of different hormonal treatment protocols used. Comparative study of the treatment regimes was carried out for comparing their effectiveness and efficacy for estrus induction and successful conception. The data obtained in the study was statistically analyzed using Duncan’s multiple range test for comparing means in an analysis of variance (p=0.05) to draw the scientific inferences.

**RESULTS AND DISCUSSION**

Postpartum anestrus in buffaloes is responsible for long calving intervals (Borghese et al., 1994). A variety of hormones are being used to treat reproductive disorders and to regulate the estrus cycle for timed breeding in buffaloes. These hormones act directly on the reproductive organs or indirectly on the pituitary gland to stimulate the release of naturally occurring hormones, which in turn act on the reproductive organs. Different hormonal protocols give satisfactory pregnancy rates, which are comparable to those achieved in animals inseminated at natural estrus (De-Rensis and Lopez, 2007).

A total of 499 buffalo with history of anestrus and infertility belonging to different agro-climatic zones of Haryana were subjected to different hormonal treatment protocols. The treated buffaloes were divided into two groups: GnRH group and CIDR + GnRH group with three sub groups in each. In the present investigation with the use of GnRH alone protocol, estrus induction and conception rate were observed to be 90.53±3.61% and 61.06±7.12% respectively (Table 1). Previously, Ramoun et al. (2012) observed 60% and 30%, estrus induction and conception rate, respectively with the administration of this protocol. They also suggested that in order to get better results with GnRH, the nutritional requirement of animal should be fulfilled for proper development of follicles for action of these hormones.

With the use of GnRH-PG protocol, estrus induction and overall pregnancy rates were observed 81.03±7.92% and 62.68±7.78%, respectively (Table 1). GnRH treatment would enhance ovulation and subsequent PGF sub treatment would induce luteolysis of CL and later on, ovulation. Hafez and Hafez (2000) suggested that
Table 1. Comparison of different hormonal treatment protocols for induction of estrus and fertility in buffaloes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Name of Hormonal Protocol Used</th>
<th>No. of animals treated</th>
<th>Animals Induced to Estrus</th>
<th>Conception at Induced Estrus</th>
<th>Conception at 2nd Estrus</th>
<th>Overall Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>n (%Mean± SE)</td>
<td>n (%Mean± SE)</td>
<td>n (%Mean± SE)</td>
<td>n (%Mean± SE)</td>
</tr>
<tr>
<td>GnRH Group</td>
<td>GnRH Alone</td>
<td>100</td>
<td>91</td>
<td>90.53±3.61 ( ^a )</td>
<td>58 61.06±7.12</td>
<td>0 0.00±0.00</td>
</tr>
<tr>
<td></td>
<td>GnRH-PG</td>
<td>153</td>
<td>120</td>
<td>81.03±7.92 ( ^{ab} )</td>
<td>86 58.95±7.27</td>
<td>5 3.73±2.31</td>
</tr>
<tr>
<td></td>
<td>Ovsynch FT AI</td>
<td>47</td>
<td>47</td>
<td>100.00±0.00 ( ^{a} )</td>
<td>29 66.23±8.68</td>
<td>2 1.79±1.79</td>
</tr>
<tr>
<td>CIDR+GnRH Group</td>
<td>CIDR-GnRH</td>
<td>58</td>
<td>54</td>
<td>98.20±1.80 ( ^{a} )</td>
<td>42 81.26±8.46</td>
<td>0 0.00±0.00</td>
</tr>
<tr>
<td></td>
<td>CIDR-GnRH-PG</td>
<td>111</td>
<td>100</td>
<td>96.87±3.12 ( ^{a} )</td>
<td>78 74.19±9.13</td>
<td>2 7.14±7.14</td>
</tr>
<tr>
<td></td>
<td>CIDR-Ovsynch</td>
<td>130</td>
<td>124</td>
<td>95.74±2.87 ( ^{a} )</td>
<td>87 71.65±7.95</td>
<td>1 2.78±2.78</td>
</tr>
</tbody>
</table>

Means with different superscripts are significantly different (P<0.05).
the administration of PGF$_{2\alpha}$ in early postpartum period would reduce the incidence of subclinical uterine infection and hasten the return to a suitable uterine environment for fertilization and pregnancy. Administration of PGF$_{2\alpha}$ at 7-10 days postpartum was effective in facilitating the uterine involution and resumption of ovarian cyclicity and improving reproductive performance (Noakes et al., 2001). The results of present study are in harmony with Hammam et al. (2009) and Yendraliza et al. (2011) who observed 60% and 100% estrus induction rates and 67% and 100% pregnancy rates respectively.

A 100% induction of estrus in treated animal was observed by the use of Ovsynch FT AI protocol (Table 1). Such a higher percentage of estrus induction might be due to the reason that GnRH – PGF$_{2\alpha}$-GnRH (Ovsynch) protocol synchronizes follicular development, luteal regression and time of ovulation, thus permitting fixed time AI after the second GnRH administration (Azawi et al., 2012). Conception rate obtained in our study (68.02±8.59 %) with Ovsynch protocol treatment in buffalo is considered high when compared to other similar studies conducted by several workers such as Irikura et al. (2003); Mialot et al. (1999) and Baruselli et al. (1999) who found lower conception rates in buffalo were 27.2, 36.1 and 42.4%, respectively. The differences in conception rates in the present study as compared to the others could be because buffaloes in our study were not specifically selected as true anestrus and also the breeding season favors the higher estrus induction and conception rates in buffaloes.

Using CIDR-GnRH protocol for treatment, estrus induction rate was observed to be 98.20±1.80% with a conception rate of 81.26±8.46% (Table 1). CIDR causes an increased circulatory concentration of progesterone resulting in increased sensitivity of hypothalamus to estrogen with subsequent increase in the intensity of estrus (Azawi et al., 2012). The conception rate in present study is higher than 20% and 66.5% reported by Azawi et al. (2012) and Hammam et al. (2009) in summer season respectively. The differences in conception rate between the present study as compared to others, could be due to the reason that the animals were treated during their normal breeding season (autumn-winter).

In the animals treated with CIDR-GnRH-PG combination, estrus induction of 96.87±3.12% and pregnancy rate of 81.33±11.01% was observed (Table 1). The results indicated that administration of GnRH after removal of CIDR showed tighter synchrony in estrus response and tended to increase the pregnancy rate in anestrus buffaloes (Naseer et al., 2011). The results of this study are in agreement with the results obtained in beef cattle (Martinez et al., 2011), dairy cows (Thatcher et al., 2006) and buffaloes (Azawi et al., 2012).

The estrus induction rates using CIDR-Ovsynch FT AI protocol were observed to be 95.74±2.87% in the present study (Table 1). These results are in agreement with Baruselli et al. (2007) who also observed that 100% estrus induction rates could be achieved in breeding season by combining CIDR with Ovsynch protocol in anestrus buffaloes. The main action of GnRH used at the start of progesterone treatment in order to synchronize emergence of a new cohort of follicles (Rhodes et al., 2003). Additionally, second injection of GnRH has the additional effect of inducing ovulation and the formation of a corpus luteum in a majority of animals, resulting in elevated concentrations of progesterone in anestrus buffaloes. GnRH synchronizes the development and occurrence of follicles and resulted in more homogenous follicular development. It also induces ovulation or luteinization of dominant follicle in non cyclic
animals. However, the induced ovulation in non-cyclic animals stimulated luteal tissue development and function resulting in the occurrence of cyclic activity (Bao et al., 2003). Inserting CIDR at the initial GnRH injection of the Ovsynch program (Ovsynch plus CIDR) and then removing the CIDR and injecting PGF$_{2\alpha}$ has been demonstrated to improve pregnancy rates in lactating buffaloes (Ravikumar et al., 2011). The conception rate in our study was observed to be 74.43±9.16% when Ovsynch protocol was supplemented with progesterone. Baruselli et al. (2007) and Azawi et al. (2012) observed 57.5 and 32% overall pregnancy rates, respectively.

Thus in the present study, it was observed that Ovsynch FT AI protocol produced maximum estrus induction rate in anestrus buffaloes under field conditions. However, inclusion of a CIDR device in combination with GnRH resulted in comparable estrus induction rate along with higher conception rate in postpartum anestrus buffaloes.

**REFERENCES**


Hafez, E.S.E. and B. Hafez. 2000. Physiology of reproduction in farm animals. *Lea &
Febiger, Philadelphia., **8**: 59-93.


Ravikumar, K., S.A. Asokan, C. Veerapandian and A. Palanisamy. 2011. Ovarian status serum progesterone (P₄) level and conception rate


FARMERS’ KNOWLEDGE ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCES OF BUFFALO UNDER SMALLHOLDER FARMING SYSTEM

B.S. Meena*, H.C. Verma and Amit Singh

ABSTRACT

Dairying farming can be cost effective when the animals rearing were directly influenced by the productive and reproductive parameters of buffalo. Productive and reproductive performances of dairy animals should be determined on the basis of average daily milk yield, lactation length, lactation milk yield, peak yield, dry period, service period, conception rate, pregnancy rate, calving interval etc. These parameters should be maintained by the farmers so that the productivity could be increased of their herd. Hence, the study was conducted to measure the productive and reproductive performances of buffalo and dairy animal and Farmers’ knowledge on these aspect in Faizabad district of Uttar Pradesh. For this purpose 150 farmers engaged in dairying were interviewed. The farmer was selected on the basis of at least completion of one lactation length of buffalo at the time of investigation and each farmer must be rearing a buffalo in combination with crossbred cow and indigenous cow at the time of investigation.

The overall Average daily milk yield (ADMY) and Lactation length (LL) was estimated to be 5.75±0.65 litre/day/animal and 276±14 days/animal. The average peak yield of buffalo in the field condition was estimated to be 8.56±0.85 litre/animals while Age at first calving (AFC) was 1288±122 days/animals respectively. The study reveals that the 41.00 percent of respondent were having high level of knowledge about productive and reproductive practices. Whilst about 30.00 percent of respondent were having medium level of knowledge. Knowledge index on productive and reproductive parameters of dairy animal was calculated and observed that respondent were possessing 73.47 and 70.21 percent knowledge in study area. Though the respondent are facing serious constraint in rearing, they were frequently updating their knowledge on productive and reproductive practices as compared to Buffalo and indigenous cow based dairy farmers.

Keywords: buffalo, dairying, farmers’ knowledge, india, productive and reproductive performance

INTRODUCTION

Dairying sector is focussing to plays a unique multi-faceted role in socio-economic development of rural households and contributes about 3.88 percent to the Gross Domestic Product and 21.58 percent to the Agricultural Gross Domestic Product in India (Annual Report, 2014-15). The 51% share of total milk production of 127.8 million tonnes (2011-12) was dominated by 105.3 million buffaloes accounting for about 51 percent of Asia’s and about 19 percent of world’s bovine population. The current market trend in livestock sector growth suggests that in
order to meet the emerging demand for livestock based products basically milk, both in domestic and global markets, there is a need to reorient the production system by enhancing the efficiency and creating quality consciousness. India ranks first in milk production which is produced by its huge bovine population (304.5 million) and little contribution from other species (Annual Report, 2014-15). But the major concerns that is troubling farmers is the low productivity per animal. Basically milk production (productivity) depends on four dimensions of animal husbandry practices i.e. breeding, feeding, health-care and management practices.

Productive and Reproductive efficiency are important parameters, which influences the economics of milk production considerably. There are a large number of productive and reproductive problem in the field condition which render the animal with losses of reproductive function. Any impairment in normal reproductive function results into infertility or sterility of animal, leading to economic losses due to widening of dry period and inter calving interval, reducing calving and lactation during lifetime of animal (Agarwal et al., 2005). About 18-40 percent of cattle and buffalo are culled and reach to abattoir mostly due to infertility (Sharma et al., 1993). Total losses due to reproductive problems in buffaloes were 39 percent of all the losses incurred by various disease conditions (Khan et al., 1995). Production and Reproduction are one of the most important considerations to determine the profitability of cattle and buffalo. Productive and reproductive performances of dairy animals should be determined on the basis of these parameters. Keeping in view these problem the following study was undertaken to find out the Productive and reproductive performance of buffaloes animals managed by farmers and to assess the knowledge of farmers on these parameters.

MATERIAL AND METHOD

The present study was purposively undertaken in the state of Uttar Pradesh, which is one of the largest state occupying first position in milk production with 23.00 percent of total buffaloes (26.44 million), producing 20.10 million tonnes of milk. Faizabad district from Uttar Pradesh was selected purposively as the buffalo population was fairly distributed in the region.

The region is not highly productive, small farmers are heavily dependent on different species of livestock for their livelihood. From the present study area six villages were selected randomly from three randomly selected blocks. The information for this study was collected from 150 farmers, 25 from each selected village. The farmer was selected on the basis of at least completion of one lactation length of buffalo at the time of investigation. The criteria for the selection of respondents was that each farmer must be rearing a buffalo in combination with crossbred cow and indigenous cow at the time of investigation. Finally, the respondents were grouped in three different dairy farming system i.e. Buffalo based farmers, Crossbreed farmers and Indigenous based farmer based on the criteria that maximum number of farmers are rearing the particular livestock. The primary data was collected by personal interview method using a structured interview schedule. The respondents were interviewed, individually and the data about animal performance and knowledge of the respondents was collected. The collected data were tabulated, scored and analyzed in the light of the set objectives.
Knowledge of farmers on productive and reproductive parameters of buffalo

English and English (1961) defined knowledge as a body of understood information possessed by an individual. Knowledge in the present study refers to which information and understanding, the respondent has about the improved dairy breeding and management practices (Productive and reproductive traits of dairy animals). A knowledge a test was developed and standardized by following the procedure described by Linquist (1951). The farmers were exposed to the test and knowledge score was obtained depending on Farmers’ recall memory. All possible care was taken to cover maximum aspect pertaining Productive and reproductive performances of dairy animal. The respondents were classified in terms of having low, medium and high knowledge level on the basis of cumulative square root frequency method.

Knowledge index

The knowledge index was measured by using following formula

\[ \text{Knowledge Index} = \sum \frac{\text{Knowledge Score}}{\text{Total Knowledge Score}} \]

RESULTS AND DISCUSSION

Productive and reproductive performances of buffaloes in dairying farming system

The current status of the production of buffalo was ascertained with respect to various production and reproduction traits considered under study were analysed and presented in the following sub-heads:

Average Daily Milk Yield (ADMY)

Average milk yield of buffalo is an important productive indicator showing average daily milk yield of upgraded buffalo in field which were reared in combination with other animals. It refers to average milk yield of buffalo during the lactation period of 305 days measured in liters/day. The result in table-1 shows that the ADMY of buffalo based farmers, cross breed based and indigenous based farmers was 6.01±0.5, 5.79±0.56, and 5.45±0.61 litre/day/animal respectively. The overall ADMY was estimated to be 5.75±0.65 litre/day/animal. The high ADMY of buffalo was due to the fact that buffalo are actually treated as milk animal and due care was taken in feeding and management of these animals.

Lactation Length (LL)

The optimum lactation length of dairy animals is one of the best productive indicator of dairy animals. LL is number of days a buffalo remain in milk from the date of calving to the date of dry. The data pertaining to present investigation the lactation length of Buffalo in different system was 281±19, 277±16, and 276±14 days/animal respectively. The overall LL of the buffalo in the area was 276±14 days/ animal. Murrah buffalo lactation length in the west Godavari was reported to be 299.91±5.01 which is quite similar to the above data (Suresh, 2013). LL was mainly influenced by the parity of lactation. Singh et al. (2011) has shown the positively correlation between the parity of lactation and lactation length in Nilli-Ravi buffaloes.

Lactation Milk Yield (LMY)

The lactation milk yield of the dairy animals has positive relation with the overall performances of an animal. It is conceptualized as the average total quantity of milk produced by an animal in its lactation period of 200 days. A cursory look on Table 1 reveal that the LMY of Buffalo reared in
the buffalo based farming was highest. It shows that the LMY was 1733.03±189, 1694.44±124, and 1487.33±117 litre/animal respectively. Hitesh et al., (2012) reported that 305 day milk yield in Murrah Buffalo was 2147.6 ± 87.06 kg The author observed these results because bufferoes been the major milk producer and due care was taken in feeding and management of the animals.

**Peak Yield (PY)**

Peak yield is measured as the highest milk produced by the milch animal in its lactation length. The data present in Table 1 reveals that the average peak milk yield of Buffalo was highest in buffalo based farming system (8.87±0.98) followed by indigenous based (8.12±0.79) and cross breed (8.64±0.85) based farming. The average peak yield of buffalo in the field condition was estimated to be 8.56±0.85 litre/animals respectively. Suresh (2013) stated that the overall peak milk yield of Buffalo was recorded as 13.97±1.13.

**Dry Period (DP)**

It refers to the number of days a cow remained dry i.e. the interval between the dates of dry to the date of next calving. It was observed from the table-1 that the dry period of Buffalo was 211±12, 230±14 and 237±16 days/animal. The average DP calculated was observed to be 226±13 days/animal respectively. Similar finding were also observed by Thiruvenkadan et al. (2010) and stated that the dry period of 250.5 ± 15.9 days in bufferoes. However Yadav et al. (2007) stated the lower dry period in the Murrah buffaloes maintained in its home tract.

**Age At First Calving (AFC)**

Late maturity and age at first calving are one of the most important reasons to losses the performances of dairy animals lower the age of first calving better the performances of dairy animals. AFC is the actual age of animals in days at the time of its first calving for bufferoes. Buffalo reared under different system the age at first calving was observed to be highest in the indigenous pattern (1308±108) followed by cross breed animals (1294±123) and least was observed in buffalo rearing system (1260±112) days/ animal respectively.

The overall AFC of buffalo in the area was stated as 1288±122 days/ animals respectively. The study conducted by Bohra et al. (2007) in Uttarakhand state found that the AFC in buffalo was about 4.6 years. In general, AFC in Indian dairy animal is much higher compared to their exotic or crossbred counterparts which is largely attributed to lack of selection for their traits from generation to generation. The Reduction in the age at first calving leads to an increase in lactation yield and helps in improving the economy of dairy farmers. Shashidhara et al (1998) stated that age at first calving of 1301-1390 days was optimum for getting maximum lactation milk yield and lifetime milk yield in buffaloes which shows that the farmers were having ideal management of bufferoes.

**Service Period**

The data pertaining to service period was presented in the Table 1 and found that the service period of Buffalo was 189±15, 198±17 and 199±18 days/animal respectively. The average service period of 139.91±2.96 days was reported in Murrah buffalo at NDRI farm (Jamuna et al., 2013). The important cause of long service period as many heat period are lost due to unavailability of breeding bulls or artificial insemination in the area. Abayawansa et al. (2011) also stated that poor detection of oestrus caused by low attention
on buffalo with first parity as they produce low milk could be one of reasons to have comparatively longer intervals.

**Service Per Conception**

It is defined as an average number of insemination or natural service required by buffalo to become pregnant. It was observed that the service per conception of buffalo was highest in buffalo based farming (1.84±0.52). Service per conception of buffalo in other system was 1.78±0.32 and 1.76±0.42 times/animal respectively. It was observed the farmers were attaining appropriate conception rate in field condition which might reflect their sound knowledge in reproductive aspect of buffalo or due to good management provided by them. Khan *et al.* (2009) observed service per conception of about 2 in Murrah buffalo at organized farm. Higher rate of service per conception may be due to un-identification of heat, post partum complication in the buffalo and may also indicative of poor postpartum management.

**Calving Interval**

Calving interval (CI) is another important parameter for performances of the dairy animals.

Table 1. Productive and reproductive performances of the Buffalo.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Buffalo Based farmers (Buffalo = 109)</th>
<th>Cross breed based farmers (Buffalo = 45)</th>
<th>Indigenous cow based farmers (Buffalo = 33)</th>
<th>Overall performance (Buffalo = 187)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Productive parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Average daily milk yield (l)</td>
<td>6.01±0.5</td>
<td>5.79±0.56</td>
<td>5.45±0.61</td>
<td>5.75±0.65</td>
</tr>
<tr>
<td>2</td>
<td>Lactation Length (Days)</td>
<td>281±19</td>
<td>277±16</td>
<td>276±14</td>
<td>276±14</td>
</tr>
<tr>
<td>3</td>
<td>Lactation milk yield (l)</td>
<td>1733.03±189</td>
<td>1694.44±124</td>
<td>1487.33±117</td>
<td>1587.60±113</td>
</tr>
<tr>
<td>4</td>
<td>Peak yield (l)</td>
<td>8.87±0.98</td>
<td>8.12±0.79</td>
<td>8.64±0.85</td>
<td>8.56±0.85</td>
</tr>
<tr>
<td>5</td>
<td>Dry period (Days)</td>
<td>211±12</td>
<td>230±14</td>
<td>237±16</td>
<td>226±13</td>
</tr>
<tr>
<td></td>
<td><strong>Reproductive parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Age at first calving (Days)</td>
<td>1260±112</td>
<td>1294±123</td>
<td>1308±108</td>
<td>1288±122</td>
</tr>
<tr>
<td>7</td>
<td>Service period (Days)</td>
<td>189±15</td>
<td>198±17</td>
<td>199±18</td>
<td>189±16</td>
</tr>
<tr>
<td>8</td>
<td>Service per Conception (no.)</td>
<td>1.84±0.52</td>
<td>1.78±0.32</td>
<td>1.76±0.42</td>
<td>1.76±0.72</td>
</tr>
<tr>
<td>9</td>
<td>Calving interval (Days)</td>
<td>495±45</td>
<td>508±47</td>
<td>513±38</td>
<td>505±39</td>
</tr>
</tbody>
</table>
Table 2. Distribution of farmers based on knowledge on Productive and reproductive practice. 

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Respondents</th>
<th>Categories</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Knowledge on productive parameters</td>
<td>Low ( &lt;17.8 )</td>
<td>43</td>
<td>28.67</td>
</tr>
<tr>
<td></td>
<td>Range (13-25),</td>
<td>Medium (17.8-21.78)</td>
<td>45</td>
<td>30.00</td>
</tr>
<tr>
<td></td>
<td>Mean (20.35)</td>
<td>High ( &gt;21.78)</td>
<td>62</td>
<td>41.33</td>
</tr>
<tr>
<td>2</td>
<td>Knowledge on Reproductive parameters</td>
<td>Low ( &lt;27.96)</td>
<td>41</td>
<td>27.33</td>
</tr>
<tr>
<td></td>
<td>Range (22-39),</td>
<td>Medium (27.96-33.60)</td>
<td>60</td>
<td>40.00</td>
</tr>
<tr>
<td></td>
<td>Mean (31.66)</td>
<td>High ( &gt;33.45 )</td>
<td>49</td>
<td>32.67</td>
</tr>
<tr>
<td>3</td>
<td>Knowledge on productive and Reproductive parameters</td>
<td>Low ( &lt;45.4 )</td>
<td>37</td>
<td>24.67</td>
</tr>
<tr>
<td></td>
<td>Range (37-62),</td>
<td>Medium (45.4-55.4)</td>
<td>58</td>
<td>38.62</td>
</tr>
<tr>
<td></td>
<td>Mean (52.07)</td>
<td>High ( &gt;55.4)</td>
<td>55</td>
<td>36.55</td>
</tr>
</tbody>
</table>

Figure 1. Knowledge index of farmers on productive and reproductive parameters.
keeping in the mind the data was properly analyzed and state that 495±45, 508±47 and 513±38 days/animals respectively. It was concluded that calving interval of buffalo was 505±39 days/animal respectively. The lower calving interval of the buffalo was due to the care taken by the farmers in their feeding and maintaining the dry period. While the study of Thiruvenkadan et al. (2010) reports the higher calving interval of 559.6±17.3 days in buffaloes.

Knowledge of Dairy Farmers on Productive and Reproductive parameters of Buffaloes

Knowledge is a body of understood information possessed by an individuals. Therefore the knowledge possessed by individual regarding the productive and reproductive practices of buffalo had impact on the profitability of farmers. Farmer’s rate of adoption was greatly influenced by the gain in knowledge. Hence an attempt has been made to study the knowledge of farmers in relation to Productive and reproductive practices of buffalo.

The finding presented in Table 2 reveals that about 41.00 percent of respondent were having high level of knowledge on productive practices of dairy animals. Whilst about 30.00 percent of respondent were having medium level of knowledge and almost 28.67 percent respondent were possessed low level of knowledge. Thus it could be concluded that respondent in the study area were having high to medium level of knowledge in productive practices of dairy animals. It is also reveals that the 40.00 percent of respondent were having medium level of knowledge on reproductive parameters of dairy animals. Whilst about 32.67 and 27.00 percent of respondent were having high and low level of knowledge on the reproductive practices of dairy animals. The study of Subhash et al. (2013) also reveals similar finding that the farmers were having highest knowledge about reproductive disorders like abortion followed by Repeat breeding, anoestrus, late maturity and retention of placenta. He also concluded that nearly 65.41 percent of respondent was considered as good knowledge on reproductive disorders of dairy animals.

Knowledge Index of Farmers on Productive and Reproductive Practices of Buffalo was calculated. The collected data was further analysed and the resulted presented in Figure 1 reveals that the overall knowledge possessed by farmers was 66.79 percent in the study area. It was further observed that those respondents were having good knowledge (68.91%) on productive practices as compared to reproductive practices (66.10%). It was also noticed that buffalo based farmers were having knowledge up to the extent of 73.47 and 70.21 percent on productive and reproductive practices of dairy animals.

The study finely concluded that majority of the farmers were having healthy knowledge about performance on productive and reproductive practices of buffalo. The buffalo based farmers were having more knowledge on productive (73.47%) and reproductive (70.21%) parameters than their counterparts. So it can be concluded from the above discussion that the performance of productive practices is comparatively better than the reproductive practices due to the more knowledge on productive practice and low knowledge on reproductive practice of farmer.

REFERENCES

Linquist, E.F. 1951. Educational Measurements Part II. American Council of Educational, Washington, USA.
STANDARDIZING PREGNANCY RATE OF INDIAN MURRAH BUFFALOES FOR HIGHER MILK YIELD

V. Jamuna*, A.K. Chakravarty, Vijay Kumar, M.A. Mir and Vikas Vohra

ABSTRACT

Study revealed that increase in milk production has lead to decline in fertility performances of Murrah buffaloes. Data pertaining to 1224 lactation records of Murrah buffaloes spread over a period 19 years were analyzed in the study. It was observed that pregnancy rate (fertility) depicted negative phenotypic association with 305 days or less milk yield (-0.08±0.04), wet average (-0.12±0.02) and test day five milk yield (-0.09±0.03). It was observed that to achieve around 2000 kg MY, 7.5 kg WA and 7.7 kg in TD 5 MY, the level of pregnancy rate varied between 30-50%. Under the present study an attempt was made to quantify the decline of fertility with the increase of milk production in Murrah buffaloes. The per unit change in fertility with respect to milk yield in Murrah buffaloes, were studied using multiple regression analysis. Increasing one kilogram milk yield in test day five (125th day) pregnancy rate reduced by about 1.3%, and increase in one kilogram milk yield per day in 305 days or hundred kilogram in 305 days of Murrah buffaloes pregnancy rate reduced by 0.9%.

Keywords: Life time, pregnancy rate, production, productivity, standardization

INTRODUCTION

India is among few countries in the world, which has contributed richly to the international livestock biodiversity and improvement of livestock genepool. India contributes about 63 percent of total world buffalo milk and 95 percent of buffalo milk in Asia comes from India. Among the various buffalo breeds in India, Murrah is the important milch breed with superior genetic potential for milk production and constitutes around 19.5% of total buffalo population in the country (FAO, 2012). The breed is versatile and has shown wide adoption for milk production across the length and breadth of the country.

A multitude of studies in dairy cattle have shown that selection for higher milk yield alone is associated with reduced health and fertility (Pryce et al., 1999). Fertility is economically important as it brings buffalo into lactation, reduces reproductive disorders and maximizes the profitability by in time calf crop. Most of the developed countries have already developed a national genetic evaluation for female fertility along with the production traits and they have used fertility traits like Pregnancy Rate (PR) and Service Period (SP) for genetic evaluation of dairy cattle (VanRaden, 2004; De Vries, 2010 and Cabrera, 2011). Pregnancy rate (PR) measures the

Animal Genetics and Breeding Division, ICAR- National Dairy Research Institute, Karnal, Haryana, India, *E-mail: jamunavalsalan@gmail.com
percentage of non-pregnant animals that become pregnant during each estrous cycle, because each estrous cycle represents only one chance for an animal to become pregnant. A comparison of pregnancy rate with other fertility traits from 14 countries has been conducted by Interbull, 2007 and the result indicated that pregnancy rate is highly correlated with other fertility traits. These correlations indicate that pregnancy rate can be expected to improve fertility in animals (Jorjani, 2007).

Conventional selection for milk has made buffaloes more profitable producers in the country and continuous selection for increased milk production has led to long service period in buffalo result in reduced pregnancy rate and decrease in calf crop and milk yield on lifetime basis. Until recently under Buffalo Improvement programme in India, no efforts have been made in using genetic selection of fertility to improve performances of buffaloes. The increase in milk production causes how much apparent decline in fertility in each lactation as well as in life time production has not been explored in Murrah buffaloes. The objective of the study was standardization of level of fertility with respect to milk production and quantifies the decline of fertility with the increase of milk production in Murrah buffaloes.

MATERIALS AND METHODS

Data Size: The breeding information of 522 Murrah buffaloes scattered over a period of 19 years from January 1993 to October 2011, maintained at Dairy Cattle Breeding Division and Livestock Research Station, National Dairy Research Institute, Karnal were collected. The basic information of Murrah buffaloes, reproduction and production traits were recorded. The reproduction traits included pregnancy rate (PR) and life time pregnancy rate (LTPR). The production traits included 305 days or less milk yield (MY), wet average (WA), test day five milk yield (TD 5 MY), life time 305 days or less milk yield (LTMY), life time wet average (LTWA) and life time test day five milk yield (LT TD 5 MY).

In the present study, the normal lactation was considered as the period of milk production by a buffalo for at least 100 days or a minimum of 500 kg milk produced and the animal calved, dried under normal physiological conditions. The information of 1224 lactation records of 522 Murrah buffaloes were subjected to standardization and normalization of traits, and subsequently 853 lactation records were obtained (distributed as 404, 230, 138 and 81 completed first, second, third and fourth lactations) for production traits and 748 records (distributed as 340, 204, 126 and 78 completed first, second, third and fourth parity) for fertility traits.

Statistical analysis

The genetic parameters for fertility and production traits were estimated by REML using Wombat software (Meyer, 2010) applying a repeatability animal model, \( Y_{ijklm} = \mu + A_i + S_j + P_k + Pa_l + AG_m + e_{ijklm} \) where, \( Y_{ijklm} \) ijk\textsuperscript{th} observation of fertility and production traits; \( m \), population mean; \( A_i \), Animal random effect; \( S_j \), Fixed effect of j\textsuperscript{th} season of calving (winter, summer, rainy and autumn); \( P_k \), Fixed effect of k\textsuperscript{th} period of calving (1 to 8); \( Pa_l \), Fixed effect of l\textsuperscript{th} parity (1 to 4); \( AG_m \), Fixed effect of m\textsuperscript{th} age group of calving (<37 months, 37-51 months, >51 months); and \( e_{ijklm} \), Random error is normally and independently distributed with mean zero and variance \( \sigma_e^2 \).
Estimation of Pregnancy Rate of Murrah buffaloes

The Voluntary Waiting Period (VWP) is the period after calving during which no inseminations occur, voluntarily left by the management for better pregnancy rate. VWP in Indian Murrah buffaloes has been standardized as 63 days (Patil et al., 2013). Pregnancy rate in each lactation was estimated as \[ PR = \frac{21}{(Service\ Period - Voluntary\ Waiting\ Period + 11)} \]. The constant factors 11 centralize the measure of possible conception within each 21 days time period.

Estimation of life time traits

An empirical estimation suggests that in buffaloes about 90.27 % was produced up to fourth lactation and thereafter milk production started to decline. Therefore, each life time performance trait was estimated as average of four parity /lactations. Life time pregnancy rate (LTPR) was estimated as average pregnancy rate of buffaloes completed four parity. Life time production or productivity was estimated as average production or productivity of buffaloes completed four lactations.

Standardization of level of fertility for optimum milk production / productivity

Standardization of fertility and production/productivity were done parity wise, overall and life time (Table 1). For standardizing the level of fertility with milk production and productivity, the pregnancy rate was classified into seven classes with the increment of 10% in all parities, overall and life time. The number of buffaloes scattered in different classes of pregnancy rate were identified and their corresponding average pregnancy rate in relation to MY, WA and TD5 MY were estimated.

To explore the per unit change in fertility with respect to milk production/productivity in Murrah buffaloes, regression analysis was done in the study using the General Linear Model (GLM) procedure of SAS (SAS Institute 2009), presented in Table 3. Following model was used: \[ Y_i = a + b_1X_1 + e_i \] where \( Y_i \) is pregnancy rate (PR), \( a \) is intercept, \( b_1 \) is regression coefficient estimated, \( X_i \) is MY/WA/TD 5 MY and \( e_i \) is Random error \( \sim NID (0, \sigma^2_\epsilon) \). The relation of change in life time pregnancy rate with respect to per month milk yield in life time was analyzed using the regression model: \[ Y_i = a + b_1X_1 + e_i \] where \( Y_i \) is life time pregnancy rate (LTPR), \( a \) is intercept, \( b_1 \) is regression coefficient estimated, \( X_i \) is per month milk yield and \( e_{ij} \) is Random error \( \sim NID (0, \sigma^2_\epsilon) \).

RESULTS AND DISCUSSION

Fertility traits of Murrah buffaloes were found influenced by period and season of calving, parity and age group of calving, while production traits were mainly influenced by period of calving and parity. Least-squares means of SP, LL and MY were estimated as 28±5.58 days, 286.06±1.72 days and 2078.20±31.21 kg, respectively. By keeping days to first service or VWP as 63 days, the average pregnancy rate (PR) and life time pregnancy rate (LTPR) of Murrah buffaloes were estimated as 0.36±0.013 and 0.38±0.03, respectively. Wet average (WA) of Murrah buffaloes was estimated as 7.29±0.06 kg. On analysis of test day milk, it was observed that TD 5 (125th day) MY had the highest phenotypic association (0.79±0.31) with MY out of eleven test day milk yields in Murrah buffaloes. The average LTMY, LTWA and LT TD 5 MY were estimated as 2188.00±41.50 kg, 7.54±0.12 kg and 8.52±0.15 kg respectively. The fertility traits were more influenced by environment and management of the herd, as pregnancy rate
encompasses buffalo’s ability to return to normal reproductive status after calving, to display visible signs of estrus, to conceive when inseminated and to maintain the pregnancy.

The heritability of PR, MY, WA and TD 5 MY were estimated as 0.02±0.005, 0.17±0.04, 0.15±0.03 and 0.12±0.04. The pregnancy rate had low but negative phenotypic associations with MY (-0.08±0.04), WA (-0.12±0.02) and TD 5 MY (-0.09±0.03). The association of pregnancy rate with life time was 0.15±0.03. The buffalo fertility had negative correlation with milk yield but is a major component of longevity, as pregnancy rate had positive association with life time.

**Level of fertility (PR) with milk production (MY) and productivity (WA/TD 5 MY) in different parities**

The pregnancy rate of Murrah buffaloes varied from less than 10% to more than 90% in each of the four parity (Table 1). The average pregnancy rate ranged from 30% to 50%. In the first parity, it was observed that with the increase of average pregnancy rate of Murrah buffaloes from 34% to 45%, the level of average production was found decreased from 2040.46 kg to 1993.32 kg, 7.15 kg to 6.58 kg in WA and 8.52 kg to 6.59 kg when TD 5 MY was considered. Almost similar trend were observed in other parities also. In the second parity, it was observed that with the increase of 35% to 46% pregnancy rate, MY reduced from 2113.40 kg to 2034.48 kg, where WA declined from 7.45 kg to 7.32 kg and TD 5 MY declined from 7.95 kg to 7.81 kg. In the third parity, with the increase of 36% to 47% pregnancy rate, MY, WA and TD 5 MY from 2008.02 kg to 1906.31 kg, 7.29 kg to 7.19 kg and 7.85 kg to 7.52 kg, respectively. In the fourth parity, though the increase of pregnancy rate was from 33% to 46%, milk yield reduced from 2118.66 kg to 1994.96 kg, however the decline of productivity was found less i.e. from 7.11 kg to 7.04 kg and 8.52 kg to 8.45 kg in WA and TD 5 MY.

**Level of fertility with milk production and productivity in overall and life time**

In overall lactations, with the increase of pregnancy rate from 34% to 45%, the corresponding production declined from 2053.98 kg to 1946.64 kg, productivity declined from 7.50 kg to 7.33 kg in WA, 7.82 kg to 7.63 kg in TD 5 MY depicted in Figure 1, 2 and 3. However, with the increase of same pregnancy rate in life time, the productivity of life time reduced from 7.12 kg to 7.00 kg and 7.60 kg to 7.33 kg in WA and TD 5 MY, respectively. The life time milk yield reduced from 2249.56 kg to 2089.65 kg, as depicted in Figure 4, 5 and 6.

From the perusal of data from different parities, overall and life time, pregnancy rate of Murrah buffaloes had been classified into three classes, i.e. the buffaloes with less than 30% pregnancy rate, 31-50% pregnancy rate and more than 50% pregnancy rate. Average milk productivity and production corresponding to three ranges of pregnancy rate in different parities as well as in over all parities has been presented in Table 2. Therefore, under Murrah Buffalo Improvement breeding programme in India, once the target is to obtain 2000 kg MY, 7.4 kg WA and 7.7 kg TD 5 MY, the level of pregnancy rate should be in between 30-50%.

**Linear evaluation of fertility and productivity / production in Murrah buffaloes**

The percent change in of fertility with the corresponding unit increase of milk productivity and production was studied in different lactations, overall and life time in Murrah buffaloes (Table 3).
The change in per unit (percent) of fertility with the corresponding increase of per month milk production in life time of Murrah buffaloes was also analyzed using simple regression model. In Murrah buffaloes, average life time was estimated as 4.10 years, average life time milk yield and average per month milk yield in life time were estimated as 2188.00 kg and 151.44 kg, respectively. The results revealed that by increasing hundred kilograms of per month milk yield in life time, the pregnancy rate will reduce by about 13% in life time.

Scanty reports were available about the literature reviewing the standardization of fertility with milk yield. According to De Vries (2010) one month life time is worth 112 lbs (approx. 50.80 kg) milk in a lactation and 1.5% daughter pregnancy rate in Holstein dairy cattle. He also reported that one percent daughter pregnancy rate is worth 73 lbs (approx. 33.11 kg) milk in 305-day lactation. Cabrera (2011) reported that improving pregnancy rate by 5% would increase economic gain of milk production by 21.41 kg per year, while De Vries (2010) reported that improving pregnancy rate by 1% will increase the milk production 7.13 kg per year, by 2.85 kg per year (Rogers and Cooper, 2011), while Hansen (2007) reported an increase of 4.26 kg per year in cattle. In India, economic estimates of percent increase of pregnancy rate with milk yield have not been explored. In present study, fertility had low negative association with milk yield. The present study quantifies the decline of pregnancy rate with increase of lactation milk yield in Indian Murrah buffaloes. However, in the absence of any direct selection pressure on pregnancy rate of Murrah buffaloes due to low heritability ($h^2$ 0.02), there has been downward trend in fertility associated with the selection for milk yield. The study emphasis the importance of fertility (pregnancy rate) along with milk yield in the genetic evaluation of Indian Murrah buffaloes and a sustainable level of fertility should be maintained for further improvement in milk yield of buffaloes.

**CONCLUSION**

In the present study, fertility had found negatively associated with production traits of Murrah buffaloes. The increase in milk production or productivity causes how much apparent decline in fertility in each lactation as well as in life time production has been revealed in the study. It was found that to achieve around 2000 kg milk yield in 305 days, 7.5 kg wet average and 7.7 kg in test day 5 (125th day) milk yield, level of fertility (pregnancy rate) should be in between 30-50%. Increasing one kilogram milk yield in test day five (125th day) pregnancy rate reduced by about 1.3%, and increase in one kilogram milk yield per day in 305 days or hundred kilogram in 305 days of Murrah buffaloes pregnancy rate reduced by 0.9%. By increasing hundred kilograms of per month milk yield in life time, the pregnancy rate reduced by about 13% in life time. In the present study, fertility had positive correlation with life time or productive life, which indicated that selection of buffaloes for fertility along with milk production increases their life time and profitability of herd. The study quantifies the decline of fertility (pregnancy rate) with the increase of milk production / productivity in Indian Murrah buffaloes. The findings of the study clearly depicts that importance should be given to fertility traits like pregnancy rate along with production traits for improving milk yield of buffaloes, thereby increasing the economic efficiency of herd.
Table 1. Parity wise, Overall and life time level of fertility (PR) with milk productivity (TD 5 MY / WA) and production (MY) in Murrah buffaloes.

<table>
<thead>
<tr>
<th>Range of PR (%)</th>
<th>N</th>
<th>PR (%)</th>
<th>MY (kg)</th>
<th>WA (kg)</th>
<th>TD 5 MY (kg)</th>
<th>N</th>
<th>PR (%)</th>
<th>MY (kg)</th>
<th>WA (kg)</th>
<th>TD5 MY (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-20</td>
<td>88</td>
<td>14</td>
<td>2239.26</td>
<td>7.12</td>
<td>8.28</td>
<td>43</td>
<td>15</td>
<td>2036.66</td>
<td>7.63</td>
<td>8.12</td>
</tr>
<tr>
<td>21-30</td>
<td>58</td>
<td>25</td>
<td>2095.98</td>
<td>7.33</td>
<td>8.23</td>
<td>22</td>
<td>25</td>
<td>2234.58</td>
<td>7.70</td>
<td>8.08</td>
</tr>
<tr>
<td>31-40</td>
<td>26</td>
<td>34</td>
<td>2040.46</td>
<td>7.15</td>
<td>7.97</td>
<td>38</td>
<td>35</td>
<td>2113.40</td>
<td>7.45</td>
<td>7.95</td>
</tr>
<tr>
<td>41-50</td>
<td>13</td>
<td>45</td>
<td>1993.32</td>
<td>6.58</td>
<td>7.42</td>
<td>14</td>
<td>46</td>
<td>2034.08</td>
<td>7.32</td>
<td>7.81</td>
</tr>
<tr>
<td>51-60</td>
<td>12</td>
<td>55</td>
<td>1961.78</td>
<td>6.55</td>
<td>7.40</td>
<td>9</td>
<td>57</td>
<td>1986.27</td>
<td>7.25</td>
<td>7.84</td>
</tr>
<tr>
<td>&gt;60</td>
<td>36</td>
<td>98</td>
<td>1821.37</td>
<td>6.44</td>
<td>6.59</td>
<td>33</td>
<td>100</td>
<td>1915.96</td>
<td>6.97</td>
<td>7.63</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| PR, Pregnancy Rate; TD 5 MY, Test day five milk yield; WA, Wet Average MY, 305 Days or less Milk Yield.
Table 2. Average milk production and productivity corresponding to pregnancy rate.

<table>
<thead>
<tr>
<th>Range of PR (%)</th>
<th>305 Days or less</th>
<th>Milk Yield (kg)</th>
<th>Wet Average (kg)</th>
<th>Test Day 5 Milk yield (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>First parity</td>
<td>Second parity</td>
<td>Third parity</td>
</tr>
<tr>
<td></td>
<td>Milk Yield (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>First parity</td>
<td>Second parity</td>
<td>Third parity</td>
</tr>
<tr>
<td>&lt;30</td>
<td>2162.78 (119)</td>
<td>2124.95 (39)</td>
<td>2431.05 (49)</td>
<td>2189.91 (25)</td>
</tr>
<tr>
<td>30-50</td>
<td>2000.31 (388)</td>
<td>2024.75 (179)</td>
<td>2165.12 (81)</td>
<td>1983.61 (64)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>1918.81 (122)</td>
<td>1856.47 (48)</td>
<td>1931.03 (57)</td>
<td>1805.54 (19)</td>
</tr>
<tr>
<td></td>
<td>7.63 (119)</td>
<td>7.24 (39)</td>
<td>7.90 (49)</td>
<td>7.71 (25)</td>
</tr>
<tr>
<td>30-50</td>
<td>7.44 (388)</td>
<td>6.98 (179)</td>
<td>7.41 (81)</td>
<td>7.25 (64)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>7.05 (122)</td>
<td>6.46 (48)</td>
<td>7.03 (157)</td>
<td>6.50 (19)</td>
</tr>
<tr>
<td></td>
<td>8.13 (119)</td>
<td>8.03 (39)</td>
<td>8.19 (49)</td>
<td>8.35 (25)</td>
</tr>
<tr>
<td>30-50</td>
<td>7.75 (388)</td>
<td>7.69 (179)</td>
<td>7.76 (81)</td>
<td>7.95 (64)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>7.37 (122)</td>
<td>6.76 (48)</td>
<td>7.34 (157)</td>
<td>7.67 (19)</td>
</tr>
</tbody>
</table>
Table 3. Parity wise regression of productivity and production on fertility (Pregnancy Rate) in Murrah buffaloes.

<table>
<thead>
<tr>
<th>Parity</th>
<th>Models</th>
<th>Intercept</th>
<th>MY b</th>
<th>WA b</th>
<th>TD 5MY b</th>
<th>$R^2$ (%)</th>
<th>Average error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>I</td>
<td>0.2646</td>
<td>-</td>
<td>-</td>
<td>-0.008</td>
<td>0.30</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.3061</td>
<td>-</td>
<td>-0.017</td>
<td>-</td>
<td>2.47</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.4112</td>
<td>-0.00003</td>
<td>-</td>
<td>-</td>
<td>0.70</td>
<td>0.08</td>
</tr>
<tr>
<td>Second</td>
<td>I</td>
<td>0.4606</td>
<td>-</td>
<td>-</td>
<td>-0.011</td>
<td>0.70</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.4634</td>
<td>-</td>
<td>-0.024</td>
<td>-</td>
<td>1.41</td>
<td>0.11</td>
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<tr>
<td></td>
<td>III</td>
<td>0.5520</td>
<td>-0.00007</td>
<td>-</td>
<td>-</td>
<td>1.90</td>
<td>-0.04</td>
</tr>
<tr>
<td>Third</td>
<td>I</td>
<td>0.4151</td>
<td>-</td>
<td>-</td>
<td>-0.011</td>
<td>0.70</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.4956</td>
<td>-</td>
<td>-0.042</td>
<td>-</td>
<td>8.89</td>
<td>0.16</td>
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<tr>
<td></td>
<td>III</td>
<td>0.5180</td>
<td>-0.00009</td>
<td>-</td>
<td>-</td>
<td>9.47</td>
<td>-0.01</td>
</tr>
<tr>
<td>Fourth</td>
<td>I</td>
<td>0.3959</td>
<td>-</td>
<td>-</td>
<td>-0.017</td>
<td>2.47</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.5802</td>
<td>-</td>
<td>-0.044</td>
<td>-</td>
<td>6.41</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.6721</td>
<td>-0.00014</td>
<td>-</td>
<td>-</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Overall</td>
<td>I</td>
<td>0.3647</td>
<td>-</td>
<td>-</td>
<td>-0.013</td>
<td>0.7</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.4192</td>
<td>-</td>
<td>-0.009</td>
<td>-</td>
<td>0.2</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.5047</td>
<td>-0.00009</td>
<td>-</td>
<td>-</td>
<td>1.47</td>
<td>0.14</td>
</tr>
<tr>
<td>Lifetime</td>
<td>I</td>
<td>0.5829</td>
<td>-</td>
<td>-</td>
<td>-0.0002</td>
<td>1.88</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.6358</td>
<td>-</td>
<td>-0.034</td>
<td>-</td>
<td>4.88</td>
<td>-0.02</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>0.8718</td>
<td>-0.023</td>
<td>-</td>
<td>-</td>
<td>15.0</td>
<td>-0.01</td>
</tr>
</tbody>
</table>

MY, 305 Days or less Milk Yield; WA, Wet Average; TD 5 MY, Test day five milk yield; b, regression co-efficient estimated; $R^2$, coefficient of determination.
Figure 1. Level of fertility (pregnancy rate) and milk yield up to four lactations of Murrah buffaloes.

Figure 2. Level of fertility (pregnancy rate) and wet average up to four lactations of Murrah buffaloes.

Figure 3. Level of fertility (pregnancy rate) and test day five milk yield up to four lactations of Murrah buffaloes.
Figure 4. Level of life time fertility (pregnancy rate) and life time milk yield in Murrah buffaloes.

Figure 5. Level of life time fertility (pregnancy rate) and life time wet average in Murrah buffaloes.

Figure 6. Level of life time fertility (pregnancy rate) and life time test day 5 milk yield in Murrah buffaloes.
ACKNOWLEDGEMENT

The authors express their sincere gratitude to Director and Vice Chancellor, National Dairy Research Institute, Karnal for providing all research facilities for successful completion of the study.

REFERENCES


ABSTRACT

The present study was conducted in the month of August, 2011 to determine the seroprevalence of brucellosis in organized herd of buffaloes with a history of abortions located in North Gujarat. For the purpose, a total of 117 serum samples were collected from buffaloes and were screened for the presence of antibodies against Brucella by Rose Bengal plate test. The overall sero-prevalence recorded as 25.64%. Age-wise prevalence indicated that 30.53% animals reacted positive to the test was of above 60 months age. However, in less than 60 months of age groups, only 4.55% animals were positive. Prevalence of brucellosis in aborted animals was 72.00% as compared to 13.04% in animals without history of abortion. The study indicated high-seropositivity of brucellosis in the farm and to prevent economic losses to the farm, it is necessary to develop and adopt various control measures.

Keywords: brucellosis, sero-prevalence, buffaloes, Rose Bengal Test

INTRODUCTION

Bovine brucellosis is caused by Brucella abortus, which is responsible for heavy economic losses to the developing countries by causing late term abortions, infertility and reduced milk production. In India, the high sero-prevalence of the disease in buffaloes has been reported as 14.61% to 65.31% by various workers (Sharma and Sani, 1995; Trangadia et al., 2010; Jagapur et al., 2013). Accurate diagnosis, quarantine and proper culling of the infected animals from the herd are required for control and prevention of the disease. Therefore, in the present study, an attempt was made to study the prevalence of brucellosis in organized herd of buffaloes with a history of abortion.

MATERIALS AND METHODS

Sample details

During the month of August 2011, a total of 117 whole blood samples were collected from Mehsani buffaloes reared in a commercial farm located in North Gujarat and the herd had a history of abortion and repeat breeding. Serum was
separated and stored at -20°C until used.

**Rose Bengal Plate Test (RBPT)**

RBPT antigen was procured from the Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh (UP), India. The test was performed according to procedure described by World Organization for Animal Health (OIE, 2008). The result was recorded after the mixture was rocked gently for 4 minutes at room temperature. Any sign of agglutination was considered as positive.

**RESULTS AND DISCUSSION**

In the present study, the seroprevalence of brucellosis was recorded as 25.64 percent in buffaloes by RBPT. In agreement to the present study, Mahajan et al. (2011) reported a prevalence of 17.64% in buffaloes by RBPT. Contrary to these findings, Sutariya et al. (2005) reported a comparatively low prevalence 7.76% in buffaloes from samples screened from the State of Gujarat. However, a higher sero-prevalence of 52.94% in buffaloes by RBPT was reported from animals reared in organized farms located in various parts of India (Trangadia et al., 2010). Similarly, Chauhan et al. (2000) also reported a higher incidence of brucellosis as 44% among buffaloes in Gujarat. Selective sampling of animals with the history of abortion, repeat breeding and retention of placenta may be responsible for the report of higher prevalence in the study (Mahajan et al., 2011).

Age-wise sero-prevalence of brucellosis was shown in Table 1. Out of 95 animals above 60 months age group, 30.53% animals (29) had shown

### Table 1. Age-wise sero-prevalence of brucellosis in buffaloes based on RBPT.

<table>
<thead>
<tr>
<th>Age of animals (months)</th>
<th>Numbers of sera tested</th>
<th>Numbers of positive reactors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-60</td>
<td>22</td>
<td>1 (4.55)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>95</td>
<td>29 (30.53)</td>
</tr>
<tr>
<td>Overall</td>
<td>117</td>
<td>30 (25.64)</td>
</tr>
</tbody>
</table>

### Table 2. Prevalence of brucellosis in buffaloes with a previous history of abortion based on RBPT.

<table>
<thead>
<tr>
<th>Previous abortion history</th>
<th>Numbers of sera tested</th>
<th>Numbers of positive reactors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>92</td>
<td>12 (13.04)</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 months gestation</td>
<td>4</td>
<td>2 (11.11)</td>
</tr>
<tr>
<td>3-6 months gestation</td>
<td>5</td>
<td>2 (11.11)</td>
</tr>
<tr>
<td>&gt;6 months gestation</td>
<td>16</td>
<td>14 (77.78)</td>
</tr>
<tr>
<td>Overall</td>
<td>25</td>
<td>18 (72.00)</td>
</tr>
<tr>
<td></td>
<td>117</td>
<td>30 (25.64)</td>
</tr>
</tbody>
</table>
positive reaction against brucellosis by RBPT, where as in the below 60 months age groups, only 4.55% (One out of 22 animals) animals reacted positively. Rahman et al. (2011) reported a high prevalence (4.92%) in >48 months age group as compared to 13-48 months age group in buffaloes by RBT (2.63%). It is possible that the higher prevalence of brucellosis among older cows may be related to their advanced age, as the organism may remain latent or chronic for an unspecified period before manifesting as clinical disease. Alternatively, aged animal have more chances of exposure to the bacteria and contracting disease. (Nicoletti, 1980).

The sero-prevalence of brucellosis with regards to the history of abortion is depicted in Table 2. The prevalence of brucellosis was recorded 72% (18/25) in aborted animals as compared to 13.04% (12/92) in animals without history of abortion. The prevalence among animals carrying pregnancy of >6 months was 77.78% (14/18) and in each of 3-6 months and <3 months of gestation was 11.11 percent (2/18). However, both the animals aborted before completing the 3 months of gestation had a history of the torsion. Similarly, Ibrahim and Habiballa (1975) reported a prevalence of brucellosis as 14.2 percent in aborted cows. Rahman et al. (2011) reported a higher prevalence of brucellosis in animals with the history of abortion as 60% than in animals with no abortion record (1.16%). Contrary to our findings, a high seropositivity was recorded (19%) in animals aborted in the 6th month of their gestations as compared to only 6 percent in the 9th month of their pregnancy (Khan and Soomro, 2013). The host mechanisms responsible for the increased susceptibility to infection in advanced pregnancy are not known, but they may be related to the differential susceptibility of placental trophoblasts during the middle and late stages of pregnancy (Samartino and Enright, 1992).

The results of the study indicated high sero-positivity of brucellosis among bovines housed in an organized farm. Such situation in the farms warrants immediate attention and preventive measures like restricted movement of animals, proper screening of animals before procurement, quarantine the animals before entry to the main herd etc. should be adopted immediately.

ACKNOWLEDGEMENT

The authors are thankful to the owner of the farm for their co-operation and help in collecting the blood samples from the buffaloes.

REFERENCES


ABSTRACT

Phyto-sources possessing different secondary metabolites are under investigation for the mitigation of enteric methane emission from livestock. Alfalfa (*Medicago sativa*) contains considerable saponin which is known for methane reduction through anti-protozoal action. Therefore, this study was undertaken to ascertain the effect of saponin containing alfalfa fodder (*Medicago sativa*; second cut) on enteric methane emission in Murrah buffaloes quantified using sulfur hexafluoride (SF$_6$) technique. Twelve male Murrah buffalo calves were randomly divided into two groups of six animals each. Buffalo calves in control group were fed on wheat straw and concentrate based diet (R: C, 60:40), while animals in test group were supplemented with saponin containing alfalfa fodder (second cut, 30%) replacing wheat straw on w/w basis. Effect of alfalfa fodder supplementation on rumen fermentation characteristics, archaeal and protozoal population were also studied. Enteric methane emission in control and test group buffaloes was reported as 78.09 and 61.39 g/d, respectively. In this study, about 21% reduction in enteric methane emission was achieved on the feeding of saponin containing alfalfa fodder at 30% level of the diet. However, dry matter intake, pH, ammonia nitrogen and total volatile fatty acid production did not differ (p>0.05) among the groups. A significant (p<0.05) decrease in acetate production was also with concomitant increase in propionate production. Results revealed a non-significant change in archaeal population, while protozoal population were adversely affected and about 20% less numbers were observed in test group. From the study it may be concluded that saponin from natural feed resources like alfalfa fodder at a level of 6.0 g/kg DM can be used for the significant enteric methane reduction.

Keywords: enteric methane, leguminous fodder, Murrah buffaloes, saponin, sulfur hexfluoride

INTRODUCTION

Current atmospheric methane concentration is 155% higher than the pre-industrial concentration (IPCC 2007). Livestock are the major contributor to anthropogenic methane wherein Indian livestock contributes substantially to global enteric methane emission. Due to the huge dispersion of methane, United Nation’s Food and Agriculture Organization recently stated livestock are the major threat for environment. As per one

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1Animal Nutrition, ICAR-National Institute of Animal Nutrition and Physiology, Bangalore, India, *E-mail: malikndri@gmail.com
2National Dairy Research Institute, Karnal, India
estimate, approximately 37% of anthropogenic methane and 12-13% of the total atmospheric methane is emitted from livestock. Asia is the harbor for 179.5 million buffaloes and India alone is possessing approximately 56.7 percent of the total (FAO, 2008). During last 10 years, the world buffalo population increased at a rate of 1.49% per year, wherein, in India and Asia, the increase was 1.53% and 1.45% per annum, respectively. Various agencies have promulgated the enteric methane emission from Indian livestock in the tune of 7.2-12.9 Tg/y (Malik et al., 2012). Buffaloes are the noteworthy emitters to this and contribute about 2.8 Tg/y (Singh, 1998). The need for curtailing the methane emission from ruminants is mandatory from global warming and dietary energy loss point of view (Malik et al., 2013).

Worldwide attempts have been made for the mitigation of livestock methane using halogenated methane analogues, antibiotics, fat and oils, organic acids, but the response from majority of these was highly variable and adoption rate due to high cost, reduced feed intake, toxicity to inhabiting rumen microbes/host animal and transitory effect was very low (Malik et al., 2012). Plant secondary metabolites such as saponin may be a potent agent in achieving the significant reduction in methane emission from livestock. So far in vitro studies have been conducted to evaluate the effect of saponin on methane production (Malik and Singhal, 2008; Wang et al., 2011), and reports for methane reduction in buffaloes on using saponin from natural sources is very limited. Keeping these facts in view, the study was under taken to assess the effect of saponin containing alfalfa fodder supplementation on enteric methane emission in buffaloes.

MATERIALS AND METHODS

Animals and feeding

Twelve male Murrah buffalo calves (BW 172.62±0.36 Kg) were selected from the herd of institute and divided into two groups of six animals each. Necessary permission from the Animal Ethical Committee was obtained for conducting the experiment. Buffalo calves were kept in a well ventilated shed having a provision for individual feeding. To keep the animals free from external and internal parasites, butox 0.5% (v/v) and albandazole (0.5 mg/kg BW) were given, respectively. Buffalo calves were fed as per the Kearl (1982) to meet the nutritional requirement. Animals in group I (control) was fed on wheat straw and concentrate based ration (60:40). Concentrate mixture comprising maize grain (33%), groundnut cake (20.2%), mustard cake (12%), wheat bran (20%), deoiled rice bran (11%), urea (0.8%), mineral mixture (2%) and common salt (1%) was offered to the animals of control group. Animals under group II received the total mixed ration consisting wheat straw, concentrate and second cut alfalfa fodder (Medicago sativa) in the ratio of 30:40:40. Concentrate mixture for the group II animals was prepared by mixing of maize grain (24%), groundnut cake (15%), mustard cake (8%), wheat bran (42%), deoiled rice bran (8%), mineral mixture (2%) and common salt (1%). Second cut alfalfa (Medicago sativa) fodder was selected for the supplementation to buffalo calves on the basis of comparatively higher saponin content among first three cuts. The saponin content of alfalfa fodder was determined as per the method of Yosioka et al. (1974). The CP was maintained about 12.0% of the diet for both control and test group.
Measuring methane emission using Sulfur Hexafluoride (SF₆) tracer technique

In vivo methane emission from Murrah buffalo calves fed on control and test diet was measured using sulfur hexafluoride (SF₆) trace technique of Johnson et al. (1994). Four successful methane gas collections from each individual buffalo were made to quantify the daily emission under control and test group. Before actual gas collection from the calves, PVC canisters were fixed around the neck of each animal for 2 days to acclimatize them. The gas was collected into PVC canister through halters tied around the head and in front of nostrils. The initial and final pressure of the PVC canisters were measured at the time of tying and removal. Brass Permeation tubes (brass, 1.25” length, 3x 16” ID) with central window were filled with SF₆ gas fitted with Teflon cap and 2µ brass frit using standard protocol. Frit was fixed to facilitate the release of SF₆ from brass permeation tube kept at room temperature. Daily weight of permeation tube was recorded until the release rate of SF₆ became constant, thereafter; the permeation tubes were placed into rumen. The SF₆ and CH₄ emitted from each animal were collected into an evacuated yoke like PVC canister through a capillary tube ending just above the nostrils of the animal (Figure 1). A similar canister was hanged in animal shed to record background concentration of CH₄. Concentration of CH₄ and SF₆ gases in collected gas samples was determined using gas chromatograph fitted with Flame Ionization Detector (FID) and Electron Capture Detector (ECD) for methane and SF₆, respectively. The methane emission rate was determined from CH₄ to SF₆ ratio using release rate of SF₆ as given in formula. Background methane was subtracted from methane concentration in the PVC canister.

\[
Q_{\text{CH}_4} = \frac{Q_{\text{SF}_6} \times \left[ (\text{CH}_4-Y - \text{CH}_4b) \right]}{\text{SF}_6}
\]

Q_{\text{CH}_4} is methane emission rate; Q_{\text{SF}_6} is the release rate of SF6; CH₄b is the concentration of methane in background sample and CH₄ Y is the methane concentration in PVC canister.

Rumen fermentation, archaeal and protozoal population

At the end of gas collection, rumen liquor samples were collected from buffalo calves for three consecutive days through stomach tube (Figure 2). Rumen liquor was stained through four layers of muslin cloth and stored into an insulated anaerobic container. Rumen liquor pH was recorded using digital pH meter just after the collection of liquor. However, ammonical nitrogen, total volatile fatty acids (TVFA) and volatile fatty acids (VFA) fractionation were determined using standard protocol of Conway (1950), Barnett and Reid (1957) and Erwin et al. (1961), respectively. Methanogenic archaea as well as protozoal population were also enumerated in rumen liquor samples collected from buffalo calves fed on control and test diet. Anaerobic media described by Ranade and Gadre (1988) for methanogens was used for enumerating the numbers while protozoal numbers was enumerated through haemocytometry microscopic counting method. Data were analyzed in SPSS 16 using one way Anova and means were compared for statistical difference.

RESULTS AND DISCUSSION

In vivo methane emission

Saponin content (DM basis) of alfalfa
Figure 1. *in vivo enteric* methane emission measurement in Murrah buffalo calves using SF$_6$ technique.

Figure 2. Rumen liquor collection from buffaloes using stomach tube.
fodder at three different cuts varied between 0.92±0.05 to 2.0±0.05 per cent with highest level at second cut (2.0%). Due to higher saponin level and results from in vitro studies (data not presented), second cut alfalfa fodder was selected for the supplementation at 30% level in wheat straw and concentrate based diet to ascertain the effect on enteric methane emission in Murrah buffalo calves. Similar to the findings of this study, Cheeke and Shull (1985) also reported highest saponin level in alfalfa fodder at second cut.

Dry matter intake (DMI) and in vivo methane emission as affected by the supplementation of saponin containing alfalfa fodder in Murrah buffaloes is depicted in Table 1 and Figure 3, respectively. Data did not reveal any significant change in dry matter intake between control and test group (Table 1). In vivo enteric methane emission in buffaloes in control and test groups is presented in Figure 3. Enteric methane emission in control and test group was 78.09 and 61.39 g/d, respectively. Enteric methane emission (g/d) in control group was significantly higher (P<0.05) than the test group. About 21% reduction in methane emission was reported in test group on the inclusion of saponin containing alfalfa fodder at 30% level replacing wheat straw in the diet (Figure 3). Similarly, in vivo methane emission on g/kg DMI was also significantly (P<0.05) lower in test group than control (18.1 vs. 12.4 g). Enteric methane emission on g/kg digestible dry matter intake basis was also significantly (p<0.05) lower in test group. Lower enteric methane emission from the test group buffaloes may be attributed to the saponin of alfalfa fodder. Average saponin intake in test group was 47 g/d or 6.03 g/kg of dry matter. Srivastava and Garg (2002) reported 19.26 g methane emission per kg of DMI in crossbred calves fed on paddy straw and fodder based diet. Mohini and Singh (2001) recorded the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control diet</th>
<th>Test diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kg/d</td>
<td>7.40±0.28</td>
<td>7.79±0.19</td>
</tr>
<tr>
<td>Kg/100kg BW</td>
<td>2.77±0.16</td>
<td>2.94±0.08</td>
</tr>
<tr>
<td>g/kgW^{0.75} BW</td>
<td>111.8±0.46</td>
<td>118.6±0.40</td>
</tr>
<tr>
<td>Fermentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.40±0.18</td>
<td>6.30±0.18</td>
</tr>
<tr>
<td>NH₃-N (mg/100ml)</td>
<td>30.92±1.37</td>
<td>28.62±1.33</td>
</tr>
<tr>
<td>TVFA (mM/l)</td>
<td>108.94±0.68</td>
<td>108.87±0.97</td>
</tr>
<tr>
<td>Acetate</td>
<td>74.13±0.34</td>
<td>67.07±0.35</td>
</tr>
<tr>
<td>Propionate</td>
<td>15.99±0.74</td>
<td>17.70±0.37</td>
</tr>
<tr>
<td>Butyrate</td>
<td>9.86±0.42</td>
<td>10.22±0.64</td>
</tr>
<tr>
<td>A:P</td>
<td>4.6:1</td>
<td>3.7:1</td>
</tr>
</tbody>
</table>

Values bearing a, b superscripts in a row differ significantly (p<0.05).
methane emission in the range of 15.97 - 18.35 g/kg DMI from buffalo calves fed on maize fodder and straw based diet. Mao et al. (2010) also found a reduction of 27.2% in methane emission from lamb on the inclusion of saponin at 4.1 g/kg level from *Camellia sinensis*. However, administration of 5 g/kg of *S. saponaria* fruits to sheep for 21 days reduced CH$_4$ emission by 7.8% only in a study of Hess et al. (2004).

Data from the study revealed that enteric methane emission in buffaloes is influenced by the saponin from alfalfa fodder. Lower methane emission in test group was attributed to the anti-protozoal action of saponin of alfalfa, which in turn puts a restriction on hydrogen transfer to methanogens (Krumholz et al., 1983). Mechanistically, saponin forms an irreversible complex with cholesterol which is an integral component of protozoal cell membrane and therefore leads to cell lysis and death.

**Effect on feed fermentability**

Dry matter intake (DMI) and fermentation characteristics as affected by the supplementation of saponin containing alfalfa fodder in Murrah buffaloes are presented in Table 1. Dry matter intake (DMI) did not show any significant change due to the supplementation (Table 1). Similarly, pH, ammonia nitrogen and total volatile fatty acid also did not affect with saponin containing alfalfa fodder supplementation. Data from the study envisaged that saponin from alfalfa fodder at 6.03 g/kg DM (total intake 47g) did not have any adverse effect on dry matter intake, and total volatile fatty acid production.

Acetate production (P<0.05) in alfalfa fodder supplemented buffaloes decreased significantly (p<0.05) by 7 units (9.5%) as compared to control (Table 1). On the other hand, propionate fraction at the cost of acetate significantly (P<0.05) increased, while butyrate remain unaffected by the supplementation. Data showed a shift in individual fatty acid production from acetate to propionate on the inclusion of alfalfa fodder. These results are in consonance of the findings of Diaz et al. (1993); Hristov et al. (1999) who also reported similar trend on the dietary incorporation of *Sapindus saponaria* and *Yucca schidigera*, respectively. Hu et al. (2006) also recorded same trend without affecting TVFA concentration on the incorporation of *Yucca* and *Quillaja saponin* in corn meal and grass based diet. From these results, it may be concluded that the saponin from alfalfa fodder to a level of 47 g/d or 6 g/kg DM did not affect the feed fermentability or dry matter intake.

**Effect on methanogen archaea and protozoa**

Results of the study revealed a non-significant effect of alfalfa fodder as such or its saponin (47g/d or 6.03g/kg DM) on rumen methanogen archaea. On the feeding of control as well as test diet, their population remained constant ~ 1.73 log CFU/ml (Figure 4), which shows that the saponin did not have any direct action on methanogen archaea and directly not accountable for methane reduction from buffalo calves. Results is in congruence of the findings of Wina et al. (2005) who did not find any decrease in methanogens number on the addition of saponin from *Sapindus rarak*. On the contrary the protozoal population was adversely affected due to alfalfa fodder supplementation (Figure 4). Protozoal population badly affected (P<0.05) in group II on the addition of saponin containing alfalfa fodder and the decrease was almost 20 per cent as compared to control group (Figure 4). Entodinimorphs are the most vulnerable to dietary changes in cattle and buffaloes (Bhatia et al., 1998) and most susceptible
Figure 3. Effect of lucerne fodder supplementation on methane emission in Murrah buffaloes.

Figure 4. Effect of supplementation on rumen methanogens and protozoa.
to the dietary saponin (Lu and Jorgenson, 1987). However, in present study no attempt was made for the characterization of various protozoal population affected by alfalfa saponin. These results are in agreement with findings of Navas-Camacho et al. (1993); Diaz et al. (1993); Klita et al. (1996), noted a significant reduction in ruminal protozoa following dietary incorporation of E. ciclocarpum, Sapindus saponaria and alfalfa root saponin, respectively. Thus, the significant reduction in methane emission in group II on the inclusion of alfalfa fodder is attributed to comparatively lesser number of protozoa badly hit by saponin. Anti-protozoal effect of saponin is depend on the presence of cholesterol in protozoal cell membrane, which possible made a selective susceptibility of ruminal protozoa to saponin and due to this reason methanogens (lacking in cell membrane cholesterol) did not directly hit by saponin.

**CONCLUSION**

It may be inferred from the study that secondary metabolite saponins from natural feed sources like alfalfa may significantly reduce enteric methane emission in buffaloes and quite safe to feed up to a level of 6.03 g/Kg of DM or 47 g/day without affecting dry matter intake. Methanogenic archaea is not affected by the alfalfa fodder supplementation, while it has an adverse action on rumen protozoa which lead to less enteric methane emission. There is a need to conduct instant research towards exploring the saponin containing phyto-sources and to optimize their safe level of inclusion in diet for the substantial methane reduction.

**REFERENCES**


Hess, H.D., R.A. Beuret, M. Lotscher, I.K.


Kearl, L. 1982. *Nutrient requirements of ruminant in developing countries*. Utah State Univ. Logam, USA.


ABSTRACT

The present research work was conducted to study distribution of Graafian and atretic follicles in buffalo ovary during different seasons of year. For this purpose, 100 ovaries of adult Murrah buffaloes during different seasons (20 in each season) of year viz; winter (November - January), spring (February - March), summer (April - June), rainy (July - August) and autumn (September - October) were collected from slaughter house. The paraffin sections of 10 µm were cut and every 20th section was stained with hematoxylin and eosin. In present study, two types of follicles were observed viz. young and mature Graafian follicles. Highest number of normal follicles was during autumn season whereas lowest number of normal follicles was during summer season. The most common type of atresia was antral. The ratio of normal to atretic follicle was maximum in summer (1: 3.10) and minimum in autumn season (1: 2.19).

Keywords: buffalo, follicular atresia, histomorphometry, ovarian follicles, seasons

INTRODUCTION

Buffaloes are known for the poor reproductive performance such as silent heat, low conception rate and long calving interval which causes heavy economic losses to farmers (Madan, 1988). Further, the embryo transfer technique in buffalo has not proved to be much successful, mainly due to very poor superovulatory response and poor recovery of viable embryo (Taneja et al., 1991). The success of in vitro production of buffalo embryos has been hampered by factors such as low quality of follicles on ovaries and poor oocyte recovery rate. In past it has been shown that environmental temperature plays an important role and buffaloes exhibit a distinct reproductive performance in different seasons (Shah, 1988). The number of graafian follicles produced on the ovary may be one of the important factor to determine the reproductive efficiency of the animal as it is affected by the environmental temperature. Although, the number of mature follicles have been counted during follicular and luteal phases of estrous cycle (Danell, 1987 and Bansal, 2002) but seasonal study on such a count is lacking. So the present research was planned to count and to correlate the number of mature follicles in each season of year.
MATERIALS AND METHODS

Collection of ovaries

Left and right ovaries of 100 adult Murrah buffaloes were collected during different seasons (20 in each season) of the year viz; winter (November - January), spring (February – March), summer (April - June), rainy (July - August) and autumn (September - October). Immediately after collection, the ovaries were fixed in 10% neutral buffered formalin (NBF).

Processing of ovaries

Ovaries were processed by acetone benzene schedule (Luna, 1968). The whole ovary was serially sectioned with a rotary microtome at a thickness of 10 µm. The serial sections were placed in a sequence on clean glass slides keeping track of section numbers and slide numbers. Every 20th section of ovaries was stained with hematoxylin and eosin (Luna, 1968). Subsequent sections were stained with Masson’s trichrome for collagen fibres, Gridley’s stain for reticular fibres and Verhoeff stain for elastic fibres.

Number of follicles

The follicles which were ≥ 1mm in diameter were observed and counted by a method reported by Danell (1987). Every 20th section of ovary was placed in an enlarging apparatus and projected 5 times larger onto normal photographic paper. After development, the photographs were arranged in a series and fixed on a white sheet. For describing the size of follicles, each particular follicle was given a similar number on all the photographs where it appeared. The mean follicle diameter was determined as the average of three measurements: the largest diameter ($D_1$), the diameter perpendicular to $D_1$ ($D_2$) and diameter perpendicular to the plane of section ($D_3$). Third diameter $D_3$ was calculated as

$$D_3 = N \times 10 \times 20$$

where

“N” represents number of sections in which the particular follicle appeared

“10” represent the thickness of section in µm

“20” represents every 20th serial section of ovary at which diameter was measured.

Actual estimated diameter was calculated by dividing the mean follicle diameter by five.

RESULTS AND DISCUSSION

Depending upon the size of antrum, two types of follicles were observed viz. young and mature Graafian follicles. Mostly the follicles contained single oocyte but occasionally 2 or 3 oocytes were also observed (Figure 1). The oocyte of the graafian follicle was round to oval with mean diameter of 71.47±8.18 µm. The nuclei were centrally or eccentrically placed. The antrum was filled with eosinophilic colloidal fluid, the liquor folliculi. The membrana granulosa was covered by basement membrane. The theca layer was completely differentiated into theca interna and theca externa (Figure 2) as reported earlier in cattle (Rajakoski, 1960) and buffalo (Bansal, 2002). Theca interna composed of mainly epithelial cells with vesicular nuclei and small amount of fibroblasts and connective tissue fibres whereas theca externa consisted of fibroblasts, connective tissue and muscle fibres. Theca interna was highly vascular as reported earlier (Danell, 1987; Bhardwaj and Roy, 1998).
The mean size of Graafian follicle was 443.18±72.1 µm. The average thickness of zona pellucida was 5.34±0.48 µm. The mean size of antrum was 395.83±39.26 µm. The membrana granulosa, which enclosed the antrum was having mean thickness of 38.22±2.52 µm. The mean thickness of theca interna and externa was 63.03±3.4 µm and 59.25±5.45 µm, respectively.

Graafian follicles (> 1 mm) were classified into five categories on basis of size viz. 1 – < 2 mm, 2 – < 3 mm, 3 – < 4 mm, 4 – < 5 mm and ≥ 5 mm and has been depicted in Figure 13. The present study revealed that the follicles falling in 1 – < 2 mm group were present in maximum number and these constituted 65.27%, 64.79%, 61.16%, 53.33% and 64.89% of the total follicles during winter, spring, summer, rainy and autumn season, respectively. These follicles represented more than half of the total number of follicles (≥ 1 mm in diameter). The findings corroborates well with the earlier findings of Danell (1987) and Bansal (2002) during different seasons.

Percentage distribution of total number of Graafian follicles (normal and atretic) in the ovaries during different seasons has been summarized in Table 1 which revealed highest number of normal follicles was during autumn season whereas lowest number of normal follicles was during summer season. Similar types of findings were observed by Sadeghinezhad and Hasanzadeh (2010) in river buffalo. Also, it was observed that although the number of normal and atretic follicles were more on the right sided ovary but the difference between distribution of follicles in left and right ovary was non-significant. The maximum per cent of atresia (75.62) was observed during summer followed by spring (72.89), rainy (71.25), winter (69.79) and autumn (68.73). These findings are almost similar to Danell (1987) who reported 70.60 per cent atresia in Surti buffalo and Bansal (2002) who reported 71.77 per cent of atresia in buffalo ovary.

The ratio of normal and atretic follicles was calculated during different seasons. It was 1:2.31 in winter, 1:2.68 in spring, 1:3.10 in summer, 1:2.48 in rainy season and 1:2.19 in autumn, respectively. The data revealed that the ratio was highest in summer season as compared to other seasons.

**Follicular atresia**

As the primordial follicles progress through different stages of development, most of them never reach maturity and thus got degenerated at certain point along the way. This process of follicular degeneration is called follicular atresia and depends upon the variable degree of susceptibility of different cells to death (Rodgers and Irving-Rodgers, 2010). In the present study, atresia was seen in follicles at all stages of development. The highest atresia was also observed in the follicles of range 1 - < 2 mm (Figure 14) as reported earlier by Danell (1987) and Bansal (2002) with maximum value of 76.35% during summer season.

The follicular atresia of large follicles was categorized as obliteratorive and cystic. The obliteratorive atresia was further subdivided into first degree and second degree as described by Danell (1987) and Bansal (2002). In first degree of atresia, a number of pyknotic nuclei were observed either in the layers of membrana granulosa close to antrum or in the antrum itself but in close proximity to the membrana granulosa whereas the cells closest to the basal lamina were tightly packed and appeared healthy (Figure 3). In some of the follicles, the upper layer of granulosa cells was detached from the underlying granulosa cells (Figures 4, 5). The pyknotic nuclei were rarely seen in the granulosa cells close to the basement membrane. The fibroblast present in the theca layer
were spindle shaped and were orientated parallel to the membrana granulosa. This type of atresia has been described as antral atresia by Irving-Rodgers et al. (2001) and is comparable to first degree of obliterator atresia described by Danell (1987) and Bansal (2002). With further advancement, there was degeneration of granulosa layers with few pyknotic nuclei in the antrum, granulosa cells and cumulus. Later on, cumulus disappeared leaving a naked oocyte followed by in growth of connective tissue in the antrum (Figures 6, 7) as described earlier by Danell (1987) and Bansal (2002) in buffalo ovary. When the antrum was completely filled with connective tissue, the degenerated follicle was termed as corpus atreticum (Figure 8).

In other type of atresia, there was destruction of the most basal layer of the granulosa cells whereas the most antral granulosa cells remained healthy and closely apposed to each other. There were very few pyknotic nuclei in the antrum or in membrana granulosa closest to antrum. The cells nearer to antrum were often flattened so that overall antral surface of membrana granulosa appeared smooth and regular. The cells in the basal layer of membrana granulosa were separated from each other and from basal lamina by intercellular spaces. With further advancement of atresia, there was degeneration of granulosa cells (Figure 9) and large apoptotic bodies (Figure 10) were observed in the antrum. The spindle shaped fibroblasts of the theca interna were arranged randomly. This type of atresia observed during present study fits well as basal atresia described earlier by Irving-Rodgers et al. (2001) and is comparable to the second degree of obliterator atresia as observed by Danell (1987) and Bansal (2002).

In cystic type of atresia, both granulosa and theca layers atrophied or only granulosa layer atrophied and theca layer may be luteinized, fibrosed or hyalinized. There was decrease in follicular size and antrum. In some of the follicles only 1-2 layers of granulosa cells was observed so giving the follicle a classic string of pearl orientation as described by Marion et al. (1968) who assumed that these follicles had begun to expand on atresia. Rodgers et al. (2001) reported that number of layers of granulosa cells decreases as follicles enlarge to reach a plateau. The number of granulosa cell layer is both a function of net rate of granulosa cell replication and the rate of antrum expansion. Thus, the string of pearls description used by Marion et al. (1968) arised by reduction in the number of layers of granulosa cells as the antrum expands during follicular growth and not during atresia. Luteinized cystic type of atresia occurred infrequently in which there was degenerated granulosa cells followed by luteinization of whole of the theca interna cells (Figure 11). Since atretic follicles produces high amount of progesterone as compared to non-atretic follicles (Westhof et al., 1991) and also dibutyryl cyclic AMP significantly stimulates progesterone production by cells of atretic follicles. The increased concentration of progesterone leads to luteinization of follicles which is accompanied by cellular hypertrophy, formation of diffusely distributed lipo-proteins and increased number of lipid droplets (Guraya, 1997). Another type of cystic atresia observed in present study was fibrosed type in which there was degeneration of granulosa layer along with increased number of fibroblasts in theca interna as reported earlier by Bansal (2002) in buffalo ovary. This type of atresia was maximum among all the types of cystic atresia. In third type of cystic atresia, theca cells were hyalinized along with degeneration of granulosa cells (Figure 12). The atresia occurs in a particular sequence as reported earlier by Guraya (1979) and Danell (1987) i.e. first...
Figure 1. Growing follicle with three oocytes (arrow). H & E stain X10.

Figure 2. Section of buffalo ovary showing layers of normal Graafian follicle; antrum (A), membrana granulosa (MG), theca interna (TI) and externa (TE). Masson’s trichrome stain X20.

Figure 3. Photomicrograph of paraffin section of ovary showing sloughing of granulosa cells (arrow) close to antrum (A). H & E stain X40.

Figure 4. Section of ovary showing loosening and sloughing of granulosa cells (arrow) close to antrum (A). Masson’s trichrome stain X40.

Figure 5. Section of ovary showing complete sloughing of granulosa cells (arrow). Masson’s trichrome stain X20.

Figure 6. Section of ovary showing degeneration of membrana granulosa (MG) cells. Masson’s trichrome stain X10.
Figure 7. Section of ovary showing connective tissue (Ct) in antrum. H & E stain X20.
Figure 8. Section of ovary showing connective tissue scar, corpus atreticum (CA). H & E stain X10.
Figure 9. Section of ovary showing wall of normal (NF) and atretic follicle (AF). Masson’s trichrome stain X20.
Figure 10. Photomicrograph of paraffin section of ovary showing apoptotic bodies (arrow) in basal type of atresia. H & E stain X 20.
Figure 11. Photomicrograph of paraffin section of ovary showing luteinization of follicle. Masson’s trichrome stain X10.
Figure 12. Photomicrograph of paraffin section of ovary showing hyalinization of follicular wall. Numerous pyknotic nuclei (arrow) can be seen. H & E stain X20.
Figure 13. Percentage of different categories of follicles (≥ 1 mm) during different seasons.
Figure 14. Number of normal and atretic follicles (≥ 1 mm) in different categories during different seasons.
Table 1. Percentage distribution of normal and atretic Graafian follicles (≥ mm) in left and right ovaries during different seasons.

<table>
<thead>
<tr>
<th>Season</th>
<th>Left ovary</th>
<th>Total</th>
<th>Right ovary</th>
<th>Total</th>
<th>Overall</th>
<th>Normal</th>
<th>Atretic</th>
<th>Normal</th>
<th>Atretic</th>
<th>Normal : Atretic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Atretic</td>
<td></td>
<td>Normal</td>
<td>Atretic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>42 (29.78)</td>
<td>99 (70.22)</td>
<td>141</td>
<td>45 (30.61)</td>
<td>102 (69.39)</td>
<td>147</td>
<td>87 (30.21)</td>
<td>201 (69.79)</td>
<td>288</td>
<td>1 : 2.31</td>
</tr>
<tr>
<td>Spring</td>
<td>36 (26.47)</td>
<td>100 (73.53)</td>
<td>136</td>
<td>41 (27.70)</td>
<td>107 (72.30)</td>
<td>148</td>
<td>77 (27.11)</td>
<td>207 (72.89)</td>
<td>284</td>
<td>1 : 2.68</td>
</tr>
<tr>
<td>Summer</td>
<td>30 (25.00)</td>
<td>90 (75.00)</td>
<td>120</td>
<td>29 (23.77)</td>
<td>93 (76.23)</td>
<td>122</td>
<td>59 (24.38)</td>
<td>183 (75.62)</td>
<td>242</td>
<td>1 : 3.10</td>
</tr>
<tr>
<td>Rainy</td>
<td>29 (28.16)</td>
<td>74 (71.84)</td>
<td>103</td>
<td>40 (29.19)</td>
<td>97 (70.81)</td>
<td>137</td>
<td>69 (28.75)</td>
<td>171 (71.25)</td>
<td>240</td>
<td>1 : 2.48</td>
</tr>
<tr>
<td>Autumn</td>
<td>53 (31.36)</td>
<td>116 (68.64)</td>
<td>169</td>
<td>53 (31.18)</td>
<td>117 (68.82)</td>
<td>170</td>
<td>106 (31.27)</td>
<td>233 (68.73)</td>
<td>339</td>
<td>1 : 2.19</td>
</tr>
<tr>
<td>Total</td>
<td>190 (28.40)</td>
<td>479 (71.60)</td>
<td>669</td>
<td>208 (28.73)</td>
<td>516 (71.27)</td>
<td>724</td>
<td>398 (28.57)</td>
<td>995 (71.43)</td>
<td>1393</td>
<td>1 : 2.5</td>
</tr>
</tbody>
</table>

Values in parentheses indicate percentage calculated from total in a row.
there was dissolution and pyknosis of granulosa cells followed by differentiation of theca interna into fibrous cells. Later on there was hyalinization and dissolution of theca interna layer.

Although atresia may occur due to excessive secretion of gonadotropins (Harman et al., 1975) or insufficient gonadotropins (Hirshfield, 1991), excessive androgens (Harman et al., 1975), excess or insufficient estradiol (McNatty, 1978) and endogenous GnRH like substance within the ovary (Birnbaumer et al., 1985), yet there are also certain other factors which regulate the cyclic appearance and atresia of dominant follicles and other follicles. These factors may include age, stage of reproductive cycle, pregnancy, lactation, hormones of extra-ovarian and intra-ovarian sources, a genetic programmed nutrition, ischemia and season (Danell, 1987). Sluss et al (1983) has suggested the possibility of FSH-binding inhibitors in the induction or propagation of follicular atresia by suppressing the responsiveness of granulosa cells to FSH. Different studies have clearly suggested that the follicles which are destined to undergo atresia in normal estrous cycle of cattle can be rescued by administration of exogeneous gonadotrophins (Driancourt, 1987). The follicles undergo atresia due to lack of gonadotrophins at a key stage (at which the amount of FSH receptors on granulosa cells and LH receptors on theca layer becomes maximum and also aromatase activity is increased) in folliculogenesis (Guraya, 1997).

Actually the balance between estrogens and androgens plays especially an important role in determining whether a follicle becomes atretic or not (Adashi, 1994). It has also been reported that metabolism of granulosa cells can be affected by any interference in the development of their LH receptors which possibly can induce follicular atresia (Guraya, 1997).

So, the most important consequences of follicular atresia is to limit the number of ovulations or select the dominant follicle (Guraya, 1997), thus reducing the number of offsprings. Secondly, follicular atresia contributes thecal type interstitial cells to the ovary which constitute the steroidogenic tissue. Atretic follicles may contribute significantly to intra-ovarian levels of androgens and progesterone which can be utilized by non-atretic follicles to enhance estradiol synthesis (Westhof et al., 1991).

REFERENCES

Driancourt, M.A. 1987. Follicular dynamics and intraovarian control of follicular development in the ewe. In Roche, J. and D.
O’Callaghan (eds.) *Follicular Growth and Ovulation Rate in Farm Animals*. Martinus Nijhoff, The Hague.


individual tertiary procine follicles \textit{in vitro}.
Possible physiologic role of atretic follicles.

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…and buffaloes (Singh et al., 1983; Shah et al., 1987; Misra, 1996; Pant et al., 2002)

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