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ABSTRACT

A unique case of idiopathic fibrinous pericarditis in a Nili-Ravi buffalo was presented. The clinical observations, diagnostic procedure and necropsy findings are discussed in detail.

Keywords: Nili-Ravi buffalo, Bubalus bubalis, fibrinous pericarditis, idiopathic

INTRODUCTION

Pericarditis is inflammation of the pericardium with assemblage of serous or fibrinous inflammatory products (Gründer, 2002) and almost always results in death of the animal (Braun, 2009). The condition is predominantly caused by traumatic penetration of the pericardium by a foreign body originating from the gastrointestinal tract (Braun, 2009). However, idiopathic pericarditis, which is frequently seen in human beings, dogs and horses, is rarely found in bovines (Jesty et al., 2005). Furthermore, among buffaloes, such a cardiac anomaly is little reported in the Nili-Ravi breed. The present communication reports an atypical case of idiopathic fibrinous pericarditis in a Nili-Ravi Buffalo.

CASE REPORT

Sumeet Sharma1, Navjot S. Gosal2 and Varun3

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3Civil Veterinary Hospital, Sahora Kalan, Pathankot, Mahatam Nagar, Fazilka, Punjab, India
TREATMENT AND DISCUSSION

The blood picture and other clinico-biochemical abnormalities were suggestive of pericarditis and cardiovascular system involvement; nonetheless, no definitive diagnosis could be made. As the owner was reluctant to consult a referral hospital, buffalo was subjected to symptomatic therapy which included parenteral antibiotics, multivitamins, intravenous fluids and NSAIDs. However, regardless of the therapy provided, the buffalo became recumbent and collapsed on day 4 of the treatment. Necropsy was conducted immediately, and generalised oedema was observed, which was more prominent in thoracic and neck region. Cardiomegaly and gross distension of the pericardial sac with offensive smelling greyish straw-coloured fluid containing flakes of fibrin was evident. Cultural examination of fluid and cardiac surface samples was positive for Corynebacterium spp. The pericardial sac was thickened and fused to pericardium by strong fibrous layer. This thick fibrinous layer had given a typical shaggy appearance to the heart. A seven-centimetre-long piece of wire was found lying in the reticulum. Except slight hepatomegaly, no other particular lesion was noticed.

The clinical observations, laboratory investigations and post-mortem lesion were suggestive of fibrinous pericarditis (Radostits et al., 2007; Saleh et al., 2008). However, as no fibrous tract connecting reticulum to pericardium was present, therefore, underlying cause of pericarditis in this case was not determined to be traumatic origin. It has also been recorded that physical penetration of the sac is not essential to the development of pericarditis as infection sometime penetrates through the pericardium from a traumatic mediastinitis (Radostits et al., 2007). Ramakrishna (2001) reported that in few cases a traumatic agent may pierce the pericardium at various sites and either goes outside of the thoracic cavity or falls

Table 1. Haematobiochemical findings in buffalo suffering from fibrinous pericarditis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>11.60</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>27.00</td>
</tr>
<tr>
<td>TLC (10^3/μL)</td>
<td>14.20</td>
</tr>
<tr>
<td>Fibrinogen (g/dL)</td>
<td>0.94</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.96</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>1.45</td>
</tr>
<tr>
<td>Total globulin (g/dL)</td>
<td>4.51</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.32</td>
</tr>
<tr>
<td>AST (u/L)</td>
<td>193.23</td>
</tr>
<tr>
<td>ALT (u/L)</td>
<td>54.87</td>
</tr>
<tr>
<td>CK (u/L)</td>
<td>224.36</td>
</tr>
</tbody>
</table>
back in to the reticulum; hence the possibility of etiological agent as of traumatic origin may not be fully ignored in this investigation. It is noteworthy that introduction of a mixed bacterial infection from the reticulum or other source causes a severe local inflammation in the pericardium, and persistence of the foreign body in the tissue is not essential for further progress of the disease (Radostits et al., 2007).

Our results are supported by the report of Saleh et al. (2008) who revealed hypoproteinemia and hyperglobulinemia in buffalos with fibrinous pericarditis. Similarly, estimated plasma enzyme profiles fell in the range described for buffaloes suffering from pericarditis (Rose et al., 2009). Moreover, increase in the activities of liver enzymes in a bovine with cardiac insufficiency (right side) merely reflects a sign of liver congestion and not primary liver disease (Braun, 2009).

REFERENCES


PERI-RECTAL CYST CAUSING RECTAL OBSTRUCTION IN A BUFFALO HEIFER

V. Sangwan¹, A. Kumar², N.S. Saini² and J. Mohindroo²

ABSTRACT

This case report describes a peri-rectal cyst in a 1.5-year-old buffalo heifer with no uro-genital involvement and causing significant rectal obstruction. The cyst was differentially diagnosed on the basis of per-rectal examination, ultrasonography, cytology, parasitological examination and biochemical tests. The cyst was successfully treated by placing a drain, percutaneously, in the cyst cavity for 10 days.

Keywords: buffaloes, Bubalus bubalis, Peri-rectal cyst, ultrasonography, surgery

INTRODUCTION

A peri-rectal cyst may be defined as a cyst in the tissues surrounding the rectum in the pelvic cavity. A few reports of Bartholin’s gland and Gartner’s duct cysts are available in cows in the peri-vaginal region causing no rectal obstruction (Fathalla et al., 2000; Bademkiran et al., 2009). Human literature cites a few clinical and pathological reports of retro-rectal cyst in patients with perineal problems of recurrent fistulas or intestinal obstruction (Goldewski et al., 2000; Sciaudone et al., 2009; Al-Khattabi et al., 2010). Previously peri-rectal abscesses have been reported to cause rectal obstruction in horses and cattle (Sanders-Shamis, 1985; Elce, 2006; Sangwan et al., 2008), but to the authors’ knowledge a peri-rectal cyst with no uro-genital involvement and causing rectal obstruction has not been reported in the veterinary literature. The present case report describes the diagnosis and treatment of a peri-rectal cyst causing rectal obstruction in a buffalo heifer with no uro-genital involvement.

CASE HISTORY AND OBSERVATIONS

A buffalo heifer aged 1.5 years weighing approximately 200 kg was presented to the teaching veterinary hospital with a history of straining while defecating for the previous 15 days. The condition worsened progressively over a fortnight, and the animal was passing scanty pasty feces. No previous history of straining or dysuria was reported by the owner. The animal was partially anorectic with normal rumination. On clinical examination, vital parameters were within normal physiological range. Per-rectal examination revealed an extraluminal
swelling of about 12 cm in diameter on the left lateral aspect of rectum (Figure 1) which was partially occluding the rectum during defecation. Per-rectal ultrasonographic examination with a 5 MHz linear transducer revealed a circumscribed anechoic cavity on the lateral aspect of the rectal wall. The urinary bladder was visible as a distinct structure cranial to the growth (Figure 2). Needle aspiration of the growth yielded clear fluid. Microscopic examination of the fluid aspirate was found negative for ova/cysts. Cytology of the fluid revealed a few squamous epithelial cells.

The urine, fluid aspirate from the growth and serum samples were examined for creatinine levels. The creatinine level of the urine was 23 mg/dL, that of the serum was 1.1 mg/dL and that of the aspirate was 0.8 mg/dL. The blood urea nitrogen was 14 mg/dL. On the basis of per-rectal examination, ultrasonography, cytology and biochemical tests the condition was diagnosed to be a sterile cyst not related to urinary or reproductive system.

**TREATMENT AND DISCUSSION**

Caudal epidural anaesthesia was achieved with 2% lignocaine for surgical drainage of the peri-rectal cyst. The area around the anus was prepared aseptically. The swelling was fixed in one hand, and a small stab incision was given on the skin lateral to the anus. A Foley catheter of size 10FG was introduced into the growth with the help of a groove director. When fluid started coming out of the catheter, the cuff of the catheter was filled with 5ml saline solution to fix the catheter within the cystic cavity. A cross mattress skin suture with silk no. 3 was applied to close the skin incision. Four additional stay sutures with the same suture material were applied to fix the catheter (Figure 3). The growth collapsed immediately following drainage of fluid contents, and the animal was able to pass feces. Post-operatively, the animal was administered antibiotic streptopenicillin 10 mg/kg twice daily, IM for 3 days and analgesic meloxicam 0.2 mg/kg OD, IM for 3 days. Drop wise fluid was seen coming out of the catheter for two more days and then later stopped. The catheter was removed on the 10th postoperative day, and the animal recovered completely. No reoccurrence of the cyst was reported up to 11 months of follow up.

The age of the animal was 1.5 years, but the present cyst was not considered a congenital anomaly as the animal had had no problem related to defecation or urination since birth. Systemic involvement was not considered in this case as the vital parameters, feed intake, rumination and general body condition of the animal was normal. Proximity of the swelling to the anus might have been the cause of difficult defecation in the present case. In veterinary science, such a history and swellings are usually related to peri-rectal abscesses or tumourous growths (Sanders, 1985; Elce, 2006; Hunt and Poucke, 2008; Sangwan et. al., 2008). Ovarian cysts can also be felt per rectally but are not reported to cause rectal obstruction. Human literature describes a few cases of retro rectal / epidermoid cysts in females with the history of pain, acute intestinal obstruction and recurrent anorectal fistula (Goldlewski et. al., 2000; Singer et. al., 2003). Endorectal ultrasonography, in the present case, was helpful to differentiate perirectal growth from the urinary bladder. Human literature also reports use of endorectal ultrasonography and other advanced techniques like CT scan and MRI to diagnose such growth/cysts (Goldlewski et. al., 2000; Singer et. al., 2003; Sciaudone et. al., 2009).

The peri-rectal space might have lesions
Figure 1. Photograph showing growth on the left side of rectum.

Figure 2. Ultrasound scan showing the cystic cavity unrelated to urinary bladder (UB).

Figure 3. Photograph showing Foley’s catheter placed in the cystic cavity through skin.
associated with the rectum or urogenital system. The peri-rectal abscess, rectal or peri rectal tumor and ovarian cyst were differentiated on the basis of per-rectal examination, ultrasonography, needle centesis and cytology. The involvement of the urinary system was ruled out using biochemical tests of different body fluids. The similarity in the creatinine levels of the growth fluid aspirate and serum and their difference from the urine creatinine confirmed no urinary involvement.

The treatment method applied in the present case using drainage with a Foley catheter No. 10 was successful with no post-drainage recurrence. There is a report of needle evacuation of a Bartholin gland cyst in a pregnant heifer and flushing it with 20 ml 2% lactogen which was found rewarding (Bademkiran et. al., 2009). Human literature describes complete surgical resection of the cyst (Flint et. al., 2004) which was not followed in this case.

In conclusion, an extraluminal swelling in the rectum must also be differentiated from any peri-rectal cyst.

REFERENCES


Successful management of a third degree vagino-cervical prolapse along with torn vulval lips in a non-descript buffalo has been recorded.

**Keywords**: buffaloes, *Bubalus bubalis*, vagino-cervical prolapse, vulval lips, tear, replacement, non-descript buffalo

**INTRODUCTION**

The vagino-cervical prolapse usually involves the prolapse of the floor, lateral walls along with roof of the vagina through the vulva which moves the cervix and uterus caudally. It occurs usually during last 2-3 months of gestation when a large amount of estrogen is secreted from the placenta (Roberts, 1971; Arthur et al., 1996). The available records report that the early attention and treatment of vagino-cervical prolapse leads to prompt recovery with few complications. But in delayed or neglected cases due to the prolonged exposure of the prolapsed mass, the mucus membranes become contaminated and necrotic; the accumulation of urine, inflammation and edema increases the size of the mass to an irreducible size; and also due to the pain, straining and movement of the animal, multiple tears and lacerations are inevitable, which further aggravates the condition and it becomes a third degree vagino-cervical prolapse. Apart from this, the adjacent structures like vulval lips and perineal region are also affected. The present report records a management and successful treatment of such a third degree vagino-cervical prolapse and tear in vulvar lips in a non-descript buffalo.

**INTRODUCTION**

A non-descript 8-month-pregnant buffalo aged 4 years was brought to the Veterinary College and Research Institute Hospital Campus, Namakkal with the history of vagino-cervical prolapse since the previous evening. The animal was treated by a local veterinarian and referred to our Hospital. On external examination it was possible to visualize a third degree vagino-cervical prolapse with tears and lacerations on the ventral floor and lateral walls of the vagina (Figure 1). It was also observed that the vulvar lips had torn on the inner side with severe lacerations and necrosis. The cervical plug was intact. The animal was showing severe and continuous straining.

**CASE HISTORY AND CLINICAL OBSERVATION**

A non-descript 8-month-pregnant buffalo aged 4 years was brought to the Veterinary College and Research Institute Hospital Campus, Namakkal with the history of vagino-cervical prolapse since the previous evening. The animal was treated by a local veterinarian and referred to our Hospital. On external examination it was possible to visualize a third degree vagino-cervical prolapse with tears and lacerations on the ventral floor and lateral walls of the vagina (Figure 1). It was also observed that the vulvar lips had torn on the inner side with severe lacerations and necrosis. The cervical plug was intact. The animal was showing severe and continuous straining.
Figure 1. Vagino-cervical prolapse with multiple tears and lacerations.

Figure 2. Scarification of necrotic tissue in the ruptured vaginal wall before suturing.

Figure 3. Vaginal tear after suturing with catgut.
TREATMENT AND DISCUSSION

The animal was given epidural anaesthesia (2% Lignocaine, 5 ml). The prolapsed mass was cleaned with normal saline and with 10 liters of 0.1% potassium permanganate solution. The urinary bladder was emptied by lifting the mass and with the use of urinary catheter. After the removal of urine, the size of the prolapsed mass was greatly reduced. The tear in the vaginal wall was scarified with a BP blade (Figure 2) and the edges were sutured with No.2 chromic catgut (simple continuous suture) (Figure 3). The mass was again washed with normal saline mixed with povidone iodine and lubricated with cetrimide cream. The prolapse was reduced by manual pushing and replaced to its original position. The tear and lacerations found in the vulval lips were repaired with No.2 chromic catgut using a simple continuous suture. Since the animal was showing continuous straining, the vulval retention suture was applied with a Gerlach needle. The animal was administered inj 5% dextrose normal saline (3 lit., I/V), inj streptopenicillin (5 gm, I/M), inj chlorpheniramine maleate (225 mg, I/M), inj meloxicam (150 mg, I/M) and inj vitamin B-complex (10 ml, I/M) and the same treatment was continued for 3 days. The straining was reduced and the feed intake became normal after the continuous treatment. The animal was discharged from the hospital after 3 days. The buffalo delivered a live male calf after 2 months.

Except in extremely severe cases of vagino-cervical prolapse, the prognosis is fair to good for both the dam and fetus but always there is a chance for recurrence during next pregnancy unless proper preventive measures are implemented (Arthur et al., 1996). In severe cases, the greatly distended bladder, increased abdominal pressure along with excessive relaxation of pelvic ligaments and vaginal muscles are the major limiting factors in replacement (Qazi Mudasir et al., 2009). In the present case, the vaginal floor and lateral walls of the vagina were severely damaged along with the tears, lacerations and necrosis in the vulvar lips. The vagina was minimally handled during the treatment and reunion was done appropriately with catgut which aided in early recovery and normal calving.

REFERENCES


AN UNUSUAL CASE OF A HUGE ABSCESS IN A BUFFALO BULL (*Bubalus bubalis*)

K.H. Hussein

**ABSTRACT**

A buffalo bull was presented to the college hospital with the complaint of the presence of a huge swelling. The animal had been infected with edematous skin disease for three months. The abscess was diagnosed as the presence of large swelling, exploratory puncture, radiographically and by ultrasoundography. This huge abscess was removed by surgical excision under the effect of sedation and local analgesia. No postoperative complications were observed during a four-month follow up period. The abscess was successfully removed by surgical excision, which resulted in better aethesis and an increase in the marketability of the animal.

**Keywords:** water buffalo, buffalo bull, *Bubalus bubalis*, abscess, edematous skin, corynebacterium

**INTRODUCTION**

An abscess is a localized suppurative inflammation limited by a wall of granulation tissue (Sasty and Rama, 2004). One of the most important causes of abscesses in Egyptian buffaloes is the lymphatic borne infection. Oedematous skin disease (OSD) is a widely distributed in Egyptian buffaloes and became endemic in Egypt (Selim, 2001). OSD is caused by *Corynebacterium pseudotuberculosis* and characterized by appearance of circumscribed nodules which gradually develop into closed abscesses containing bloody up to pus tinged with blood or pure creamy pus along the course of lymphatic vessels of the forelimbs, abdomen, and thigh (Sayed *et al*, 2007). In this paper, a huge abscess at an atypical site, i.e. lateral to the mandible of a buffalo bull, and its and successful treatment by surgical excision have been reported.

**HISTORY AND CLINICAL SIGNS**

A 1-year-old, male buffalo bull was admitted to the veterinary teaching hospital, Assiut University, for presence of a large swelling the size of a large water melon lateral to the right branch of mandible and decrease of appetite. The swelling was reported to have been present for more than three months. At this time, the limbs were edematous and the temperature was 40.5°C. The animal was treated by a local veterinarian using high dose of penicillin and streptomycin in addition to hydrocortisone, which led to disappearance of the edema from the limbs but the abscess continued to develop.

On admission the buffalo bull appeared in bad body condition with normal clinical parameters and the presence of a large, hot, painful,
fluctuating and sharply circumscribed swelling the size of large melon lateral to the right mandible branch (Figures 1 and 2). Aspiration revealed the presence of bloody pus with offensive odor (Figure 3). Ultrasonographic examination revealed the presence of hypoechoic fluid with hyperechoic dots in addition to a thick capsule (1.6-2 cm thickness) (Figure 4). Radiographic examination revealed the presence of radiopaque content (Figure 5). A sample of the aspirated exudate was sent to the lab for detection of the causative agent.

**TREATMENT AND DISCUSSION**

The abscess was removed by surgical excision under the effect of xylazine HCl 0.1 mg /Kg Bwt and local analgesia by lidocaine HCl 2%. The bull was in recumbent position and the surgical area was prepared aseptically. An elliptical incision was made around the base of the abscess, the subcutaneous tissue and fascia were carefully dissected bluntly. The larger blood vessels were double ligated and severed while hemostasis of the small blood vessels was done by pressure or crushing using mosquito artery forceps. Then the abscess was removed and the subcutaneous tissue was sutured with 3-metric catgut in a simple continuous suture pattern. The skin was closed with silk in a simple interrupted suture pattern.

Postoperatively, ciprofloxacin and hydrocortisone were administrated. The skin sutures were removed after 10 days. The physical examination of the bull on the 10th and 30th days postoperatively revealed a healthy animal without any postoperative complications. The surgical correction resulted in a return to normal food intake and increase of body weight. Long-term follow-up obtained four months after surgery revealed a sound bull with good cosmetic appearance.

The weight of the abscess after removal was 5 kg while the volume of the bloody pus was 4100 ml (Figures 6 and 7).

The diameter of the wall was 1.5 cm on average (Figure 8). Laboratory diagnosis indicated that *Corynebacterium pseudotuberculosis* was the causative agent.

Buffaloes are more highly susceptible than cows for OSD, and the disease is more common in the age group of 8 months up to 3 years. Insects play a major role in transmission of the disease, so the disease is more prevalent during summer months in Egypt. The disease was diagnosed for first time in April 1960 (El-Sawalhy, 1999).

In the present case, recurrence was not reported. Total extirpation is possible if the abscess is accessible with a well developed capsule and no surrounding cellulites (Flower, 1998). It can be stated that the ultrasonographic examination offers an excellent method for measurement of the abscess capsule and the relationship with the surrounding tissues, which help the surgeon in detection of the preferred treatment technique.

**REFERENCES**


*Continued on page 188*
ABSTRACT

A bladder worm of the tapeworm *Echinococcus granulosus* is called a hydatid cyst. The adult is found in the dog, fox, wolf and other carnivores is characterized by having only 4 proglottids, and is 1.0 cm in length. For the present study, the livers of a total number of 510 buffaloes brought to the Cantonment Board Slaughter House, Mhow (M.P) for slaughter were examined for hydatidosis. The present study indicated that out of the total buffaloes examined, only six animals showed hydatidosis in the liver, which constituted about 1.19% incidence in the total examined animals.

Keywords: buffaloes, *Bubalus bubalis*, *Echinococcus granulosus*, hydatidosis, liver

INTRODUCTION

The buffalo is the predominant domestic animal for milk and meat production. On average, buffaloes are about four times as productive as indigenous cows in India. India has the world’s best dairy buffalo breeds and provides superior buffalo germplasm to several countries of the world (Kaikini, 1992). In our country, there are 93.8 million buffaloes (Anon, 2000), which contribute to more than half of the total buffalo population (164.9 million) in the world. Recently, India has emerged as the largest milk producer in the world. In spite of the huge buffalo population, animal husbandry and dairy sectors do not provide greater percentage of total agricultural income as low productivity of buffaloes is considerably affected by liver diseases such as hydatidosis of liver. The present investigation was carried out to assess the health of the livers of buffaloes.

MATERIALS AND METHODS

The materials for the present study was obtained from buffaloes brought from the different parts of the Malwa region as the source of meat slaughtered at the Cantonment Board Slaughter House, Mhow (M.P). The affected livers of a total of 510 buffaloes ranging from 3 to 12 years of age were examined in-situ for gross abnormalities, if any. After this, these livers were collected, brought to the laboratory for a careful examination of pathoanatomical abnormalities, where ever present. The observations were recorded, and the affected livers were preserved in 10% formalin. After 48 to 72 h, formalin-preserved tissue was washed overnight in running tap water, dehydrated
in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax of 60-62°C melting point. Sections of 4-6 micrometer thickness were cut through a Spencer’s rotary microtome and stained with H & E as per the standard procedure recommended by Lillie (1954).

RESULTS AND DISCUSSION

Out of the affected livers of a total of 510 buffaloes, hydatidosis was observed in six cases.

Gross lesion:

The colour of the livers infected with *Echinococcus granulosus* was too dark as compared to the normal liver, with greater size and thickness. The edges of the liver lobes were more rounded than the normal liver. There were multiple cysts in the liver (Figure 1). In one case, eight cysts were present in the liver. These cysts were full of yellowish and greenish fluids and contained many scolices. In one liver, there was a hydatid cyst as large as a tennis ball. This cyst was whitish yellow in colour. When it is incised, it contained jelly-like fluid (Figure 2). The liver containing hydatid cysts was pale in colour.

Microscopic lesion:

Microscopically, within the laminated cyst wall, light eosinophilic material representing cystic fluid was seen. In few cases, the germinal layer and brood capsules containing scolices were seen lining the wall (Figure 3). Fibrous tissue proliferation around the cyst wall along with the infiltration predominantly mononuclear cells were the main changes noticed in these lesions. Peripherally connective tissue proliferation resulted in the formation of usually thick capsules around the cyst (Figure 4).
The occurrence of hydatidosis in the liver of buffaloes was noticed in six cases, which constituted about 1.19% incidence in the total examined animals. Similar findings were reported by Tavasoli (1996) with a 1.54% incidence rate. This finding was not much lower. Hassieb et al. (1995) reported a much lower incidence rate—only 0.16%. During the present study, such cases were mostly seen in abattoir animals having poor conditions, and therefore, malnutrition might have a significant role to play in hydatidosis. Husain et al. (1992) reported the highest incidence of hydatidosis ranging from 9.8%-34.88%.

**REFERENCES**


*Continued from page 185*


ABSTRACT

The effect of retention of fetal membranes following abortion (n=10) or parturition (n=12) on the serum progesterone profile at days 0, 30 and 45, and the occurrence of postpartum first estrus and fertile estrus was studied in relation to normal parturition (n=15). The mean serum progesterone concentrations on days 0, 30 or 45 did not reveal any significant differences among the groups. The values varied from 0.41 ± 0.18 to 0.80 ± 0.14 ng/ml on day 0 and the range was still narrow on days 30 and 45 postpartum between the groups. Among the followed up cases, the occurrence of postpartum first estrus in buffaloes with retained fetal membranes after parturition (RFM/AP) and after abortion (RFM/AA) was found to be longer as compared to buffaloes not retaining fetal membranes (control group). The appearance of fertile estrus postpartum in RFM/AA group was significantly (P<0.05) delayed in comparison with the control group, but the values of control and RFM/AP group did not differ significantly. The number of services per conception in RFM/AA group was found to be significantly (P<0.05) higher than that of control group; however, the values of control and RFM/AP groups did not differ significantly. It is concluded that the breeding efficiency of buffaloes decreases significantly following RFM. This calls for better nutritional management during advanced pregnancy and at parturition to reduce RFM and to maintain normal breeding efficiency of dairy bovines.

Keywords: buffaloes, retained fetal membranes, serum progesterone, breeding efficiency

INTRODUCTION

The productive and reproductive performance of the buffalo is negatively influenced by the calving related reproductive disorders, especially retention of fetal membranes (El-Wishy, 2007). Retention of placenta causes stress and trauma to the reproductive tract that results in significant reduction in milk yield in the ensuing lactation. This malady also causes reduction in the fertility due to endometritis, metritis and delayed uterine involution, which lead to a reduction in pregnancy rate, increases number of consultations per conception and consequently longer calving interval (Bella and Roberts, 2007). The higher occurrence of metritis after RFM has been identified as the main reason for reduced fertility of cows with RFM (Grohn and Rajala-Schultz, 2000). The incidence of retention of fetal membranes is increased by abortion, premature birth, dystocia, hypocalcemia, twin birth, high environmental...
temperature, senility, induction of parturition, placentitis and nutritional disturbances (Han and Kim, 2005). Information on the postpartum endocrinology in buffaloes is limited (Prakash et al., 2005), especially in buffaloes with calving disorders like retention of fetal membranes. Therefore, the present study was planned to evaluate serum progesterone concentration and the reproductive efficiency of Surti buffaloes calving without and with retention of fetal membranes (RFM), under field conditions.

**MATERIALS AND METHODS**

The study was conducted on 37 animals of farmers around Anand as well as of the University farm. The selected animals were divided into three groups:

- **Group1**: Buffaloes (n=12) retaining FM for more than 12 h after parturition (RFM-AP)
- **Group2**: Buffaloes (n=10) retaining FM for more than 12 h after abortion (RFM-AA)
- **Group3**: Buffaloes (n=15) which expelled FM within 12 h of parturition (Normal Control)

These animals were examined per-rectally at regular intervals of 15 days for the confirmation of cyclic changes in accordance with the owner’s history so as to know the occurrence of postpartum first estrus, fertile estrus (days) and the number of services per conception. AI/NS was performed only after 45 days of calving. Pregnancy was confirmed by rectal examination 45 days after breeding.

Blood samples were collected aseptically with sterile needle from the jugular vein on days 0, 30 and 45 of parturition/abortion. Samples were allowed to clot for 6 h and the serum samples separated out by decanting the vials/tubes were stored in deep freeze (-20°C) until analyzed for progesterone profile using standard RIA technique of Kubasic et al. (1984). The sensitivity of the assay was 0.1 ng/ml, and intra- and inter-assay coefficients of variation 5.4 and 9.1 percent, respectively. The data were analyzed using completely randomized design and critical difference test (Snedecor and Cochran, 1986).

**RESULTS AND DISCUSSION**

The analysis of number of services per conception required for the three groups of buffaloes followed up for 6 months postpartum revealed that it was significantly (P<0.05) higher in the RFM/AA group than the control group (2.33 vs 1.64); however the values of the control and RFM/AP groups (1.64 vs 1.88) did not differ significantly (Table 1). This was similar to the observations of Pandey et al. (1994) and Gaafar et al. (2010) in cows and of Nakhushi et al. (2006) and Khan et al. (2009) in buffaloes.

The intervals for the postpartum first estrus (days) in the three groups of buffaloes, viz. RFM/AP, RFM/AA and control (71.13±6.45, 76.50±7.45 and 58.91±5.50, respectively) did not vary statistically. The value of the control group was non-significantly lower than the RFM groups. These findings are in agreement with those of Martin et al. (1986); Sabry et al. (1997) in cows and of Suthar and Kavani (1992); Nakhushi et al. (2006); Khan et al. (2009) in buffaloes. The mean number of days required for the fertile estrus or conception in the RFM/AA group was significantly (P<0.05) higher than in the control group (147.83±16.55 vs. 93.82±12.12 days), but the values of the control and RFM/AP groups did not differ significantly (Table 1). Nakhushi et al. (2006) reported a similar increase in the fertile estrus interval for RFM
buffaloes.

The mean serum progesterone levels obtained on days 0, 30 and 45 were insignificantly lower in the control group than those in the RFM/AP and RFM/AA groups (Table 2). The effect of group, period or group × period interaction was found to be non-significant for this trait. Sabry et al. (1997), Kaczmarowski et al. (2006) and Amjad Ali et al. (2009) reported higher progesterone levels in cows and buffaloes with RFM. The progesterone level drops at normal parturition, which may explain the low values obtained in the control group. Higher progesterone levels on day 0 in the RFM/AA and RFM/AP (almost double) groups indicate that luteal function was disturbed prematurely and that probably estrogen levels were also low at this stage. Progesterone causes the uterine smooth muscles to be in relaxed state, which may be the reason for the retention of fetal membranes.

**CONCLUSION**

It is concluded that the breeding efficiency of buffalo decreases significantly following RFM though it did not influence the serum progesterone levels. This calls for better nutritional management at advanced pregnancy and at parturition to reduce RFM and to maintain normal breeding efficiency of dairy buffaloes.

**ACKNOWLEDGEMENT**

We gratefully acknowledge the help of the authorities of ARDA, Amul, Anand for providing the technical support through their field veterinarian in sampling and follow up of animals.

Table 1. Mean (±SE) number of services/conception and interval to first postpartum estrus and fertile estrus in Surti buffaloes with and without retention of fetal membranes (RFM).

<table>
<thead>
<tr>
<th>Group</th>
<th>RFM/AP (n=8)</th>
<th>RFM/AA (n=6)</th>
<th>Control (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Services/conception</td>
<td>1.88±0.19ab</td>
<td>2.33±0.22b</td>
<td>1.64±0.16a</td>
</tr>
<tr>
<td>First postpartum estrus</td>
<td>71.13±6.45</td>
<td>76.50±7.45</td>
<td>58.91±5.50</td>
</tr>
<tr>
<td>Fertile estrus</td>
<td>122.50±14.33ab</td>
<td>147.83±16.55b</td>
<td>93.82±12.12a</td>
</tr>
</tbody>
</table>

Means with different superscripts within the row vary significantly (P<0.05) from each other.

Table 2. Mean (±SE) serum progesterone levels (ng/ml) on different days postpartum in Surti buffaloes with and without retention of fetal membranes (RFM).

<table>
<thead>
<tr>
<th>Group</th>
<th>Days after parturition/abortion</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>RFM/AP (n=12)</td>
<td>0.80±0.14</td>
<td>0.53±0.13</td>
</tr>
<tr>
<td>RFM/AA (n=10)</td>
<td>0.55±0.15</td>
<td>0.52±0.14</td>
</tr>
<tr>
<td>Control (n=15)</td>
<td>0.41±0.12</td>
<td>0.59±0.12</td>
</tr>
<tr>
<td>Pooled (n=37)</td>
<td>0.58±0.08</td>
<td>0.55±0.07</td>
</tr>
</tbody>
</table>

Group, Period and group x period interaction were found to be non-significant.
REFERENCES


ABSTRACT

The present research was planned to study follicular development and initiation of post-partum ovarian activity ultrasonographically and per-rectally in Murrah buffaloes having retained placentas. A total of 36 buffaloes with retained placentas were divided into three groups of twelve. In the buffaloes from Groups 1 and 2, placentas were removed manually and Furea boluses were kept intra-uterine (3 boluses per day I/U for 3 consecutive days post-partum) while Inj. GnRH (5 ml) and Inj. PGF2 alpha (5 ml) were administered intramuscularly on day 14 post-partum to Groups 1 and 2, respectively. The buffaloes from Group 3 were kept as control. The follicular development and initiation of ovarian activity were monitored on days 14, 21 and 28 post-partum in the experimental buffaloes. Multiple small follicular activity and good follicular activity were not seen on day 14 post-partum. On day 21 post-partum, 17 (47.22%) ovaries showed small follicular development and five (13.88%) ovaries showed multiple follicular activity when observed ultrasonographically. On day 28 post-partum, 11 (30.55%) ovaries indicated good follicular development observed per-rectally while 13 (36.11%) ovaries indicated good follicular development when observed ultrasonographically. The post-partum ovarian activity was initiated in an average of 3.54 weeks in the GnRH treated buffaloes (Group 1) of 3.54 weeks in the PGF2α treated buffaloes (Group 2) and in 4 weeks in the control group.

Keywords: Murrah buffaloes, Bubalus bubalis, placenta, follicular development

INTRODUCTION

Growing human population, increasing urbanization and rising per capita income are predicted to double the demand for and supply of livestock products in developing countries over the next two decades (Delgado et al., 1999). Livestock production is growing faster than any other sub-sector and it is predicted that by 2020, livestock will produce more than half of the total global agricultural output in value terms. The buffalo occupies an important place in the livestock economy of Asia as well as of India. Buffaloes are valued for milk, meat and draught power. Hence the importance of buffaloes to the economy of this country by way of milk, meat and draught power is considerable and cannot be underestimated.

Retention of fetal membranes (RFM) is one of the common maladies during puerperium in buffaloes (Sane et al., 1982). The consequences of RFM are an increase in the calving to service interval, increase in the number of services per conception.
and consequently longer calving intervals (Halpren et al., 1985). The major significance of RFM is in the mediation of more severe conditions. Thus cows that suffered RFM were at a significantly higher risk for developing metritis (Bartlett et al., 1986) and even subsequent abortion in the following pregnancy (Grohn et al., 1990). It is estimated that milk production decreases by 8.8% during the first 5 days of lactation due to RFM (Deluyker et al., 1991), thereby causing considerable economic loss at the herd level (Laven and Peters, 1986). The impact of RFM on bovine fertility has been examined by several researchers (Martin et al., 1986). Magnitude of effect varies widely from study to study. Borsberry and Dobson (1989) had found that uncomplicated RFM cases can lead to an increase in calving interval. However, other studies have shown no direct effect of the RFM condition on fertility (Nakao et al., 1992). The deleterious effect on fertility usually associated with RFM is in the pathogenesis of metritis (Mellado and Reyes, 1994), which may be up to 19 times more likely than after a normal calving (Curtis et al., 1985). Infection of uterus invariably causes damage to the endometrial epithelium; thus, the uterus becomes unable to secrete luteolytic pattern of PGF₂α, and hence the corpus luteum is retained and self perpetuating infection results (Parkinson, 2001).

Retention of fetal membranes has adverse effects on reproduction (i.e. metritis, slower uterine involution and reduced conception rates) in cattle (Roberts, 1971). Clinical examination often show a close relation between an early start of follicular activity in the ovaries after parturition in an undisturbed puerperal period, while cows with retained fetal membranes have delays and problems with recommencement of normal ovarian activity (Halpern et al., 1985). GnRH injection in early post-partum period (on day 14) in cows with retained fetal membranes contributed to early involution, stimulated ovarian activity and increased conception rates (Mori et al., 1988).

Prostaglandin plays major role in the regulation of reproductive cyclicity (Singh and Madan, 1985). The reproductive cyclicity and its rhythm in terms of its reawakening during the early post-partum period has been linked to temporal changes of hormones, mainly prostaglandin (Perera et al., 1981). Lindell et al., (1980) reported that PG metabolites increased at the time of parturition and remained high for 8 to 16 days post-partum. So delay in involution of uterus was due to a short period of high prostaglandin F2 alpha metabolite release. Whereas, long duration of PGF₂α release resulted in a short period for completion of uterine involution (Lindell, 1981). It has also positive effect on uterine musculature tone (Lindell and Kindahl, 1983). So PGF₂α injection in early post-partum period (day 14) enhances the uterine involution and reproductive efficiency in normal calved buffaloes (Nazir et al., 1994) and also in retained placentas cows (McClary et al., 1989). Keeping this view the present study was planned to study follicular development and initiation of post-partum ovarian activity ultrasonographically and per-rectally on day in early post partum period in retained placentas Murrah buffaloes treated with GnRH and PGF₂α on day 14 post-partum.

**MATERIALS AND METHODS**

The present investigation was carried out using 36 post-partum Murrah buffaloes at M/S B.G. Chitale Dairy, Research and Development Farm, Bhilawadi in Sangli district, over a period of ten months.
**Housing and management**

The buffaloes were housed in a loose housing barn with four groups of twenty-four buffaloes. The buffaloes were kept indoors and there was no open paddock in the barn. Each lot had twenty-six resting places (1.2X 2m) on one side and a manure alley with Delta Master™ manure scraper (Delaval AB, Sweden) on the other hand side positioned towards the feed rack. The feed rack had twenty-six standing places (each 1.2 m wide) and was without any locking arrangement. Each lot had one automatic concentrate feeding station (AFS) and nine valve-controlled automatic water bowls. Concentrate feeding was done at milking and milking was controlled with the Alpro™ system (Delval AB, Sweden) where a central processor received the milking and concentrate feeding data of all the buffaloes in the herd. Manure scrapers were turned on twice in the morning and afternoon before milking, while the workers raked down the dung from the cubicles. A micro sprinkler was fitted above each buffalo’s standing place and was turned on between 12.00 to 14.00 h every day. Buffaloes also received a shower before being milked two times a day at 7.00 and 17.00 h in tandem parlor. Temperatures were recorded every fourth hour. Indoor and outdoor temperatures ranged between 19 and 36°C.

**Feeding**

The ordinary routine in the barn was to provide roughages three times a day for *ad libitum* feeding. The leftovers were marginal, and very little feed was left in the troughs before the morning cleaning. The roughages fed during the experiment consisted of fresh cut and chopped sugarcane, alfalfa, napier grass, green maize and jowar straw which were chopped and transported to the barn in tractor trolley and dispensed manually into the feed troughs. A pre-calculated quantity of concentrate mixture was fed to each buffalo based on milk yield, body weight and pregnancy status. Concentrate was fed through the automatic concentrate feeding station (AFS) in the barn. If the pre calculated amount was not consumed, the residual was transferred to the next feeding. Residual amounts at the end of a 24 h period were transferred to the next 24 h period. During milking, an in-parlor feeding (IPF) system supplied a fixed amount of concentrates. The buffaloes were provided mineral mixture according to their milk production and body weight.

**Health care of buffaloes**

All the buffaloes were appropriately vaccinated against foot and mouth disease and haemorrhagic septicemia. They were also tested annually to detect possibilities of brucellosis, Johne’s disease and tuberculosis and the positive reactors were suitably disposed of. The fecal samples and blood smears were also screened periodically for detection of parasitic infestations and protozoan parasites, respectively. As a routine, all buffaloes were dewormed biannually.

**Selection of buffaloes**

Total 36 Murrah buffaloes with second to seventh lactation were selected. These buffaloes were divided into three groups and following treatments were given.

**Group 1**

This group consisted of 12 Murrah buffaloes with retained placenta. The placentas were removed manually and Furea boluses were kept intra-uterine (3 boluses per day I/U for 3 consecutive day’s post-partum). All the buffaloes from this group were treated with Inj. GnRH 5 ml
intramuscularly on day 14 post-partum.

**Group 2**

This group consisted of 12 Murrah buffaloes with retained placenta. The placentas were removed manually and Furea boluses were kept intra-uterine (3 boluses per day I/U for 3 consecutive days post-partum). All the buffaloes from this group were treated with Inj. PGF2 alpha 5 ml intramuscularly on day 14 post-partum.

**Group 3**

This group consisted of 12 Murrah buffaloes with retained placenta. The placentas were removed manually and Furea boluses were kept intrauterine (3 boluses per day I/U for 3 consecutive days post-partum) (Control group for Groups 1 and 2). All the buffaloes from this group were untreated.

All the 36 buffaloes in the above groups were observed for ovarian activity on days 14, 21 and 28 post-partum per-rectally and ultrasonographically. The ovarian follicular development, corpus luteum development and regression were noted. The ovarian follicular activity noted per-rectally was classified as

A. SFD: Slight follicular development
B. GFD: Good follicular development
C. NPS: No palpable structure
D. F: Presence of follicle
E. CL: Presence of corpus luteum
F. RCL: Regressing corpus luteum
G. PCL: Persistent corpus luteum

The ultrasound imaging of ovaries in the 36 post-partum buffaloes revealed ovarian activity with respect to follicular growth right from the first day of examination (day 14 post-partum). The ovaries were characterized by growth and regression of several small (up to 5 mm) and medium sized (> 5 and < 10 mm in diameter) follicles until the detection of first post-partum dominant (≥ 10 mm)
and/or ovulatory follicle during the study period i.e. from day 14 to 28 post-partum.

Statistical analysis
The data pertaining to follicular development and initiation of post partum ovarian activity was analyzed following statistical methods described by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Follicular development
A total 424 observations on ovaries were carried out by per-rectally and ultrasonographically. The manual observations were compared with the ultrasonographical observations to study the comparative detectability of ovarian follicles by palpation per-rectum and by ultrasonography. The observations for the same are presented in Table 1.

On perusal of the table, it can be seen that three (8.33%) ovaries indicated small follicular development when observed per-rectally while nine (25%) ovaries showed small follicular development when observed ultrasonographically on day 14 post-partum. Multiple small follicular activity and good follicular activity were not seen on day 14 post-partum.

On day 21 post-partum, 14 (38.88%) ovaries indicated small follicular development when observed per-rectally while 17 (47.22%) ovaries showed small follicular development and five (13.88%) ovaries showed multiple follicular activity when observed ultrasonographically. Whereas, four (11.11%) ovaries indicated good follicular development when observed per-rectally while seven (19.44%) ovaries indicated good follicular development when observed ultrasonographically. On day 28 post-partum, 17 (47.22%) ovaries indicated small follicular development when observed per-rectally while 11 (30.55%) ovaries showed small follicular development and nine (25%) ovaries showed multiple follicular activity when observed ultrasonographically. Whereas, 11 (30.55%) ovaries indicated good follicular development when observed ultrasonographically.

Table 1. Ovarian activity in retained placenta buffaloes monitored by per-rectally and ultrasonographically.

| Groups | Follicular Development | Day 14 PP | | Day 21 PP | | Day 28 PP |
|--------|------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|        |                        | Per-rectally | Ultrasonographically | Per-rectally | Ultrasonographically | Per-rectally | Ultrasonographically |
| Group 1 | SFD                    | 2         | 3         | 6         | 6         | 7         | 4         |
|         | MSF                    | -         | 0         | -         | 2         | -         | 3         |
|         | GFD                    | 0         | 0         | 3         | 4         | 4         | 5         |
| Group 2 | SFD                    | 1         | 4         | 5         | 6         | 7         | 5         |
|         | MSF                    | -         | 0         | -         | 2         | -         | 3         |
|         | GFD                    | 0         | 0         | 1         | 3         | 4         | 4         |
| Group 3 | SFD                    | 0         | 2         | 3         | 5         | 3         | 2         |
|         | MSF                    | -         | 0         | -         | 1         | -         | 3         |
|         | GFD                    | 0         | 0         | 0         | 0         | 3         | 4         |

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development observed per-rectally while 13 (36.11%) ovaries indicated good follicular development when observed ultrasonographically.

**Initiation of post-partum ovarian activity**

Following parturition, there is an early resumption of sequential but transient FSH increase of 2-3 days duration in cows. The first increase results in the emergence of the first post-partum follicular wave and decline in FSH results in selection of dominant follicle. The ovulatory fate of this dominant follicle depends on LH pulse frequency. In the post-partum dairy cows, growth of 6 to 8 mm follicles begins within 7 to 10 days of calving and from this group of follicles; a single dominant follicle emerges and ovulates in 75-80 percent of cows between days 10 to 30 after calving. Cows ovulating after, but not generally before, days 20 post-partum are more likely to have a short cycle (8-13 days duration) where the first dominant follicle ovulates (Roche and Boland, 1991).

On perusal of table it is seen that the post-partum ovarian activity was initiated with average 3.54 weeks in GnRH treated buffaloes (Group 1) and 3.54 weeks in PGF$_2$$_\alpha$ treated buffaloes (Group 2). One buffalo each from these two groups did not shown initiation of follicular activity till day 28 post-partum. Whereas, in Group 3, (Control group) the post-partum ovarian activity was initiated in an average of 4 weeks. Eleven buffaloes from the control group did not show the initiation of ovarian activity up to 4 weeks post-partum. The results are non significant at P<0.05 and P<0.01. Though statistically there was no significant difference between the control group and the treatment groups, the numerical values indicated that the two treatment groups had beneficial effect on initiation of post-partum ovarian activity in buffaloes with retained placentas. The onset of ovarian activity during the post-partum period is a complex phenomenon, which constitutes the regression of pregnancy corpus luteum (CL), uterine involution and resumption of ovarian follicular activity. A delay in the resumption of post-partum ovarian activity represents an important factor contributing to a prolonged calving to conception interval in the suckled buffaloes. It is important for optimal reproductive efficiency in buffaloes that they calve at 12-13 month intervals. This is achieved only when the buffaloes conceive by 80 days after calving. Therefore it is desirable that post-partum ovarian activity is initiated as early as possible. In the present study, trans-rectal ultrasonography proved to be a better tool for studying initiation of post-partum ovarian activity.

**CONCLUSION**

It can be concluded that rectal palpation is a fairly good technique to diagnose good follicular activity or prominent follicular growth, but it is possible that slight follicular development or deeply

Table 2. Average week of initiation of post-partum ovarian activity in retained placenta buffaloes.

<table>
<thead>
<tr>
<th>Buffalo No.</th>
<th>Group 1 (GnRH)</th>
<th>Group 2 (PGF$<em>2$$</em>\alpha$)</th>
<th>Group 3 (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>3.54± 0.15</td>
<td>3.54±0.14</td>
<td>4* NS</td>
</tr>
</tbody>
</table>

NS at P<0.05

NS at P<0.01

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Figure 1. Follicular development in experimental buffaloes.
situated follicles could be missed. Thus, rectal palpation is a fairly good indicator of ovarian activity only for routine observation. But ultrasonography should be considered as a tool when daily/frequent monitoring of follicular activity is required. Thus ultrasonographical ovarian scanning should be considered especially as a research technique and for therapeutic purposes.

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ABSTRACT

Use of ultrasonography for early pregnancy diagnosis as well as studying various reproductive disorders in bovines is a recent development. Real-time ultrasonic transrectal scanning of the uterus appears to offer rapid, safe, accurate and practical method of early pregnancy diagnosis. The main principle of early pregnancy diagnosis is to detect open (non-pregnant) buffaloes as early as possible and rebreed the nonpregnant animals by adopting suitable treatment to maintain optimum calving interval. In the present study, it is observed that due to the confirmatory signs of pregnancy and non-pregnancy at 37 days of gestation by ultrasonography proves this technique to be highly accurate for early pregnancy diagnosis at this stage for field use with 100% accuracy.

Keywords: buffaloes, Bubalus bubalis, pregnancy, ultrasonography, diagnosis, uterus

INTRODUCTION

The use of real-time, B-mode diagnostic ultrasound has been increasing as an imaging modality in dealing with the bovine reproduction cycle and its concurrent disorders and pregnancy diagnosis. The reproductive performance and productive efficiency of the animals are directly related to each other. Diagnosis of pregnancy at an early stage is essential for effective reproductive management. Ultrasonography has been found to be the most reliable method for early pregnancy diagnosis in buffaloes among other methods of pregnancy diagnosis. In this method, presence of the conceptus and viability of embryo can be detected (Tiwari et al., 2002). This will not only helpful in ruling cases of early embryonic mortality but also for diagnosing different reproductive problems during scanning. This is essential to take appropriate remedial measures.

The purpose of early pregnancy diagnosis is to identify all “open animals”: so that these animals can be re inseminated and become pregnant as early as possible before they are designated as reproductive culs. This will helpful for achieving an optimum calving-to-conception interval of 85-110 days as well as an ideal calving interval of 14-16 months in buffaloes. Early pregnancy diagnosis will also help to improve the reproductive efficiency as well as to develop strategies for adopting suitable remedial measures for non-pregnant animals. Therefore, the present study was undertaken to assess the earlier day for detection of early pregnancy in buffaloes by ultrasonography.
MATERIALS AND METHODS

Housing and management of experimental buffaloes

Four groups of 24 buffaloes were housed in a loose housing barn. They were kept indoors, and there was no open paddock in the barn. Each lot had 26 resting places (1.2 X 2 m) on one side and a manure alley with Delta Master™ manure scraper (Delaval AB, Sweden) on the other hand side positioned towards the feed rack. The feed rack had 26 standing places (each 1.2 m wide) and was without any locking arrangement. Each lot had one automatic concentrate feeding station (AFS) and nine valve-controlled automatic water bowls. Concentrate feeding was done at milking, and milking was controlled with the Alpro™ system (Delval AB, Sweden) where a central processor received the milking and concentrate feeding data of all the buffaloes in the herd. Manure scrapers were turned on twice in the morning and afternoon before milking, while the workers raked down the dung from the cubicles. A micro sprinkler was fitted above each buffalo’s standing place and was turned on between 12.00 to 14.00 h every day. Buffaloes also received a shower before being milked twice a day, at 7.00 and 17.00 h in a tandem parlor. Temperatures were recorded every fourth hour. Indoor and outdoor temperature ranged between 19 and 36°C.

Feeding

The ordinary routine was to place rounphages for ad lib feeding in the barn three times a day. The leftovers were marginal, and very little feed was left in the troughs before the morning cleaning. The roughages fed during the experiment consisted of fresh, cut and chopped sugercane, alfalfa, napier grass, green maize and jowar straw, which were chopped and transported to the barn in tractor trolley and dispensed manually into the feed troughs. A pre-calculated quantity of concentrate mixture was fed to each buffalo based on milk yield, body weight and pregnancy status. Concentrate was fed through the automatic concentrate feeding station (AFS) in the barn. If the pre-calculated amount was not consumed, the residual was transferred to the next feeding. Residual amounts at the end of a 24 h period were transferred to the next 24 h period. During milking, an in-parlor feeding (IPF) system supplied a fixed amount of concentrates. The buffaloes were provided mineral mixture according to their milk production and body weight.

Modus oprendi of ultrasonography

Early pregnancy diagnosis was attempted in 72 buffaloes on days 21, 28 and 37 day post insemination. The machine (SD-900 Aloka Co. Ltd., Japan) with 7.5 MHz rectal transducer was used to scan the pregnant uterus. The buffalo was restrained in standing position in travis. The buffalo was backracked by evacuating the rectum. The contact jelly was applied over the transducer before insertion. After backracing, the transducer was positioned in close proximity to the dorsal surface of the uterine horn, ipsilateral to the ovary containing CL. After initial orientation, ipsilateral uterine horn was scanned on its dorsal and then on lateral surface for signs of pregnancy.

Positive diagnosis of pregnancy was based on the presence of a non-echogenic round area of varying size in the lumen of an echogenic uterine horn representing the fluid filled allantoic cavity termed the embryonic vesicle (Pieterse et al., 1990). The presence of an embryo within the
embryonic vesicle was confirmed by observing an echogenic (white) area with rhythmic pulsation representing heartbeats (Pierson and Ginther, 1984). Correct diagnosis in this study was defined as either (1) an animal diagnosed pregnant with ultrasound examination and subsequently confirmed pregnant during palpation per rectum on day 60 post-service, or (2) an animal diagnosed non-pregnant with ultrasound examination and subsequently confirmed non-pregnant during palpation per rectum or returned to estrus at a later date. An incorrect diagnosis was defined as either (1) an animal diagnosed pregnant with ultrasound examination and subsequently confirmed non-pregnant during palpation per rectum or returned to estrus at a later date, or (2) an animal diagnosed non-pregnant with ultrasound examination and subsequently confirmed pregnant during palpation per rectum.

Diagnostic accuracy was defined as the percentage of correct diagnoses out of the total number of ultrasound examinations. Sensitivity of the method was defined as the percentage of animals found pregnant by ultrasound scanning out of the total number of animals found pregnant by palpation per rectum. Specificity was defined as the percentage of non-pregnant animals diagnosed non-pregnant by ultrasound scanning and later confirmed non-pregnant by rectal palpation or returned to estrus at a later date. The positive predictive value was defined as the percentage of actual pregnant animals out of the total number of animals diagnosed pregnant through ultrasound scanning. The negative predictive value was defined as the percentage of actual non-pregnant animals out of the total number of animals diagnosed non-pregnant through ultrasound scanning (Badtram et al., 1991).

**Statistical analysis**

The data pertaining to uterine involution, ovarian activity, post-partum exhibition of oestrus, early pregnancy diagnosis, conception rate and blood biochemical profiles in the six different groups were suitably tabulated and analyzed following statistical methods described by Snedecor and Cochran (1989).

**RESULTS AND DISCUSSION**

**Early pregnancy diagnosis through ultrasound scanning on day 21 of gestation**

Seventy-two buffaloes were scanned by trans-rectal ultrasonography on day 21 after insemination. Out of these eight buffaloes were diagnosed as pregnant and 64 as non-pregnant. The buffaloes which were diagnosed pregnant showed thin elongated anechoic areas of 3-4 mm in length. These areas indicated areas of fluid accumulation and were found in the horn, ipsilateral to the corpus luteum. An embryonic vesicle could be noticed with hypoechoic endometrium indicating increased circulation. It was not possible to locate an embryo and heart beats in all cases. These observations regarding early pregnancy diagnosis corroborate Pawashe et al. (1994) and Rane et al. (2002) who observed the presence of the embryonic vesicle as early as day 19.0±1.69 and day 24, respectively in buffaloes. However, Glatzel et al. (2000) detected pregnancy as early as the 25th day after breeding in water buffalo heifers by detection of fluid in the apex of the uterine horn and the presence of CL.

**Early pregnancy diagnosis through ultrasound scanning on day 28 of gestation**

Seventy-two buffaloes were scanned on day 28 post insemination. Out of them, 14 were
diagnosed pregnant and 58 were diagnosed non pregnant. The buffaloes which were diagnosed pregnant showed expansion of the embryonic vesicle with a diameter of 6-8 mm. In 14 buffaloes, an embryo was detected in the embryonic vesicle and in four buffaloes heartbeats were observed. The most peculiar characteristic of the image during this stage of gestation was compartmentalization of the embryonic vesicle. These compartments were seen as hypoechoic images of the fluid sac interrupted in places by hyperechoic folds projecting in the hypoechoic lumen. This created a pseudo-ampullar image as observed in cattle by Kahn (1989). Typically two to three anechoic sections were visible through the chorionic vesicle. Similar observations regarding compartmentalization of the embryonic vesicle was reported in buffaloes by Bhosrekar and Hangare (2000). An embryo was noticed in one of these compartments in all the cases. The echogenicity of the embryo was little more intense than that of the neighbouring endometrium. The embryo was seen projecting from the wall into the anechoic uterine lumen. A flickering echo of the heartbeats confirmed the presence of live embryo. A few millimeters away from the embryo a very thin, arched, hyperechoic amniotic vesicle surrounded the embryo was observed in all pregnant buffaloes. Limb buds were also clearly seen. A total 72 buffaloes were scanned during this stage. Out of them, 20 were diagnosed as pregnant. In all these buffaloes, embryos along with heartbeats were observed. These results were further confirmed with per-rectal examination at 60 days after insemination. The observations regarding presence of amnion and limb buds corroborate those reported by Pawshe et al. (1994); Rane et al. (2002); Mali (2006) in buffaloes.

**Early pregnancy diagnosis through ultrasound scanning on day 37 of gestation**

This period is most suitable for the confirmation of early pregnancy as the vesicle is further expanded and compartmentalization was observed. The compartments were seen as hypoechoic images of the fluid sac interrupted in places by hyperechoic folds projecting in the hyperechoic lumen. A flicking echo of the heartbeats confirmed the presence of live embryo. A few millimeters away from the embryo a very thin, arched, hyperechoic amniotic vesicle surrounded the embryo was observed in all pregnant buffaloes. Limb buds were also clearly seen. A total 72 buffaloes were scanned during this stage. Out of them, 20 were diagnosed as pregnant. In all these buffaloes, embryos along with heartbeats were observed. These results were further confirmed with per-rectal examination at 60 days after insemination. The observations regarding presence of amnion and limb buds corroborate those reported by Pawshe et al. (1994); Rane et al. (2002); Mali (2006) in buffaloes.

**Accuracy of early pregnancy diagnosis through ultrasound scanning**

In the scanning the buffaloes which were confirmed pregnant ultrasonographically as well as by rectal palpation were classified as “diagnosis pregnant correct” (a). The buffaloes diagnosed pregnant ultrasonographically but confirmed non-pregnant by rectal palpation were classified as “diagnosis pregnant incorrect” (b). The buffaloes confirmed non-pregnant ultrasonographically as well as by rectal palpation were classified “diagnosis non pregnant correct” (c). The buffaloes diagnosed non-pregnant ultrasonographically but confirmed pregnant by rectal palpation were classified as “diagnosis non-pregnant incorrect”
(d). These observations were used to calculate sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of pregnancy.

A total of 72 buffaloes were scanned for early pregnancy diagnosis from 21 to 37 days of gestation. This period was divided in three stages (day 21, day 28 and day 37) depending upon the stage of gestation. These buffaloes scanned in the respective sage of gestation by ultrasonography and confirmed with rectal palpation at 60 days after insemination. The data regarding these observations for the respective stage of gestation are represented in Table 1.

The above table reveals that the overall percentage of accuracy of early pregnancy diagnosis was 85.71%, 92.30% and 100% on days 21, day 28 and day 37 of gestation in buffaloes. The data regarding sensitivity, specificity, positive predictive value, negative predictive value and percent accuracy for the respective stage of gestation are presented in Table 2.

Although the specificity was 100 percent in all the three stages (21, 28 and 37) of ultrasound scanning, the sensitivity was lower on day 21 than on day 28, being 40 and 70 per cent, respectively.

Although positive predictive value of early pregnancy was 100 percent in all three stages (21, 28 and 37 day) of ultrasound scanning, the negative predictive value of early pregnancy diagnosis was lower on day 21 than on day 28, being 84.21 and 90.62 percent, respectively. Whereas, sensitivity, specificity, positive and negative predictive value were 100 percent on day 37 of gestation. Awasthi

Table 1. Accuracy of early pregnancy diagnosis in different stages of gestation.

<table>
<thead>
<tr>
<th>Stage of gestation</th>
<th>No. of scans</th>
<th>USG P</th>
<th>USG NP</th>
<th>Rectal P</th>
<th>Rectal NP</th>
<th>Overall accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>On day 21</td>
<td>72</td>
<td>8</td>
<td>64</td>
<td>20</td>
<td>52</td>
<td>85.71%</td>
</tr>
<tr>
<td>On day 28</td>
<td>72</td>
<td>14</td>
<td>58</td>
<td>20</td>
<td>52</td>
<td>92.30%</td>
</tr>
<tr>
<td>On day 37</td>
<td>72</td>
<td>20</td>
<td>52</td>
<td>20</td>
<td>52</td>
<td>100%</td>
</tr>
</tbody>
</table>

P- Pregnant, NP- Non-pregnant

Table 2. Overall accuracy percentage of early pregnancy diagnosis by ultrasonography.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>On day 21</th>
<th>On day 28</th>
<th>On day 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis pregnant correct (a)</td>
<td>8</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Diagnosis pregnant incorrect (b)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diagnosis non-pregnant correct (c)</td>
<td>64</td>
<td>58</td>
<td>52</td>
</tr>
<tr>
<td>Diagnosis non-pregnant incorrect (d)</td>
<td>12</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity (100x a/(a+d))</td>
<td>40</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (100 c/(c+b))</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value (100x a/(a+b))</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value (100x c/(c+d))</td>
<td>84.21</td>
<td>90.62</td>
<td>100</td>
</tr>
<tr>
<td>Overall accuracy percentage</td>
<td>85.71</td>
<td>92.30</td>
<td>100</td>
</tr>
</tbody>
</table>
(2004) who observed 87.5 percent specificity and 93.33 percent positive predictive value which were lower than results of present study whereas the negative predictive was slightly higher (95.45 per cent) than results of present study on day 28 of gestation in Surti buffaloes. Mali (2006) reported 100 percent specificity, positive predictive value and overall accuracy, which were higher than the present study, below 25 days of gestation in buffaloes. The variation in the findings depends upon a variety of factors including type of ultrasound used (sector or linear), frequency of transducer selected, age and parity animals, stage of gestation at which examination was carried out and the operator’s skill.

CONCLUSION

Strictly adhering to the principles of calculation, in the present study, it can be emphatically said that the confirmatory signs

Figure 1. Initiation of compartmentalization and embryo with vesicle.
of pregnancy and non-pregnancy at 37 days of gestation by ultrasonography proves this technique to be highly accurate for early pregnancy diagnosis at this stage for field use with 100% accuracy.

REFERENCES


Efficacy of different harvesting techniques on oocyte retrieval from buffalo ovaries

M. Mutha Rao* and Y. Uma Mahesh

ABSTRACT

A study was undertaken to assess the comparative efficacy of three harvesting techniques viz., the aspiration, puncture and slicing methods on oocyte recovery in buffalo ovaries obtained from a local abattoir. Effect of ovarian status (luteal phase vs. non-luteal phase), ovarian volume (<1.5 cm³ vs. >1.5 cm³), side of ovary (right vs. left) and number of visible follicles (No follicles vs. 1-5 follicles vs. 6-10 follicles) on oocyte yield and quality were also analyzed. Among the three collection methods, the slicing technique yielded the highest number of total and good quality oocytes, respectively (7.98±0.70 and 3.23±0.30) followed by the puncture (3.46±0.31 and 1.25±0.17) and the aspiration methods (2.38±0.19 and 0.84±0.10). Irrespective of the method employed, a little over 1/3 of recovered oocytes were of poor quality (32.46-34.34%). Luteal phase ovaries (having CL) yielded lower numbers of oocytes compared to non-luteal phase (no CL) ovaries. Both left and right ovaries contributed equally to total as well as different quality grades of oocytes in all the methods. Ovarian volume non-significantly affected the oocyte yield, which was slightly higher in ovaries with a mean volume >1.5 cm³. The results indicate that slicing method is superior to the other two methods employed in this study to harvest a greater number of good quality as well as culture grade oocytes from buffalo ovaries.

Keywords: buffaloes, Bubalus bubalis, harvesting technique, oocyte recovery, visible follicles, ovarian volume

INTRODUCTION

Availability of large number of culture grade oocytes is an essential prerequisite to realise a greater number of pre implantation embryos in in-vitro embryo production programmes. With the slaughter of more than 2 million buffaloes annually in India (Agnihotri, 1992), the ovaries obtained from this source can provide the cheapest and the most abundant source of primary oocytes. However, the oocyte yield varies with the harvesting technique employed. Though follicle aspiration is a widely used method due to its speed of operation, the oocyte yield is compromised in this method. Hence, apart from this conventional method of follicle aspiration, other methods of oocyte retrieval like follicle dissection, follicle puncture and ovarian slicing have been attempted in many domestic species with equivocal results (Carolan et al., 1994; Pawshe et al., 1994; Das et al., 1996; Wani et al., 1999). The aim of the present study...
was to assess the comparative efficacy of follicular aspiration, follicle puncture and ovarian slicing on oocyte yield and to evaluate retrospectively the influence of various factors like ovarian status, ovarian volume, side of ovary and the number of visible follicles on the recovery of different quality grades of oocytes in slaughter derived buffalo (*Bubalus bubalis*) ovaries.

**MATERIALS AND METHODS**

Buffalo ovaries (n=144) collected from a local slaughter house were transported within two hours to the laboratory in a flask containing 0.9% normal saline at room temperature. In the laboratory, they were washed twice in sterile PBS with antibiotics to remove superfluous tissue, bursa and blood clots. The weight (g) of each ovary was measured on a precision electronic balance (monopan) and biometry (cm) was recorded using vernier calipers separately for length, width and thickness. The volume of the ovary was deduced by the formula length x width x thickness x 0.523 as per (Amir Lass and Peter Brindson, 1999). The number of follicles visible to the naked eyes were counted and recorded. The ovaries were then placed in oocyte collection medium (TCM 199, Sigma, USA plus gentamycin sulphate 50μg/ml and heparin 10 IU/ml). Each ovary was processed individually and oocytes were collected by one of the following methods.

i) Aspiration of visible follicles (2-6 mm diameter) on the ovaries using a 20 gauze needle attached to a 5 ml sterile disposable plastic syringe containing 2 ml oocyte collection medium (OCM). Aspirated follicular fluid was then transferred to a sterile 35 mm Petri dish.

ii) Puncture of whole ovarian surface by a sterile 18 gauze hypodermic needle while the ovary is held completely submerged in OCM in a 90 mm Petri dish.

iii) Slicing of ovaries - in this method a hemostat was attached to the base of the ovary to hold it firmly in place and 2-3 mm deep incisions were made across the whole ovarian surface using a sterile scalpel blade. The ovary was swirled vigorously in a beaker containing OCM. The medium was then placed in a 90 mm Petri dish.

The Petri dish was kept undisturbed for 5 minutes to allow the cumulus-oocyte-complexes (COC) to settle. The Petri dish was then examined twice by at least two persons under stereo zoom microscope and the oocytes were scanned. The scanned oocytes were separated into a 35 mm Petri dish for grading at 63x magnification and then assessed as good, fair and poor based on cumulus corona investment and the homogeneity of ooplasm (Chauhan et al., 1998). The number and quality of oocytes were recorded for each ovary. Good and fair quality oocytes were considered as culturable grade. Statistical analysis to compare the efficacy of different collection methods and to assess the affect of various factors on oocyte recovery was performed by student’s t-test and one way anova as per (Snedecor and Cochran, 1989).

**RESULTS AND DISCUSSION**

The mean oocyte recovery in the present study was 4.60±0.33 of which 1.77 ± 0.14 (38.46%), 1.28±0.10 (27.75%), 1.55±0.12 (33.79%) and 3.05±0.23 (66.21%) were of good, fair, poor and culture grade oocytes, respectively (Table 1). The total as well as culture grade oocytes recorded in this study were much higher than earlier findings (Madan et al., 1994; Jamil et al., 2008) in buffaloes.
Table 1. Effect of harvesting technique on the quantity and quality of oocytes recovered from buffalo ovaries.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Harvesting technique</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspiration</td>
<td>Puncture</td>
</tr>
<tr>
<td>No. of ovaries used</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>No. of oocytes recovered</td>
<td>114</td>
<td>166</td>
</tr>
<tr>
<td>Mean No. of oocytes</td>
<td>2.38±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.46±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean good oocytes</td>
<td>0.84±0.10&lt;sup&gt;a&lt;/sup&gt;(35.08)</td>
<td>1.25±0.17&lt;sup&gt;b&lt;/sup&gt;(36.14)</td>
</tr>
<tr>
<td>Mean fair oocytes</td>
<td>0.77±0.09&lt;sup&gt;a&lt;/sup&gt;(32.46)</td>
<td>1.02±0.11&lt;sup&gt;b&lt;/sup&gt;(29.52)</td>
</tr>
<tr>
<td>Mean poor oocytes</td>
<td>0.77±0.09&lt;sup&gt;a&lt;/sup&gt;(32.46)</td>
<td>1.19±0.14&lt;sup&gt;b&lt;/sup&gt;(34.34)</td>
</tr>
<tr>
<td>Mean culture grade oocytes</td>
<td>1.61±0.14&lt;sup&gt;a&lt;/sup&gt;(67.54)</td>
<td>2.27±0.22&lt;sup&gt;b&lt;/sup&gt;(65.66)</td>
</tr>
</tbody>
</table>

Means with different superscripts within a row vary significantly (P<0.01).
Figures in parentheses indicate percentage.

Table 2. Factors affecting oocyte recovery and quality in buffalo ovaries.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>No. of oocytes</th>
<th>Oocyte recovery (Mean ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>I) Ovarian status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL present</td>
<td>111</td>
<td>3.00±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL absent</td>
<td>552</td>
<td>5.16±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>II) Side of ovary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>318</td>
<td>4.42±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Left</td>
<td>345</td>
<td>4.79±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III) Ovarian volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 1.5 cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>424</td>
<td>4.61±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Greater than 1.5 cm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>239</td>
<td>4.60±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV) Visible Follicles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No follicles</td>
<td>46</td>
<td>2.88±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 - 5 follicles</td>
<td>452</td>
<td>4.07±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 - 10 follicles</td>
<td>112</td>
<td>9.71±1.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different superscripts within a column for an attribute differ significantly (P<0.01).
Figures in parentheses indicate percentage.
Among the three harvesting techniques, the slicing method appeared to be superior in terms of both total recovery and number of culture grade oocytes. This method yielded a significantly (P<0.01) higher number of oocytes (7.98±0.70) per ovary compared to the puncture (3.46±0.31) and aspiration methods (2.38±0.19). This higher oocyte yield in the slicing method was also reflected in a greater number of mean good, fair, poor quality and culture grade oocytes. Similar findings have been reported in buffaloes (Das et al., 1996; Jamil et al., 2008) and cattle (Carolan et al., 1994). Higher oocyte recovery in ovarian slicing may be due to their release from both surface follicles as well as from deeper cortex (Das et al., 1996). Our study also revealed higher oocyte yield in the puncture method compared to aspiration. While it may be difficult to aspirate oocytes from small and medium sized follicles before cumulus expansion (Ball et al., 1983) the extra pressure applied during puncture may release oocytes from these follicles.

Comparison of oocyte recovery rate in buffaloes with that of cows revealed that in the latter species as many as 11-15 oocytes per ovary were recovered by follicle aspiration (Hamano and Kuwayama, 1993; Carolan et al., 1994) and about 30-45 by various surface cutting techniques (Stringfellow, 1993; Carolan et al., 1994). In agreement with our results earlier workers also reported a considerably lower oocyte recovery rate from buffalo ovaries compared to those of cows (Zoheir et al., 2007; Amer et al., 2008).

Distribution of different quality grades indicates that approximately 1/3 oocytes each were of good, fair and poor quality in all the methods. On the other hand, Das et al. (1996) recorded only 11% good quality oocytes and ≥ 50% poor quality oocytes in bubaline ovaries. Age, season, nutritional status (body condition) and cyclicity of animals at the time of slaughter, size and functional status of follicles, method of oocyte retrieval etc. are some of the factors that might contribute to recorded variation in oocyte quality (Nandi et al., 2001; Zoheir et al., 2007; Amer et al., 2008).

Retrospective analysis of various factors that might influence oocyte recovery revealed that non-luteal phase ovaries yielded significantly higher number of oocytes compared to luteal phase ovaries (5.16±0.41 vs.3.00±0.34). Also a greater number of usable oocytes could be obtained from ovaries not bearing CL (Table 2). Similar findings were reported in buffaloes (Amer et al., 2008; Jamil et al., 2008) and cows (Moreno et al., 1993). Side of ovary and ovarian volume did not affect the oocyte yield in this study. Further it was observed that, ovaries having more than five surface follicles produced a greater number of total as well as usable oocytes than those having either no follicles or 1-5 follicles. However, only 11.81% ovaries employed in this study contained more than five follicles and thus it is unlikely that greater oocyte production would be from this group of ovaries in buffaloes.

The results demonstrate that collection of oocytes from non-luteal phase ovaries by the slicing technique produces a greater number of total as well as culture grade oocytes. The presence of a greater number of surface follicles would be a fringe benefit to ensure additional oocyte recovery from buffalo ovaries.

REFERENCES


ABSTRACT

The pharmacokinetics of flunixin after single intramuscular injection 2.2 mg/kg in six male buffalo calves was studied. Plasma flunixin concentration was analysed using a sensitive LC-MS/MS method with emtricitabine as internal standard. The limit of quantification for the method was 0.1 μg/ml. A set of eight calibrants ranging from 0.100 μg/ml to 19.996 μg/ml was used to plot a standard linear curve for quantifying flunixin with r² value 0.9964. Pharmacokinetic parameters were determined for each buffalo calf using non-compartmental analysis and were calculated using WinNonlin.software Analysis of plasma showed rapid absorption, extensive distribution and slow elimination of flunixin following intramuscular administration in buffalo calves. Mean peak plasma flunixin concentration of 7.00±1.82 μg/ml occurred at a mean time of 0.67±0.11 h.

Keywords: buffaloes, Bubalus bubalis, pharmacokinetics, intramuscular, flunixin, LC-MS/MS

INTRODUCTION

Flunixin is an nonsteroidal anti-inflammatory drug approved in 1977 for exclusive use in animals by the USFDA. It inhibits the synthesis of cyclooxygenase derived eicosanoid inflammatory mediators (McKellar et al., 1989, 1991; Robinson, 1989). Flunixin has anti-inflammatory, analgesic and antipyretic properties. It can be administered by various routes (intravenous, intramuscular, oral). These pharmacological and pharmaceutical features have resulted in its extensive use to treat a number of conditions in various animal species viz., mastitis in cows (Rantala et al., 2002); endotoxaemia in cows (Anderson et al., 1986) and mares (Daels et al., 1991); colic in horses (Boothe, 2001); arthritis, shock, ophthalmic and other inflammatory conditions in dog; pain, shock, trauma in birds (Sandhu and Rampal, 2006) Its pharmacokinetics has been studied in lactating cattle (Anderson et al., 1990), sheep (Cheng et al., 1998), camels (Oukessou, 1994.), goats (Konigsson et al., 2003), pigs (Zu-gong et al., 2007) and birds (Baertand, 2003). Flunixin has been used for over three decades in veterinary practice in Europe and America However, in India, it has been made available only recently (Flugesic, flunixin meglumine injection USP, Cipla Ltd, Indore, India) for veterinary clinical use and it is intended to be used in buffaloes. In view of unavailability of pharmacokinetic data in buffaloes the present study was planned.
MATERIALS AND METHODS

Animals
Six male buffalo calves (5-6 months old) weighing 90 to 115 kg included in the study were clinically examined before flunixin administration to assure their health and soundness. They were managed as per standard animal husbandry practices.

Drug administration
The buffalo calves were administered a single intramuscular dose of flunixin (Flugesic, flunixin meglumine injection USP, Cipla Ltd, Indore, India) into the neck muscles 2.2 mg/kg. The behaviour of animals immediately following drug administration indicated slight irritation. However, no tissue reaction was observed on subsequent clinical examination.

Blood sampling
Blood samples were collected aseptically from the jugular vein at 0, 0.16, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24 and 36 h into heparinised tubes. At each time point approximately 5 ml blood was collected and centrifuged at 3000 g for 15 minutes. The plasma was stored at -20°C and transported on dry ice to the analytical laboratory.

Analytical assay
Flunixin was analysed using an LC-MS/MS (HPLC by Agilent of 1200 series. MS/MS by MDS SCIEX with model no. API 3200. Column: Zorbax SB-C8, 50 × 4.6 mm, 5 μm by Agilent). For extraction of flunixin, 10 μl emtricitabine (IS) (Batch no. AECBVSP10010110; Macleods Pharmaceuticals Ltd, India) was added as an internal standard to 200 μl of buffalo calf plasma aliquot and vortexed for 10 seconds. To this, 1.5 ml of acetonitrile was added followed by vortexing for 2 minutes and centrifugation at 5000 g for 10 minutes. The supernatant was injected into LC-MS/MS. The limit of quantification for the method was 0.1 μg/ml. A set of eight calibrants ranging from 0.100 μg/ml to 19.996 μg/ml was used to plot standard linear curve for quantifying flunixin with r² value 0.9964. Accuracy and precision at the lower limit of quantification, lower quality control, middle quality control, higher quality control and upper limit of quantification were 86.60 and 7.50, 109.22 and 7.62, 103.44 and 6.49, 94.72 and 2.16, 105.60 and 6.78 %, respectively. For system suitability % coefficient of variation (%CV) of area ratio of flunixin/emtricitabine was 4.06%.

Pharmacokinetic calculations
Pharmacokinetic parameters were determined for each buffalo calf (Table 1) using non-compartmental analysis and were calculated using WinNonlin software. Maximum concentration (C_max) and time at which it occurred (T_max) were read directly from the data. Various pharmacokinetic parameters calculated were elimination half-life (t½ el); the area under curve (AUC) and, apparent overall first order elimination constant (K_oa), apparent volume of distribution during terminal phase (Vz) and total body clearance (Cl).

RESULTS AND DISCUSSION
Analysis of plasma showed rapid absorption (Figure 1) and slow elimination of flunixin following intramuscular administration in buffalo calves. Observed mean C_max of 7.00±1.82 μg/ml occurred at mean T_max 0.67±0.11 h. Mean plasma flunixin concentration declined below the
Figure 1. Mean plasma concentration of flunixin from buffalo calves (n = 6) following single intramuscular (2.2 mg/kg) administration.

Table 1. Pharmacokinetic parameters in plasma following single intramuscular administration of Flunixin 2.2 mg/kg bw.wt.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Animal</th>
<th>Animal</th>
<th>Animal</th>
<th>Animal</th>
<th>Animal</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>μg/ml</td>
<td>2.94</td>
<td>4.12</td>
<td>4.11</td>
<td>6.52</td>
<td>9.61</td>
<td>14.70</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>hr</td>
<td>0.50</td>
<td>1.00</td>
<td>1.00</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>hr</td>
<td>6.69</td>
<td>9.16</td>
<td>6.01</td>
<td>6.10</td>
<td>2.93</td>
<td>6.59</td>
</tr>
<tr>
<td>K&lt;sub&gt;e&lt;/sub&gt;</td>
<td>hr&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>0.10</td>
<td>0.08</td>
<td>0.12</td>
<td>0.11</td>
<td>0.24</td>
<td>0.11</td>
</tr>
<tr>
<td>AUC (0-t)</td>
<td>hr, μg/ml</td>
<td>15.33</td>
<td>14.88</td>
<td>22.84</td>
<td>45.68</td>
<td>41.00</td>
<td>53.99</td>
</tr>
<tr>
<td>AUC (0-∞)</td>
<td>hr, μg/ml</td>
<td>16.38</td>
<td>22.01</td>
<td>24.06</td>
<td>50.26</td>
<td>41.42</td>
<td>60.65</td>
</tr>
<tr>
<td>V&lt;sub&gt;z&lt;/sub&gt;</td>
<td>ml/kg</td>
<td>1295.21</td>
<td>1320.19</td>
<td>792.72</td>
<td>385.20</td>
<td>224.48</td>
<td>344.86</td>
</tr>
<tr>
<td>Cl</td>
<td>ml/hr/kg</td>
<td>134.27</td>
<td>99.94</td>
<td>91.94</td>
<td>43.77</td>
<td>43.77</td>
<td>36.27</td>
</tr>
</tbody>
</table>

SEM= Standard error of mean, C<sub>max</sub>=maximum concentration, T<sub>max</sub> = time for C<sub>max</sub>, T<sub>1/2</sub>= half life, AUC (0-t)=Area Under Curve up to last sampling, AUC (0-∞)=total area under curve from 0 to ∞, V<sub>z</sub>= Apparent volume of distribution, Cl= Total body clearance.
The apparent pharmacokinetic parameters of flunixin are shown in Table 1. There was highly individual variation in peak plasma flunixin concentrations. Individual buffalo calf plasma flunixin concentrations showed humps, which could be because of redistribution of flunixin from storage site. Enterohepatic circulation of flunixin has been demonstrated in cats (Horii et al., 2004). Mean elimination half-life of flunixin in buffalo calf was 6.25±0.81 h, which was less than cattle (8.12 h, Boothe, 2001) but more than horse (1.6-2.5 h, Semrad et al., 1985), dog (3.67 h, Boothe, 2001) and goat (2.6-7.1 h, Konigsson et al., 2003). The increase in the clinical use of flunixin following its recent launching in India is expected to augment documents on its clinical utility in buffalo in future.

ACKNOWLEDGMENTS

The authors thank Mayfair Clinical Education and Research, Thane for LC-MS/MS analysis of flunixin.

REFERENCES


ABSTRACT

The pharmacokinetic study of *Withania somnifera* (Ashwagandha) was investigated after single dose administration of 500 mg/kg, orally in six non-descript healthy buffalo calves. Estimation of concentration of *Withania somnifera* in plasma was carried out by microbiological assay technique (Agar gel diffusion technique) by using *E. coli* (ATCC 25922) as the test organism. Following a single oral dose of *Withania somnifera* in healthy buffalo calves, the mean peak plasma concentration at 0.75 h was 248.16±16.12 μg/ml and was detected up to 3 h with a mean plasma concentration of 6.55±0.12 μg/ml.

The mean therapeutic concentration (≥ 0.1 mg/ml) of *Withania somnifera* was maintained from 10 minutes to 3 h in plasma of healthy buffalo calves. The mean elimination half life (t₁/₂ β) of *Withania somnifera* was observed to be 0.92 ± 0.032 h. The mean value of area under curve in plasma (AUC) and area under first moment curve (AUMC) were found to be 181.44 ± 8.84 μg/ml.h and 246.26 ± 17.66 μg/ml.h². The total body clearance (Clβ) ranged from 2.26 to 3.09 L/kg/h with a mean value of 2.78 ± 0.12 L/kg/h.

**Keywords:** pharmacokinetics, *Withania somnifera*, Ashwagandha, buffalo calves

INTRODUCTION

WHO has predicated that microorganisms are becoming resistant to most antibiotics and by 2020 antibiotics (the so-called wonder drugs of the 20th Century) will lose their effectiveness and no longer be used to cure diseases in man and animals. Most antibiotics are bacteriostatic in nature and as such they do not kill the bacteria; rather, they suppress their growth and the bacteria have to be killed by the body’s defense mechanisms named the phagocytic system through macrophages. WHO has also advised its all member countries to explore and use traditional wisdom for the health management. Due to these facts (harmful chemical residues and antibiotic resistance) there is a race among healthcare personnel/scientists throughout the world to find suitable and sustainable methods of treating ailments. Now the attention of the international scientific forum has been diverted towards alternative therapies.

*Withania somnifera* (*W. somnifera*), commonly known as Ashwagandha or Indian ginseng, has been an important herb in indigenous medicinal systems (Ayurveda) for over 3000 years. Among all parts of this plant, the *W. somnifera* root is considered to be the most active for therapeutic purposes like strengthening the body and for helping to prevent disease. *W. somnifera* is used in several indigenous drug preparations for maintaining health.
as well as treatment of several disease conditions. Its main use is as an immunomodulator and as an antistressor. The roots of *W. somnifera* contain several alkaloids, withanolides, a few flavanoids and reducing sugars. *W. somnifera* contains number of phytoconstituents, withanolides as the major constituent. It is one of the most commonly used drugs as a natural antimicrobial agent (Jaffer and Jawad, 1998). *W. somnifera* commonly used Indian medicinal plant for antimicrobial activity since the ancient time. The antibacterial activity of *W. somnifera* is now approaching for evaluation of its therapeutic efficacy and valuable use as an antibacterial agent in the present study. Its therapeutic use should be based on the correlation between antibacterial activity and its concentration achieved in vivo. Among various factors that determine the variation in intensity and duration of pharmacological effects, dosage, route of administration and disease status of animal are of much importance.

The majority of the population, particularly those living in villages, depends largely on herbal medicine. Scientific data on a good number of medicinal plants investigated is well documented. However, only very few drugs of plant origin have reached clinical use and the National Formulary could not adopt even a dozen plant medicines. For this reason, a special effort is needed for development of herbal drugs having therapeutic utility.

There is no data available for the kinetic study of *W. somnifera* in any species of animal so far. Therefore, the study was conducted in expectation to enhance to a remarkable extent the use of *W. somnifera* judiciously in animal practices, and also to consider species variations due to differences in pharmacokinetics of antibacterial agents. Hence, the present study was undertaken, to investigate the pharmacokinetics of *W. somnifera* in healthy buffalo calves.

**MATERIALS AND METHODS**

Six clinically healthy male buffalo calves of non-descript breed between 6 to 8 months of age and 100-150 kg body weight were used. The experiment was approved by the institute ethical committee and the synopsis committee of Madhya Pradesh Pashu Chikitsa Vigyan Vishwavidyalaya, Jabalpur, Madhya Pradesh, India as a part of post graduate degree programme of the first author. These buffalo calves were housed in an animal shed and maintained on dry fodder and greens as well as routine grazing for at least 4-5 h a day. Clean drinking water was available ad libitum.

The roots of *W. somnifera* were obtained from the Department of Aromatic and Medicinal Plants, Agriculture College, J.N.K.V.V., Jabalpur. The roots of *W. somnifera* were shed dried and crushed in a mixer and grinder to prepare a fine powder. 100 g of *W. somnifera* powder was dissolved in 1 L of sterile triple distilled water for 24 h to make a cold aqueous extract of *W. somnifera*. The cold aqueous extract of *W. somnifera* was administered at a dose rate of 500 mg /kg body wt. orally by drenching tube in each of the six healthy buffalo calves. Before collection of blood, the sites around the jugular vein on either side of the neck of the animals were aseptically prepared. The sites were sterilized prior to each collection with rectified spirit. Blood samples (approx. 1 ml) were withdrawn from jugular vein into heparinized glass centrifuge tubes at 0, 10, 15, 20, 30, 45 minutes and 1, 1.5, 2, 2.5, 3, 4 and 6 h after administration of the drug. Plasma was separated by centrifugation at 3,000 r.p.m. for 15 minutes at
room temperature and kept at -4°C until analysis. For preparation of standards, normal plasma prior to drug administration was also collected.

Aqueous extract of *W. somnifera* was prepared as 100 g of *W. somnifera* powder with 1 L of sterile triple distilled water; after 24 h, this solution was used for preparation of stock solution of 100 mg/ml of *W. somnifera*. One millilitre of stock solution (100 mg/ml) was dissolved in 1 ml of triple distilled water under constant stirring to obtain 50 mg/ml and also 50 mg/ml was diluted in triple distilled water to make different strengths viz., 25, 12.50, 6.25, 3.13, 1.56 and 0.78 mg/ml in water. From each standard solution of *W. somnifera* in water, 50 μl was added to a centrifuge tube containing 450 μl of plasma collected prior to drug administration. This yielded *W. somnifera* standards of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 mg/ml in plasma. Blank plasma containing no *W. somnifera* was also prepared.

The test organism used for the microbiological assay technique (agar gel diffusion technique) of *W. somnifera* was *E. coli* (ATCC 25922). The organism was grown on the slant of culture tube containing nutrient agar slants at 37°C for overnight. Then it was stored under refrigeration. The organism was transferred weekly to fresh media to maintain its normal activity (Arora et al., 2004).

Pharmacokinetic analysis of *W. somnifera* after single oral administration was calculated from a semi-logarithmic scale as a plot of plasma drug concentration versus time curve. The log plasma drug concentration versus time profile showed a non-linear curve and hence, non-compartmental analysis was done through statistical moment approach as described by Singh (1999). The mean therapeutic concentration (≥ 0.1 mg/ml) of *W. somnifera* was maintained from 10 minutes to 3 h in plasma of healthy buffalo calves as stated by Arora et al. (2004) who reported the minimum inhibitory concentration (MIC) of *W. somnifera*, which came out to be 0.1 mg/ml for *S. typhimurium* and *E. coli*.

**RESULTS AND DISCUSSION**

Plasma concentrations of *W. somnifera* at various time intervals following a single oral dose of 500 mg/kg in healthy buffalo calves are shown in Figure 1.

The mean plasma concentration of the drug at 0.16 h was found to be 6.39 ± 0.11 mg/ml and the value ranged from 6.13 to 6.88 mg/ml. The drug was detectable in all six animals up to 3 h with the mean plasma concentration was 6.55 ± 0.12 mg/ml. The drug was not detectable in any of six animals after 4 h. The peak concentration of *W. somnifera* was found at 0.75 h with mean concentration of 248.16 ± 16.12 mg/ml as shown in the Figure. 1.

Plasma drug concentration versus time profile has shown non-linear curve. Hence, kinetic parameters were derived from the formula of non-compartmental analysis through statistical moment approach. The values of different kinetic parameters calculated by the above noted non-compartmental analysis. The elimination rate constant (β) ranged from 0.67 to 0.82 h⁻¹ with a mean value of 0.74 ± 0.025 h⁻¹. The mean elimination half life (t₁/₂ β) values of the drug were observed to be 0.92 ± 0.032 h as shown in Table 1.

There is no data available for the kinetic study of *W. somnifera* in any species of animal so far; therefore, the kinetic parameters calculated in the present study are discussed as follows: Plasma concentration of *W. somnifera versus* time disposition curves after oral administration
were best fit to non compartmental analysis in all six buffalo calves, which is in accordance with results reported for pharmacokinetics of oral administration of sulphur mustard decontaminant CC-2 in rats (Lal et al., 2003).

Following a single oral dose of *W. somnifera* in healthy buffalo calves, mean peak plasma concentration at 0.75 h was 248.16 ± 16.12 mg/ml and was detected up to 3 h with a mean plasma concentration of 6.55 ± 0.12 mg/ml.

Most of the kinetic parameters of *W. somnifera* (500 mg/kg, orally) as elimination rate constant (β) was calculated 0.74 ± 0.025 h⁻¹ which suggested that slightly faster rate of elimination of *W. somnifera* when administered orally. This is best supported by elimination half-life (t½ β) which were noted 0.92 ± 0.032 h in healthy buffalo calves.

The high values of AUC∞, AUMC∞ and MRT reflect that most of the body area is covered with the drug concentrations. The AUC∞ value was calculated 181.44 ± 8.84 mg/ml.h in healthy buffalo calves. Similar to this AUMC∞ value was 246.26 ± 17.66 mg/ml.h² and MRT values 1.34 ± 0.045 h were found. That clearly indicated that the maximum area covered by drug *W. somnifera* after

![Figure 1. Plasma concentrations of *W. somnifera* following a single oral dose of 500 mg/kg in healthy buffalo calves.](image)

<table>
<thead>
<tr>
<th>Parameter (Unit)</th>
<th>Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>β (h⁻¹)</td>
<td>0.74 ± 0.025</td>
</tr>
<tr>
<td>t½ β (h)</td>
<td>0.92 ± 0.032</td>
</tr>
<tr>
<td>AUC∞ (mg/ml.h)</td>
<td>181.44 ± 8.84</td>
</tr>
<tr>
<td>AUMC∞ (mg/ml.h²)</td>
<td>246.26 ± 17.66</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.34 ± 0.045</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>3.68 ± 0.12</td>
</tr>
<tr>
<td>ClB (L/kg/h)</td>
<td>2.78 ± 0.12</td>
</tr>
</tbody>
</table>
oral administration in the body of buffalo calves.

The relatively high value of Vdss (3.68 ± 0.12 L/kg) was observed in healthy buffalo calves. A large volume of distribution (>1 L/kg) indicates wide distribution throughout the body or extensive tissue binding or rapid excretion of a drug or combination of all the above. A high value of Vdss obtained in the present study showed the wide distribution of *W. somnifera* in the body of buffalo calves.

The total body clearance (Clb) value of *W. somnifera* in healthy buffalo calves was 2.78 ± 0.12 L/kg/h which showed slightly increased clearance from the body of buffalo calves, which is in accordance with results of Clb = 2.45 ± 0.21 L/kg/h after oral administration of sulphur mustard decontaminant CC-2 in rats (Lal et al., 2003).

Herbs are the backbone of therapeutic strategies. India, having huge wealth of plant biodiversity, holds excellent potential for herbal treatment. After evaluating the efficacy and pharmacokinetics of medicinal plants, extracts will be recommended for clinical trials in animals under controlled conditions. The ethano medicinal data on indigenous plant *W. somnifera* will serve as useful tool to pharmacologists and clinicians for development of herbal preparations of indigenous plants. Pharmacokinetics of *W. somnifera* will provide valuable clues to the clinician for its large scale use in future.

REFERENCES


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

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