ICAR PUBLICATIONS

D. K. M. A.

636 (05) CODEN : IJLAA4 **70** (10) : 999-1102 (2000) II ISSN 0367-8318

Dedicated to INDIAN SCIENCE CONGRESS

The Indian Journal of Animal Sciences

Indian Science Congress 2001

ICAR

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
NEW DELHI

10 *Indian J Anim Sci* Vol. **70** No. 10 pp. 999-1102 New Delhi October 2000

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THE INDIAN JOURNAL OF
ANIMAL SCIENCES
Previous Issue : Vol. 70, No. 9, pp. 893-998

Vol. 70, No. 10 October 2000

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Indian Journal of Animal Sciences **70** (10): 1001-1007, October 2000

Fractionation and immunoreactivity of *Mycobacterium bovis* AN5 sonicated antigens

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Received: 8 November 1999; Accepted: 7 April 2000

ABSTRACT

Mycobacterium bovis AN5 sonic extract was fractionated using get permeation and anion exchange chromatography. The immunoreactivity of fractionated antigens was evaluated using *M. bovis* AN5 serum in ELISA. Out of several fractionated regions obtained from the sonic extract, the more immunogenic regions were heterogenous polypetides of 68 and 71kDa and 79 and 81kDa and homogenous polypeptides of 56 and 48kDa molecular masses. These 4 fractionated antigens showed a greater reactive response to anti *M. bovis* serum in ELISA. Immunoblotting on one diamensional SDS PAGE separated sonic extract revealed 8 seroreactive bands of 81, 71, 65, 56, 28, 22, 18 and 14kDa. Most of the fractionated antigens showed cross reactivity with other *Mycobacterium* spp. in ELISA except 56kDa antigens which showed less cross reactivity in ELISA. It is concluded that these antigens might be possessing immunodominant epitopes for the humoral and cellular responses.

Key words: ELISA, *Mycobacterium bovis* AN5, Tuberculosis, Sonic extract

For highly specific antibody assay specific antigen would be needed to be developed due to extensive antigenic cross reactivity between mycobacterial species (Young *et al.* 1992, Gupta *et al.* 1994 a, b). Many antigens of *M. bovis* have been isolated and sequenced (Young *et al.* 1992). Apart from the well known cross-reactivity of the non-protein antigens it was found that many of the major protein antigens share homologies within the mycobacterial genera and with a wide number of other bacterial species. Use of a largely *M. bovis* specific protein (MPB70) in an ELISA for bovine tuberculosis has greatly improved the specificity of the assay (Fifis *et al.* 1994).

Purification and characterization individual antigens, and their testing for immunological reactivity (Roberts *et al.* 1972, Wright *et al.* 1972, Closs *et al.*, 1980, Gupta *et al.* 1994 a, b). The greatest part of work is difficult to relate to many other studies because of lack of a widely adopted cross-referencing system. Little is known about the protective antigens of mycobacteria as these have proved difficult to fractionate by conventional means. Polysaccharide antigens tend to induce strong humoral responses which may have taxonomic and diagnostic importance (Daniel 1989) but provide little or no protection to the host (Reggiardo and Middlebrook 1974). Protein antigens tend to induce cellular responses, some of them may be protective (Brennan 1989) while others may even be deleterious (Dennenberg and Tomashafsky 1988). Some of these proteins may be immunodominant, possessing epitopes which trigger strong T-Cell responses both *in-vitro* (Young *et al.* 1986, Lamb *et al.* 1989, Gupta *et al.* 1994).

Limited specificity and sensitivity are still major problem in the development of a serological test of bovine tuberculosis (Ivanyi *et al.* 1988), although purified protein from sonicated *Mycobacterium tuberculosis* and from culture medium have been used (Daniel and Debanne 1987). The present study was therefore undertaken to isolate antigen(s) from *M. bovis* AN5 sonicate which might prove to be more specific.

MATERIALS AND METHODS

Bacteria

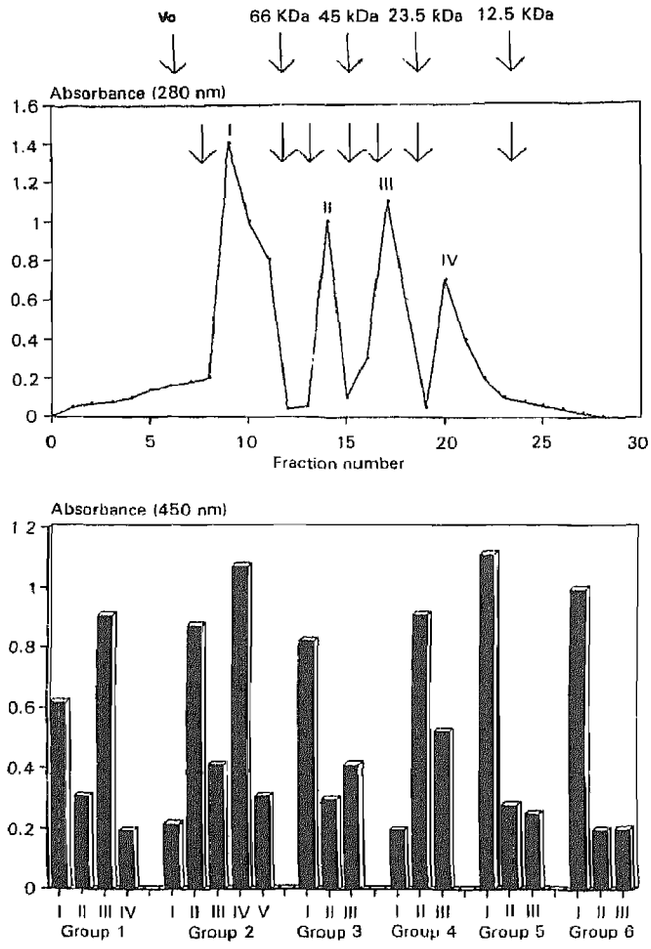
Mycobacterium bovis AN5 was obtained from the Biological Products Division, Indian Veterinary Research Institute, Izatnagar, India. *M. tuberculosis, M. simae, M. fortuitum, M. intracellulare, M. kansasii, M. phlei, M. smegmatis, M. xenopi* and *M. chelonei* were obtained from the Central Jalma Institute for Leprosy, Agra (India).

Culture of M. bovis *AN5*

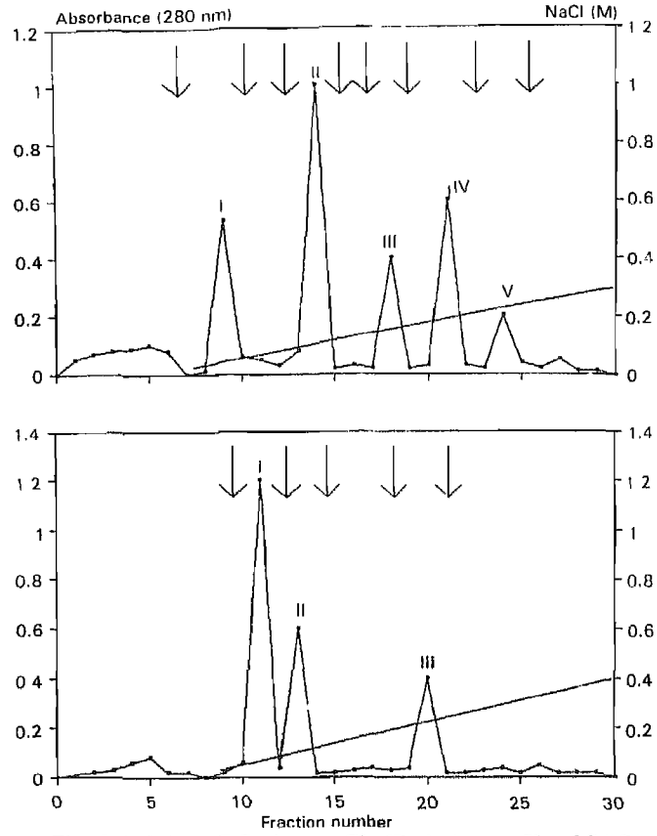
M. bovis AN5 were grown in Sauton's liquid medium composed 2 mM Mg_2SO_4, 10 mM citric acid, 2.5 mM K_2SO_4,

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[2]Senior Scientist and Incharge, Immunology Section.



Figs 1-2. **1.** Sephacryl S-200 elution profile of *M. bovis* AN5 sonicated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 7.5 containing 0.1M CaCl. **2.** Reactivity of different regions in ELISA (with *M. bovis* AN5 antiserum) obtained in Sephacryl S-200 and DEAE-Sepharose CL-6B elutions of *M. bovis* AN5 sonicated antigens.



Figs 3-4. **3.** DEAE-Sepharose CL-6B elution profile of SAG1 of *M. bovis* AN5 sonicated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.3M. **4.** DEAE-Sepharose CL-6B elution profile of SAG2 of *M. bovis* AN5 sonicated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM Tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.4M.

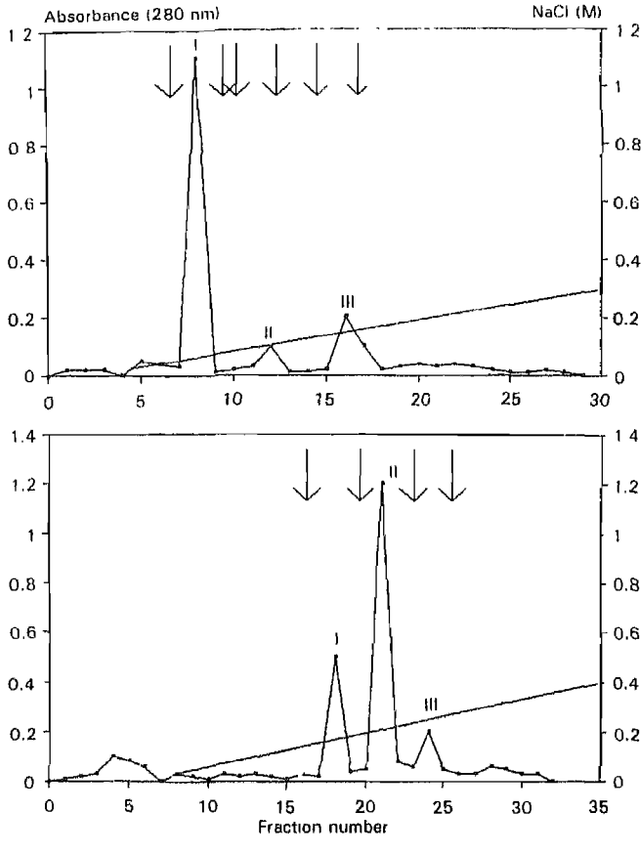
30 mM asparagine, 0.1 mM ferric ammonium citrate and 830 mM glycerol (final pH 7.4) in Hopkin's flask at 37°C. Bacteria were harvested in saline after 4 to 5 weeks when uniform confluent growth was obtained. Bacterias were killed by putting Hopkin's flask at 70°C for 2 hr in a water-bath. Residual activity of bacteria was checked by reinoculating the bacteria into the same medium.

Preparation of M. bovis *AN5 sonicated antigen*

Washed cells of *M. bovis* AN5 harvested in saline, were suspended to 250 mg wet weight/ml in 0.85% of saline and disrupted by disintegration in a MSE ultrasonicator using a titanium probe (12.7 mm diameter) operating at 10-15mm peak to peak. The sonication vessel was immersed in an ice water slurry and the contents were subjected to 15 min sonication at 30s intervals, to reduce heating. Sonicated antigenic extracts were centrifuged at 10000 rpm for 30 min at 4°C. The supernatent fluid was filter sterilized using 0.45mm filter and stored at –20°C. The protein content of sonicated soluble antigen was determined as per Lowry (1951) and found to be 12 mg/ml. Thiomersal (0.01%) was used as preservative.

Gel filtration chromatography

M. bovis AN5 sonicated antigens (SA) were fractionated by gel filtration on a 1.5 cm × 80 cm column bed of Sephacryl S-200 having a total volume (vt) of 138 ml. Void volume (Vo) of the column bed was 43 ml. The column was precaliberated with standard protein molecular weight markers viz-bovine serum albumin (MW=66,000 Da), egg albumin (MW=44,000 mw), chymotypsinogen A (MW=23,500 Da) and cytochrome C (MW=12,500 Da). Concentrated sonicated antigen (2ml) containing 100 mg total protein was eluted @ 20 ml / hour in 6 ml fraction each with 30 mM Tris buffer (pH 7.5) containing 0.1 M sodium chloride. Absorbance of elutes was monitored at 280 nm. Fraction thus

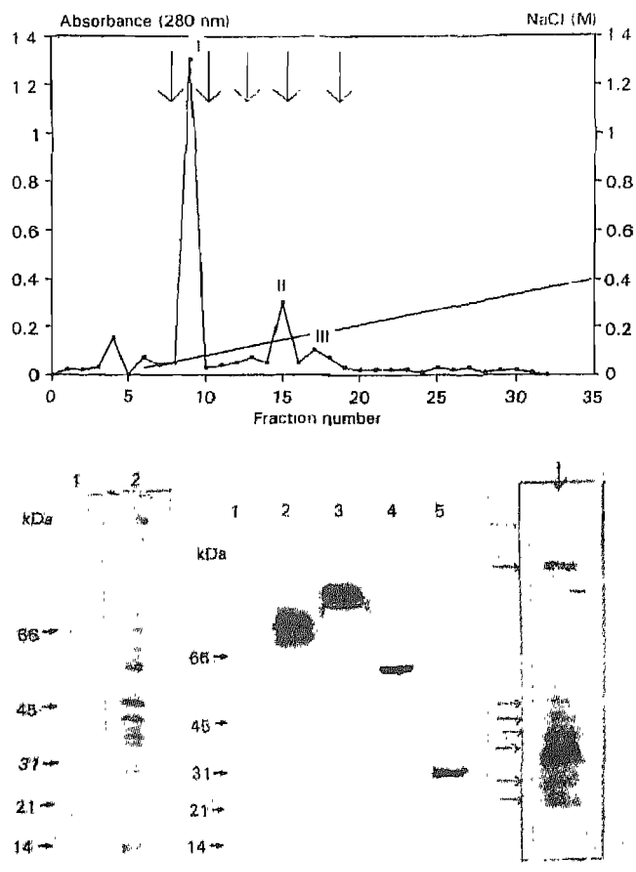


Figs 5-6. **5.** DEAE-Sepharose CL-6B elution profile of SAG3 of *M. bovis* AN5 sonicated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.3M. **6.** DEAE-Sepharose CL-6B elution profile of SAG2D1 of *M. bovis* AN5 sonicated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM Tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.4M.

obtained in each region according to optical density at 280 nm were pooled and were dialysed against distilled water at 4°C. All the pooled regions were concentrated by vacuum concentrator and filter sterilized through a membrane (0.22mm) and stored at -20°C.

Anion exchange chromatography

The sephacryl S-200 fractionated regions reacting intensely in ELISA were subjected to DEAE-Sepharose CL-6B chromatography. The proteins 5 mg to 20 mg of different regions dialysed against 30 mM Tris buffer were loaded on a column of DEAE-Sepharose CL-6B (Size 1.5 cm × 30 cm) and eluted with same buffer (pH 8.7) containing 3 M urea, using a linear gradient of sodium chloride from 0 to 0.3 M. Flow rate was 18 ml / hour and fractions of 5.5 ml were collected. Fractions under each region according to absorbence at 280 nm were pooled. Regions showing high seroreactivity in an ELISA were further rechromatographed

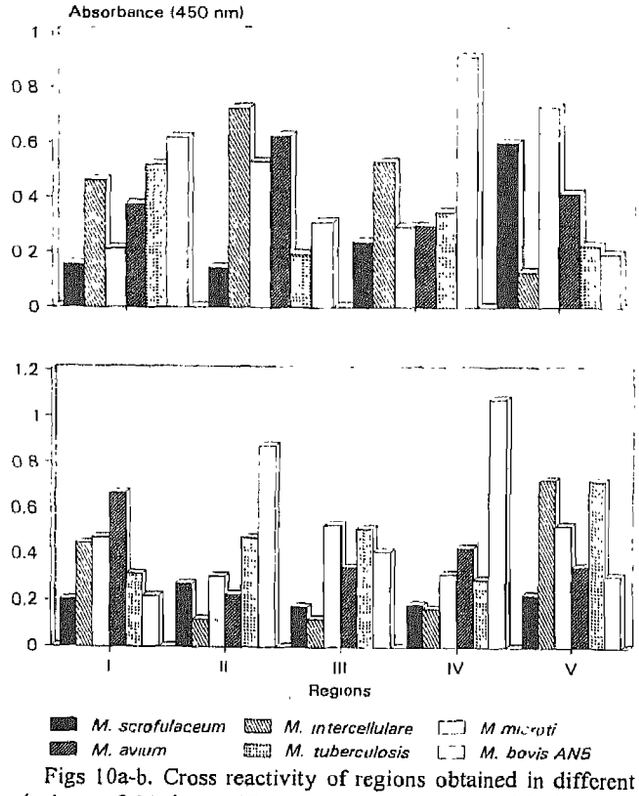


Figs 7-9. DEAE-Sepharose CL-6B elution profile of SAG3D2 of *M. bovis* AN5 sonicated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.4M. **8a.** Commasie brilliant blue stained SDS-PAGE (10% slab gel) showing polypetides of *M. bovis* AN5 sonicated antigens : Lane1-SDS-PAGE molecular weight markers; lane2- polypeptides of sonicated antigens. **8b.** Commasie brilliant blue stained SDS-PAGE (10% slab gel) showing polypeptides of *M. bovis* AN5 sonicated antigens : lane1-SDS-PAGE molecular weight markers; lane2-polypeptides of SAG1D4; lane3-polypeptides of SAG1D2; lane4-polypeptides of SAG2D1d1; lane5-polypeptides of SAG3D2d1. **9.** Western immunoblot analysis of *M. bovis* AN5 sonicated antigens with *M. bovis* AN5 antiserum. Figures against arrows indicate mw in kDa.

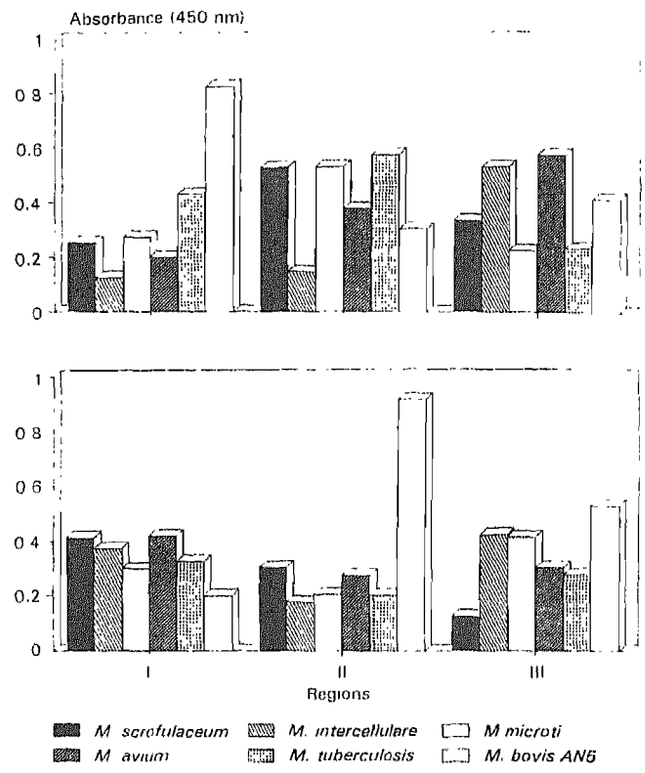
on the same column using 0.0 to 0.4M sodium chloride gradient. All the fractions were vacuum concentrated filter sterilized (0.22m m membrane), and stored at-20°C.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed using the method of Engvall and Pearlman (1971). Triplicate wells of 96 well flat bottom plates were coated with 50 ml antigen containing 20 mg / ml protein. All plates were blocked at 37°C for 3 hr using 150 ml of 3% (w/v) bovine serum albumin, pH 7.4 in each well and washed in PBS + Tween 20 (0.05%) once. Rabbit anti *M.bovis* AN5



Figs 10a-b. Cross reactivity of regions obtained in different elutions of *M. bovis* AN5 sonicated antigens with different mycobacterial antisera by ELISA: **10a**. Regions obtained in sephacryl S-200 elution; **10b.** Regions obtained in DEAE-sepharose CL-6B elution of SAG1.



Figs 10c-d. **10c.** Regions obtained in DEAE-sepharose CL-6B elution of SAG2; **10d.** Regions obtained in DEAE-sepharose CL-6B elution of SAG3.

serum was used as the source of first antibody and the plates were incubated at 37°C for 60 min. After washing thrice with PBS + Tween 20,100 µl of substrate solution (O-phenylene diamine dihydorchloride, freshly prepared) was added to each well and incubated for 30 min in dark. Reaction was stopped by adding 25 ml of 5N H_2SO_4 to each well. Absorbance was recorded at 450 nm by ELISA reader.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

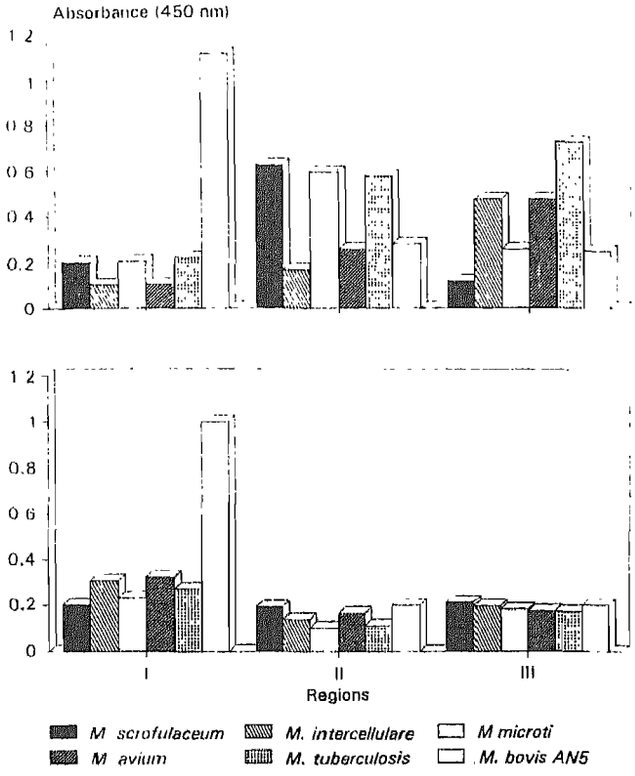
M. bovis AN5 sonicated antigen (SA) and their purified and partially purified fractions were analysed by SDS-PAGE (Laemmli 1970) with some modification (Hames 1981). A slab gel electrophoresis system was used with 10% polyacrylamide gel containing 0.1% SDS. The samples were diluted in loading buffer (11% of v/v glycerole, 1% of w/v SDS, 0.01% of w/v bromphenol blue, 26% of v/v 0.5 M Tris-HCl buffer, pH 6.8) to which 5% v/v betamercaptoethanol was added so that the quantity of protein was 20 µg / track for crude antigens while 10 µg / track for purified and partially purified antigens. The samples were heated at 100°C for 3 min before loading. Gels were run at 40 volts for 18 hr and then the protein bands stained using coomassie brilliant blue. Calculation for the determination of molecular weight was based on the distance the polypeptide migrated in the gel compared to polypeptide markers o known molecular weights.

Western immunoblotting

Sonicated antigen (SA) of *M. bovis* AN5 was separated on 10% (w/v) polyacrylamide slabs using gel electrophoresis under reducing conditions in the presence of SDS (Laemmli 1970). The separated antigens were then transferred electrophoretically to a nitrocellulose membrance (NCM) in tris/glycine/methanol buffer (Towbin *et al.* 1979) and the membrance was immersed overnight at room temperature in PBS containing 3% BSA and 0.05% of Tween-20. After thorough washing in washing buffer the membrane was incubated for 2 hr at 37°C in 30 ml of 1:20 diluted rabbit anti *M. bovis* AN5 serum and washed thoroughly. The membrane was incubated with 30 ml of 1:1 000 diluted goat antirabbit HRPO conjugate for 2 hr at 37°C. The NCM was washed like the previous step assuring complete removal of the unbound conjugate followed by incubation in substrate solution (diamino benzidine in tris buffer) with gentle shaking. Nitrocellulose membrane was washed after development of desirable colour reaction avoiding excessive background colour.

Cross-reactivity of different regions

The cross-reactivity of different regions obtained in gel



Figs 10e-f. **10e.** Regions obtained in DEAE-sepharose CL-6B elution of SAG2D1; **10f.** Regions obtained in DEAE-sepharose CL-6B elution of SAG3D2.

filtration and anion exchange chromatography was tested by using ELISA. ELISA was performed according to the method of Engvall and Pearlman (1971). Rabbit anti *M. tuberculosis, M. kansasii, M. phlei, M. intercellulare, M. xenopi* and *M. chelonei* sera were used as source of first antibody. Goat anti rabbit IgG HRPO conjugate was used as the second antibody and the test was read at 450 mm by ELISA reader.

RESULTS AND DISCUSSION

Our understanding of immune responses in bovine tuberculosis has largely been adopted from studies on tuberculosis in man and laboratory animals. Complexity of interactions between mycobacteria and their host (Chaparas *et al.* 1970, Edwards and Krikpatrick 1986, Maufmann 1988) is well illustrated. Clearly, both antibody and cell-mediated immune responses can be induced following mycobacterial infection. The present study was conducted with the aim of characterizing *M. bovis* AN5 antigens, since a detailed understanding of their immunoreactivity will aid in the development of more specific and sensitive diagnotic test. In this study, antigen purification was based on the physical characteristics of the antigen with respect to, size and charge. Each fractionated region has been given a separate nomenclatene (Table 1).

Gel filtration chromatography of sonicated antigen (SA)

Table 1. Nomenclature of different fractionated regions of *M bovis* AN5 sonicated antigens

Elution	Regions	Name given
Sephacryl s-200 elution	I	SAGI
	II	SAG2
	III	SAG3
	IV	SAG4
DEAE-sepharose CL-6B	I	SAGIDI
elution of sephacryl	II	SAGID2
S-200 eluted I region (SAGI)	III	SAGID3
	IV	SAGID4
	V	SAGID5
DEAE- sepharose CL- 6B	I	SAG2DI
elution of sephacryl	II	SAG2D2
S-200 eluted II region	III	SAG2D3
(SAG2)		
DEAE- sepharose CL-6B	I	SAG2D1d1
elution of DEAE-sepharose	II	SAG2D1d2
CL-6B eluted I region (SAG2D1)	III	SAG2D1d3
of sephacryl s-200		
eluted III region (SAG3)		

revealed viz. SAG1, SAG2, SAG3 and SAG4 regions (Fig.1). All 4 regions obtained were subjected to their immunoreactivity test *in vitro* using ELISA (Fig.2). First 3 regions, viz. SAG1, SAG2 and SAG3 had considerable reactivity in ELISA. Out of them SAG3 had highly reactive followed by SAG1 and SAG2. All 3 regions (SAG1 SAG2 and SAG3) were further fractionated by anion exchange chromotography on DEAE-Sepharose CL-6B. On anion exchange chromatography of SAG2 revealed 3 regions (Fig.4) out of which SAG2D1 showed higher reactivity followed by SAG2D3 and SAG2D2 in ELISA (Fig.2). Similarly SAG3 was resolved into 3 regions (Fig.5) out of which SAG3 D2 showed higher reactivity in ELISA (Fig.2). SAG2D1 and SAG3D2 regions showing higher reactivity were further fractionated by anion exchange chromatography (Figs 6, 7). Both regions revealed 3 regions. Out of these, SAG2D1d1 and SAG3D2d1 showed more reactivity in ELISA (Fig. 2).

Strong immunogenecity is a condition for a component to be of value in immunologic studies, particularly in the development of diagnostic reagents in relation to mycobacterial studies, particularly in the development of diagnostic reagents in relation to mycobacterial diseases. SDS-PAGE analysis of *M. bovis* AN5 sonicated antigen revealed 37polypeptides, out of whcih 15 were major (81, 76, 71, 65, 58, 48, 46, 42, 38, 34, 30, 28, 23, 21.5, 12.5 kDa mw) and 22 were minor (102, 84, 79, 76, 72, 62, 59, 50, 48.5, 47, 44.5, 43, 41, 35, 30.5, 26, 24, 22, 18, 16.5 and 14.5 kDa) ranging from 102 to 14.5 kDa mw (Fig.8a) SDS-PAGE analysis of region SAG1D4 and SAG1D2 showing more immuno-reactivity revealed polypeptides of 79 and 81 kDa and 68

and 71 kDa, respectively, while other 2 immunoreactive regions SAG2D1d1 and SAG3D2D1 were appeared to be homogenous and possess polypeptides of 56 kDa and 28 kDa, respectively (Fig. 8b). These antigens isolated from *M. bovis* AN5 sonicate satisfied the criteria of strong immunogenecity and were highly immunoreactive in the initial tests of its immunoreactivity.

Various workers have isolated and purified several *M. bovis* antigens using classical methods exploiting a range of antigen characteristics. (Nagai *et al.* 1981, Haslov *et al.* 1987, Abou-Zeid *et al.* 1986, 1987, Fifis *et al.* 1994; Gupta *et al.* 1994). In our previous study of *M. bovis* BCG sonicated antigen fractionation we reported a 71 kDa mw protein antigen purified from *M. bovis* BCG sonicate possessed immunodominant determinants for both humoral and cellular immune responses (Gupta *et al.* 1994). Western immunoblot analysis of sonicated antigen revealed 8 bands of 81, 71, 65, 56, 28, 22, 18 and 14 kDa mw (Fig.9). This indicates that these are the major polypeptides against which humoral response is mainly directed. The 12 kDa (Minden *et al.* 1984) and 70 kDa (Britton *et al.* 1986) antigens were purified from culture filtrate and sonicate antigens of *M. tuberculosis*. Two *M. tuberculosis* antigens, the 30 kDa (Young *et al.* 1992, Kadival 1987, Worsaac *et al.* 1987) and the 14 kDa (Mathews *et al.* 1985) also purified by the same methods. Verbon *et al.* (1990) isolated protein from 12, 25 and 35 days culture supernatant and sonicates of *M. tuberculosis* and characterized by gel permeation chromatography and SDS-PAGE. The major protein bands having mw of 24 and 12 kDa found in supernatant of heat treated culture possessed epitopes. They considered them to be *M. tuberculosis* specific and partially valuable for development of serological tests (Verbon *et al.* 1990).

All fractions of SA isolated were examined for possessing corss-reactive epitopes among different mycobacterial species (Fig.10a-f). SAG1D4 cross-reacted with *M. microti* and *M. avium* in ELISA, SAG1D2 cross reacted with *M. tuberculosis* and *M. avium* in ELISA, SAG2D1d1 was found less cross-reactive to other mycobacterial species in ELISA, while SAG3D2d1 reacted with *M. tuberculosis*, *M. avium* and *M. intercellulare* antisera in ELISA. All other regions reacted with one and the other mycobacteria included in this study. This could be due to sharing of certain common epitopes between mycobacteria. The sharing of epitopes among *M. bovis* AN5, *M. tuberculosis*, *M. avium* and *M. scrofulaceum* was also confirmed by immunoblot analysis. That antigen of 84, 47, 28 and 22 kDa mw of *M. tuberculosis*, 15.5 and 14 kDa of *M. avium*, 24 and 18 kDa of M. scrofulaceum possess cross-reactive epitopes to *M. bovis* AN5 sonicated antigens. Among 14 species of mycobacteria examined antigen 5 was identified only in *M. tuberculosis* and *M bovis* (Daniel *et al.* 1979). Daniel and Demuth (1977) reported antigen 6 as a major protein of *M. tuberculosis* and it was also present in culture filtrate of *M. szulgi*.

This can be interpreted that some of our purified and partially purified antigens could be used as potent candidate for development of serological assay in conjunction with a cellular assay in future to improve the overall sensitivity of bovine tuberculosis diagnosis. These results also indicated that very few antigens of *M. bovis* AN5 sonicate could be used in a comparatively sensitive serological test without removal of cross-reactive epitopes.

ACKNOWLEDGEMENTS

We thank Director, Indian Veterinary Research Institute, Izatnagar for providing necessary research facilities. This work is partly funded by USIF-IN-AES 572.

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Indian Journal of Animal Sciences **70** (10): 1008-1014, October 2000

Fractionation and immunoreactivity of *Mycobacterium bovis* AN5 culture filtrate antigens

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Received: 8 November 1999; Accepted: 7 April 2000

ABSTRACT

Protein secreted by *Mycobacterium* species have been suggested as major immune targets. Four weeks old *M. bovis* AN5 culture filtrate protein antigens were fractionated by gel permeation and anion exchange chromatography. These fractionated antigens were evaluated for their reactivity to anti *M. bovis* AN5 antiserum in ELISA. Out of several fractionated antigens, the more reactive antigens were heterogenous polypeptides of 46,42,40 and 38 kDa and homogenous polypeptides of 48 and 17.4 kDa molecular masses. Western immunoblot analysis of crude culture filtrate antigens revealed 9 reactive bands of 74,48,46,43, 38, 23,21.4,17.4 and 14 kDa molecular masses. Most of the fractionated culture filtrate antigens showed cross- reactivity with other *Mycobacterium* species in ELISA except 17.4 kDa antigen that showed species specificity to *M. bovis* AN5. It is concluded that some of the culture filtrate antigens are highly immunogenic for humoral response.

Key words: Culture filtrate antigens, ELISA, *Mycobacterium bovis* AN5

The characterization of mycobacterial antigens has been vigorously pursued, since Seibert's work (Seibert 1934, Seibert and Glenn 1941) with the hope of producing well defined and specific reagents for diagnostic tests and potential vaccines. The study of *M. bovis* components has led to a better understanding of the course of disease development and the immunological responses in infected animals (Young *et al.* 1990). The *M. bovis* AN5 produces a number of complex glycoprotein, glycolipids, polysaccharide and lipoprotein antigens. Most of the protein antigens are released in media by actively growing bacteria. Very little is known about how many of these secreted antigens induce the immune response in the infected host. The intradermal tuberculin test lacks both specificity and sensitivity partially due to the complex nature of the antigen (PPD) used which includes several cross-reactive proteins common to a range of other mycobacteria (Ivanyi 1988). If a new and more sensitive *in-vitro* test could be developed such test would include well characterized and highly specific *M. bovis* antigen. For specific antigen it is generally thought that protein antigens are the principal candidate, as carbohydrate and lipid molecules are known to be largely cross-reactive (Krambovitis 1987). In present study efforts were made to isolate specific antigen present in *M. bovis* AN5 culture filtrate and also to investigate the cross reactivity pattern of culture filtrate antigen with various mycobacteria.

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MATERIALS AND METHODS

Bacteria

Mycobacterium bovis AN5 was obtained from the Biological Products Division, Indian Veterinary Research Institute, Izatnagar. *M. tuberculosis, M. simae, M. fortuitum, M.intercellulare, M. kansasii,. M. phlei, M.smegmatis, M. xenopi* and *M. chelonei* were obtained from the Central JALMA Institute on Leprosy, Agra (India).

Extraction of M. bovis *AN5 culture filtrate antigen (CFA)*

Mycobacterium bovis AN5 was cultured in Sauton's medium consisting of 2 mM Mg_2SO_4, 10 mM citirc acid, 2.5 mM K_2HPO_4, 30 mM asparagine, 0.1 mM ferric ammonium citrate and 830 mM glycerol (final pH 7.4) in flask at 37 °C. Culture filtrate obtained after harvesting bacterial cells after 4 week of growth was precipitated with 80% ammonium sulphate saturation and collected by centrifugation at 10 000 × g for 15 min at 4 °C.The pellet was dissolved and dialyzed against tris (hydroxymethyl)- amino methane hydrochloride buffer (Tris buffer,pH 8.7) and was passed through a membrane filter of pore size 0.45 mμ to remove any bacterial cells. Protein content of the sample was estimated as per

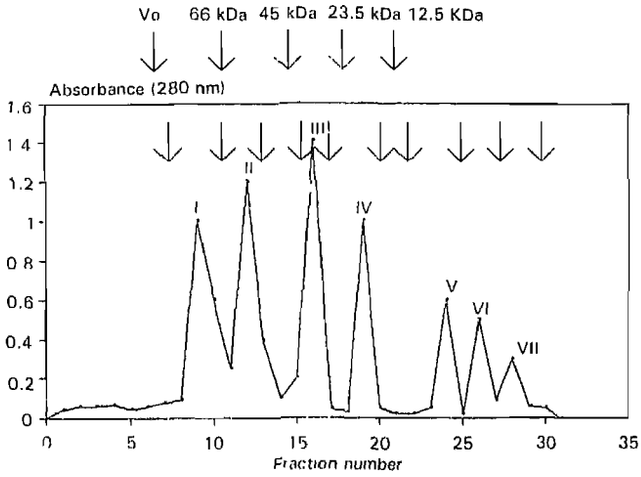


Fig.1. Sephacryl S-200 elution profile of *M. bovis* AN5 culture filtrate antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM Tris buffer, pH 7.5 containing 0.1M NaCl.

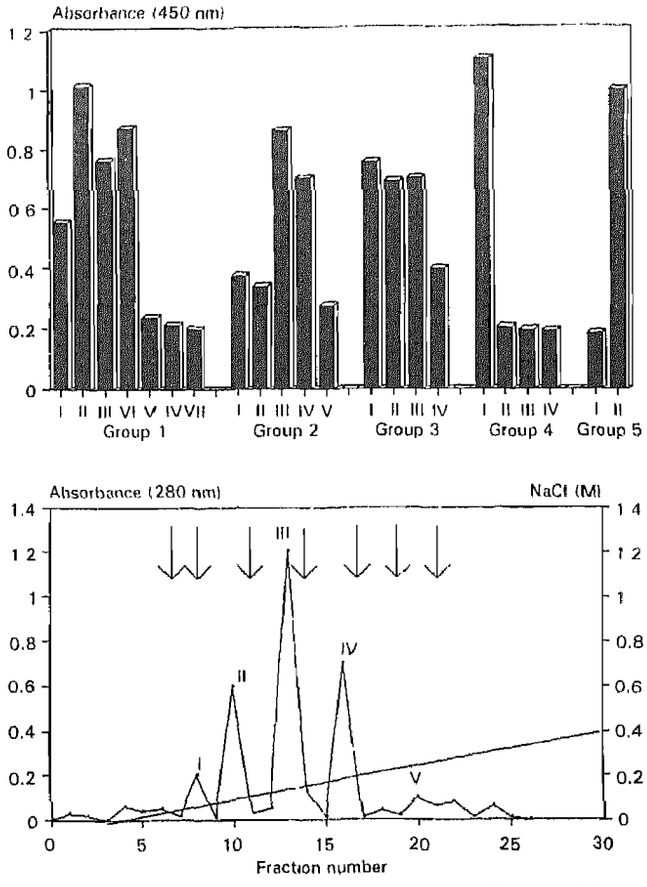
Lowry *et al.* (1951) and was 4 mg/ml. Thiomersal (0.01%) was added to the final preparation and it was kept frozen at 20 °C until further use.

Gel filtration chromatography

The culture filtrate of *M. bovis* AN5 was desolved by gel filtration using Sephacryl S-200 column (80cm × 1.5 cm). Void volume (Vo) of the column bed was 42 ml. The column was precalibrated with standard protein molecular weight markers viz bovin serum albumin, egg albumin, chymotrypsinogen A and cytochrome C having molecular weights of 66 kDa, 45 kDa, 23.5 kDa and 12.5 kDa, respectively. The culture filtrate (40 mg protein in 2 ml) was eluted at @18 ml / hr with tris buffer (30 mM, pH 7.5) containing 0.1 M sodium chloride. Absorbance of elutes was monitored at 280 nm. All fractions under each region according to absorbance at 280 nm were vacuum concentrated by vacuum concentrator. The pooled materials were filter sterilized through a membrane filter (0.22 mμ) and stored at -20°C.

Anion exchange chromatography

The sephacryl S-200 resolved regions depicting the higher reactivity in ELISA and DTH were subjected to anion exchange chromatography. The sample (5 mg to 20 mg in 30 mM tris buffer, pH 8.7) were charged on DEAE- sepharose CL-6B column (1.5 cm ×30 cm) equilibrated with 30 mM tris buffer (pH 8.7), and then eluted with 30 mM tris buffer (PH 8.7) containing 3 M urea, using a linear gradient of sodium chloride from 0 to 0.3 M. Flow rate was 16 ml/hr and fractions of 5.5 ml were collected. All fractions were pooled under each peak according to absorbance at 280 nm, vacuum concentrated and filter sterilized (0.22 mμ membrane). The regions showing higher immunoreactivity in ELISA were



Figs 2-3. **2.** Reactivity of different regions in ELISA (with *M. bovis* AN5 antiserum) obtained in Sephacryl S-200 and DEAE-sepharose CL-6B elutions of *M. bovis* AN5 culture filtrate antigens. **3.** DEAE-sepharose CL-6B elution profile of CFG2 of *M. bovis* AN5 culture filtrate antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.4M.

further rechromatographed on the same column using 0.0 to 0.4M NaCl gradient.

Enzyme linked immunosorbent assay (ELISA)

Each region was tested for its seroreactivity as per Engvall and Pearlman (1971). Flat bottom 96 well plates were coated with 50 μl antigen containing 20 microgram protein / ml crude CFA or 10 mg partially purified or purified protein CFA in triplicate. Rabbit anti *M. bovis* (AN5 serum) was used as the source of first antibody. Goat anti rabbit Ig HRPO conjugate was used as the second antibody. The substrate (O-phenylene diamine dihydrochloride) was added and the test was read at 450 nm in ELISA reader.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Mycobacterium bovis AN5 crude and fractionated

culture filtrate antigens were analyzed by SDS- PAGE employing the method of Laemmli (1970) with some modifications (Hames 1981). A slab gel electrophoresis system was used with 10% polyacrylamide gels containing 0.1% SDS. The samples were diluted in loading buffer (11% v/v glycerol, 1% w/v SDS,0.01 of w/v bromophenol blue, 26% of v/v 0.5 M Tris- HCl buffer,pH 6.8), to which 5% v/v beta mercaptoethenol was added so that the quantity of protein was 20 mg / track for crude CFA while 10 mg for fractionated antigen. The samples were heated at 100 °C for 3 min before loading. Gels were run at 40 volts for 18 hr and then stained with Coommassie brilliant blue.The calculations for the determination of molecular weight was based on the distance the polypeptide migrated in the gel *vis* a *vis* to polypeptide markers of known molecular weight.

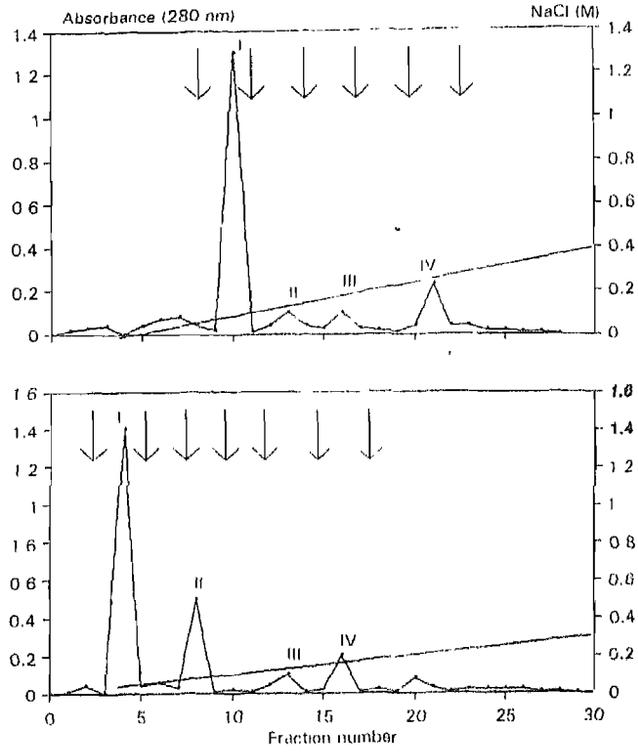
Western immunoblotting: The crude and fractionated CFA were separated on 10% polyacrylamide slab gel electrophoresis under reducing condition in the presence of SDS (Laemmli 1970). The separated antigens were then transferred electrophoretically to a nitrocellulose membrane (NCM) in Tris/glycine/methanol buffer (Towbin *et al.* 1979) and the membrane was immersed over night at room temperature in PBS containing 3% BSA and 0.05% Tween - 20. After thorough washing in washing buffer the membrane was incubated for 2 hr at 37 °C in 30 ml of 1.50 diluted rabbit anti *M. bovis* AN5 serum, washed thoroughly and incubated with 30 ml of 1: 1 000 diluted goat antirabbit IgG HRPO conjugate for 2 hr at 37 °C. The NCM was washed like previous step assuring complete removal of the unbound conjugate and incubated in substrate solution (diamino benzidine in tris buffer) with gentle shaking. NCM was washed after development of desirable colour reaction avoiding excessive background.

Cross-reactivity of different regions

The cross reactivity of different regions obtained in gel filtration chromatography and anion exchange chromatography was tested by ELISA according to Engvall and Pearlman (1971). Rabbit anti *M. tuberculosis, M.simae, M. fortuitum, M. intercellulare, M. kansasii, M. phlei, M. smegmatis, M xenopi* and *M. chelonei* sera were used as source of first antibody. Goat anti rabbit Ig HRPO conjugate was used as the second antibody and the test was read at 450 nm by ELISA reader.

RESULTS AND DISCUSSION

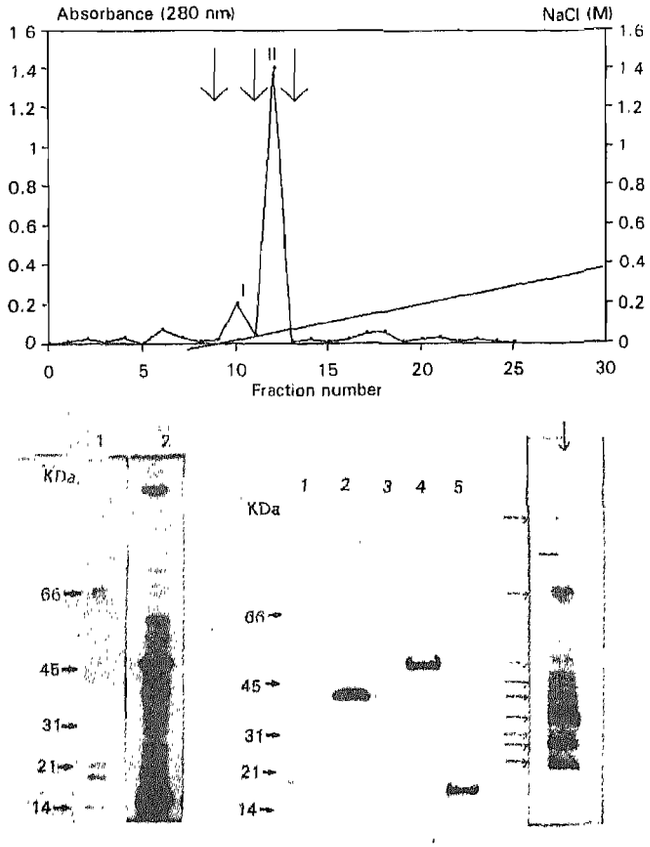
Gel filtration chromatography of CFA revealed 7 regions (Fig.1). Each fractionated region has been given a separate nomenclature (Table 1). All 7 regions obtained were subjected to their immunoreactivity test using ELISA (Fig.2).CFG2 representing higher mw had found to have more antigenic reactivity that was closely followed by CFG4, that seems to be having molecules of low molecular weight, in



Figs 4-5. 4. DEAE-sepharose CL-6B elution profile of CFG4 of *M. bovis* AN5 culture filtrate antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.3M. 5. DEAE-sepharose CL-6B elution profile of CFG2D3 of *M. bovis* AN5 culture filtrate antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.4M.

ELISA. CFG2 and CFG4 were further fractionated by anion exchange chromatography, on which CFG2 resolved into 5 regions (Fig.3). Of these regions CFG2D3 had strong antigenic reactivity when tested in ELISA. CFG4 further revealed 4 regions on anion exchange chromatography (Fig.4). Of these regions CFG4d1 exhibited more antigenic reactivity in ELISA (Fig.2). CFG2D4 and CFG4D1 were further resolved to 4 and 2 more regions, respectively, on anion exchange chromatography (Figs 5, 6). Of these regions CFG2D4d1 and CFG4D1d2 showed more antigenic reactivity in ELISA (Fig. 2). These regions showing antigenic reactivity indicated presence of immunodominant polypeptides.

SDG-PAGE analysis of *M.bovis* AN5 CFA revealed 13 major (81,74,58,48,46,43,38,32,30,23,21.4,19,5,17,4 kDa) and 9 minor (105,86,77.73,67,66,44,33, and 18 kDa) structural polypeptides (Fig 7a). On SDS-PAGE analysis CFG2D3 was heterogenous polypeptide with 38 kDa prominent polypeptide.Other regions like CFG2D4d1 and CFG4D1d2 were homogenous on SDS-PAGE containing 48 and 17.4 kDa mw respectively (Fig. 7b). Although the new



Figs 6-8. **6.** DEAE-sepharose CL-6B elution profile of CFG4D1 of *M. bovis AN5 culture filtrate antigens.* Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.4M. **7a.** Commasie brilliant blue stained SDS-PAGE (10% slab gel) showing polypeptides of *M. bovis* AN5 culture filtrate antigens: Lane1-SDS-PAGE molecular weight makers; lane2-polypeptides of culture filtrate antigens. **7b.** Commasie brilliant blue stained SDS-PAGE (10% slab gel) showing polypeptides of *M.bovis* AN5 culture filtrate antigens: Lane1-SDS-PAGE molecular weight markers; lane2-polypeptides of CFG2D3; lane3-polypeptides of CFG2D4d1; lane4-polypeptides of CFG4D1d2. **8.** Western immunoblot analysis of *M.bovis* AN5 culture filtrate antigens with *M. bovis* AN5 antiserum. Figures against arrows indicate mw in kDa.

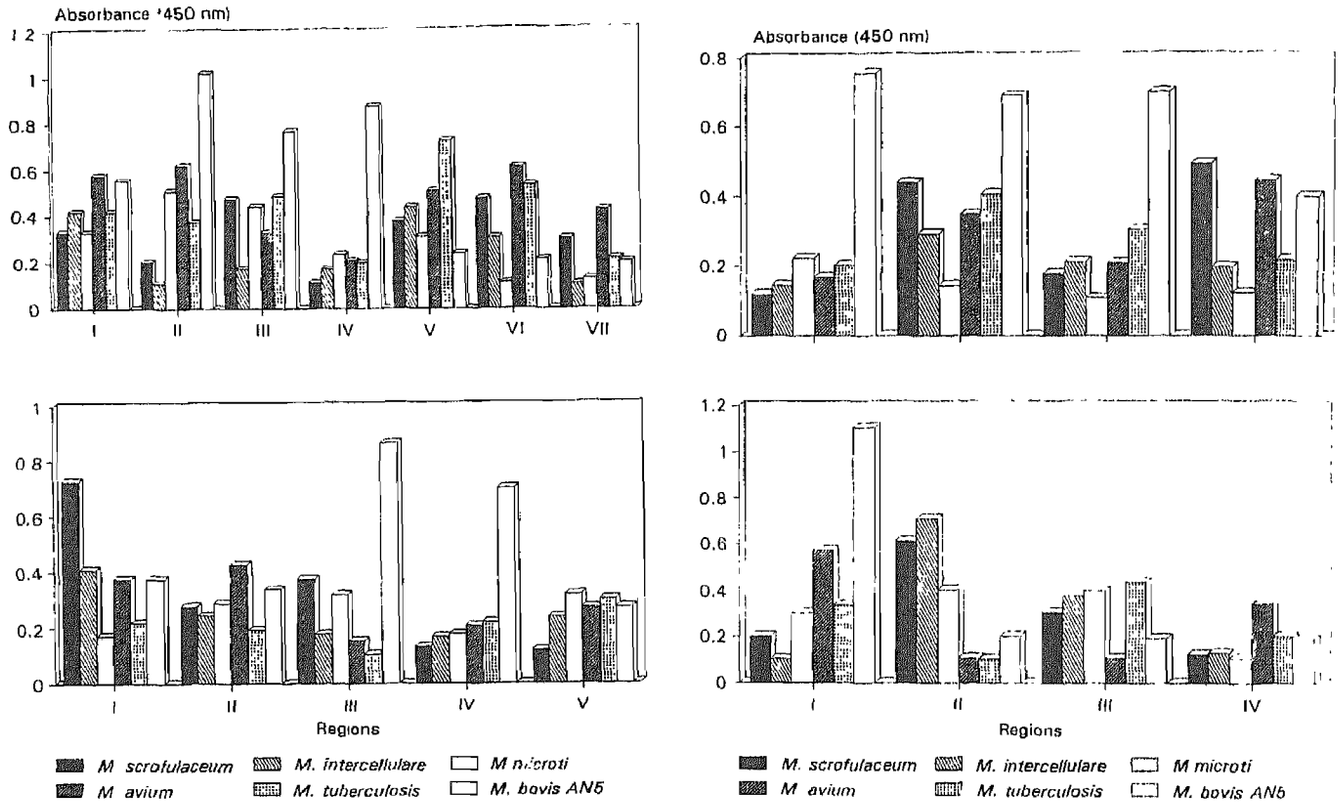
technologies of monoclonal antibodies production and recombinant DNA gene expression provided the means for the purification of large amounts of antigens, only limited reports using these techniques were forthcoming (Ivanyi *et al.* 1988). The 12 kDa (Minden *et al.* 1984) and 70 kDa (Britton *et al.* 1986) antigens of *M. bovis* have been purified using affinity purification from culture filtrate on monoclonal antibody columns, the first using on *M. bovis* specific mAb,the second using a mAb raised against *M. leprae. M. tuberculosis* antigens, the 38kDa (Young *et al.* 1986; Kadival *et al.* 1987, Worsaae 1987) and the 14 kDa (Matthews *et al.* 1985) were purified by the same method. However, only sufficient

Table 1. Nomenclature of different fractionated regions of *M. bovis* AN5 culture filtrate antigens

Elution	Regions	Name given
Sephacryl s-200 elution	I	CFG1
	II	CFG2
	III	CFG3
	IV	CFG4
	V	CFG5
	VI	CFG6
	VII	CFG7
DEAE-sepharose CL-6B	I	CFG2D1
Elution of sephacryl	II	CFG2D2
S-200 eluted II region (CFG2)	III	CFG2D3
	IV	CFG2D4
	V	CFG2D5
DEAE- sepharose CL-6B	I	CFG4D1
Elution of sephacryl	II	CFG4D2
S-200 eluted IV region	III	CFG4D3
(CFG4)	IV	CFG4D4
DEAE- sepharose CL-6B	I	CFG2D4d1
Elution of DEAE-sepharose	II	CFG2D4d2
CL-6B eluted IV region (CFG2D4)	III	CFG2D4d3
of sephacryl s-200	IV	CFG2D4d4
eluted II region (CFG2)		
DEAE- sepharose CL-6B	I	CFG4D1d1
Elution of DEAE-sepharose	II	CFG4D1d2
CL-6B eluted I region (CFG4D1)		
of sephacryl s-200		
eluted IV region (CFG4)		

amounts of antigen for analytical work can be purified by mAbs affinity chromatography (Young 1988).Needless to say that large scale immunological studies, substantial amounts of the purified antigens are required. Recombinant gene expression can produce large quantities of antigens and *M. bovis* and *M. tuberculosis* antigens were produced in this manner (Kingston *et al.* 1987, Thole *et al.* 1987). However, problem with solubility and purification of the expressed antigen are often encountered. There is also the possibility that recombinant antigens might not be antigenically identical to their native counterpart. Recombinant *M. bovis* antigens are not necessarily recognized by the immune system of some infected animals to the same degree as the native antigens (Collins *et al.* 1994).

In present study, western immunoblot analysis of CFA revealed 9 bands of 74,48,46,43,38,23,21.4,17.4, and 14 kDa (Fig. 8). These antigens may play their role in humoral response against *M. bovis* AN5 in infected cattle. Many researchers have isolated 4 antigens of 22(a), 24, 30 and 32 kDa, also known as MPB 70, MPB64, MPB 59 and P32 using classical methods from *M. bovis* CF (Harboe *et al.* 1986,



Figs 9a-d. Cross reactivity of regions obtained in different elutions of *M. bovis* AN5 culture filtrate antigens with different mycobacerial antisera by ELISA: **9a.** Regions obtained in sephacryl S-200 elution. **9b.** Regions obtained in DEAE-sepharose CL-6B elution of CFG2 **9c.** Regions obtained in DEAE-sepharose CL-6B elution of CFG4 **9d.** Regions obtained in DEAE-sepharose CL-6B elution of CFG2D4.

Wiker *et al.* 1986 a, b, De Bruyn *et al.* 1987). 15.3 kDa antigen was isolate from *M. bovis* BCG culture filtrated using classical methods (Gupta *et al.* 1994 b). Ten mycobacterial proteins of 39, 32, 30, 25, 24, 22 (a and b forms), 19, 15 and 12 kDa molecular mass have been purified from *M. bovis* CF using several chromatographic methods (Fifis *et al.* 1991).However, cross reactivity of these purified CF antigens were not studied in detail.

All fractions isolated by fractionation of CFA in this study were examined for cross-immuno reactivity with other mycobacteria by ELISA (Fig. 9). CFG4D1d2 did not cross-react with other mycobacteria used in this study suggesting this molecule to be species specific. However, all other fractionated CFA tested in the present study were cross reactive to one or other mycobacterial species in ELISA. No other resolved regions except CFG4D1d2 (17.4 kDa mw) seems to be species specific. Serodiagnosis, in terms of specificity is a major problem. It has long been known that the different species of mycobacteria have a considerable number of antigens in common (Gupta *et al.* 1994 a, b) some of these antigens are also common to *Nocardia* and *Corynebacterium* (Ridell 1975, Ridell 1977). Among 14 species of mycobacteria examined,antigen 5 was identified only in *M. tuberculosis* and *M. bovis* (Daniel and Janicki 1978, Daniel and Debanne 1987). Daniel and Demuth (1977) reported antigen 6 to be a major protein of *M. tuberculosis* but it was also present in culture filtrate of *M. szulgi*.

The presence of carbohydrate in antigen 5 has been attributed to contamination by lipoarabinomannan (Daniel 1989). The cross reactivity observed is most likely due to some weak recognition of sugar structure on the 38 and 39

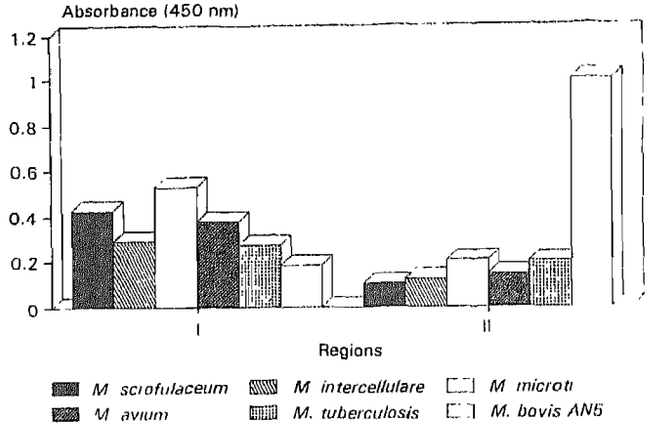


Fig. 9e. Regions obtained in DEAE-sepharose CL-6B elution of CFG4D1.

kDa antigens that simulates the lipoarabinomannan (LAM) antigenic determinant as the sugar monomer composition of both LAM and the carbohydrate moiety on the 39 kDa is mainly mannose (Fifis *et al.* 1991).

In this study, CFG4D1d2 (17.4 kDa) seems to be species specific to *M. bovis* AN5. Nagai *et al.* (1981) isolated on antigen with molecular weight of 18 kDa from culture filtrate of *M. bovis* BCG and named it as MPB 70. MPB 70 was reported to be an immunodominant antigen at least in mice (Wood *et al.* 1988).This 17.4 kDa isolated in this study could be a possible candidate to be used in the diagnosis of bovine tuberculosis. In a study,MPB70 and 11 other purified antigens were used in serological studies (Fifis *et al.*1989,1994).

Since the cross-reactivity of most of the fractionated regions in this study was unacceptably high, the future studies should strive to develop any serological test would be inclusion of only *M.bovis* specific epitopes or antigens.

ACKNOWLEDGMENTS

We thank Director, Indian Veterinary research Institute, Izatnagar, for providing necessary research facility. This work is partly funded by USIF-IN-AES 572.

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Indian Journal of Animal Sciences **70** (10): 1015-1020, October 2000

Fractionation and immunoreactivity of membrane associated proteins of *Mycobacterium bovis* AN5

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Received: 8 November 1999; Accepted: 7 April 2000

ABSTRACT

The membrane associated antigen was extracted from the pellet of *M.bovis* AN5 sonicate with 2% SDS and 2% mercaptoethanol. The extracted antigen was fractionated on gel filtration and anion exchange columns. The fractionated regions were checked for their reactivity to anti *M. bovis* AN5 serum in ELISA. Among several fractionated regions obtained, the highly immunoreactive antigens were heterogenous polypeptides of 66 and 68 kDa and 26 and 28 kDa and a homogenous polypeptide of 38 kDa. These 3 antigens were highly reactive in ELISA to anti *M. bovis* AN5 serum. The immunoblot analysis of crude membrane associated antigens revealed 6 reactive bands of 68,45,38,32,26,and 14 kDa. All the fractionated antigens showed cross- reactivity with other *Mycobacterium* species in ELISA.

Key words: ELISA,*Mycobacterium bovis* AN5, Membrane associated antigens

Many reports illustrated that the interaction between mycobacteria and the host is complex (Closs *et al.* 1980, Daniel *et al.* 1978). Clearly, both antibody and cell-mediated immune responses can be induced following mycobacterial infection. The cell wall framework of *M. tuberculosis* Erdman, the so called covalent skeleton (Barnes *et al.* 1994) isolated by classical means (Draper 1971) or by a procedure involving sequential removal of known entities demonstrated protein T cell immunoreactivity (Young *et al.* 1992). Other have reported that dehydrated mycobacterial cell walls, but not soluble protoplasm were protective against *M. tuberculosis* infection in mice (Ribi *et al.* 1982). The serological distinction of integral (detergent soluble) membrane protein as a class of mycobacterial antigens, using *M. fortuitum* as a model has been reported (Mehrotra *et al.* 1995). These framework or membrane associated protein antigens generate active interest particularly in view of their possible role in the design and development of newer and more different diagnostic tests and vaccines against bovine tuberculosis. Nonetheless, since these membrane associated proteins have mostly been picked up from crude extracts or sonicate by physicochemical means (Gupta *et al.* 1994a,b) it is quite likely that many predominant membrane associated antigens were missed. This realization has led us to a comprehensive search for immunodominant membrane associated antigens which can possibly be used in development of newer diagnostic test in future.

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MATERIALS AND METHODS

Bacteria

Mycobacterium bovis AN5 was obtained from the Biological Products Divisions, Indian Veterinary Research Institute, Izatnagar. *M.tuberculosis, M.simae,M. fotuitum, M. intercellulare, M. kansasii, M. phlei, M. smegmatis, M. xenopi* and *M. chelonei* were obtained from the Central JALMA Institute for Leprosy Agra.

Preparation of M.bovis *AN5 membrane associated antigens (MAA)*

M. bovis AN5 was grown in Sauton's liquid medium composed of 2mM Mg_2SO_4, 10mM citric acid, 2.5 mM K_2HPO_4, 30 mM asparagine, 0.1 mM ferric ammonium citrate and 830 mM glycerol (Final pH 7.4) in flask at 37 °C. Bacteria were harvested after 4 to 5 weeks of culture in early logarithmic phase after heat killing at 70 °C for 2 hr in a water-bath. Cells were washed in normal saline solution (NSS) and suspended to 250 mg wet weight per ml in NSS and disrupted by disintegration in a MSE ultrasonicator using a titanium probe (12.7 mm diameter) operating at 10-15 mm peak to peak. The sonication vessel was immersed in an ice water slurry and the contents were subjected to a total of 15 min sonication at 30s intervals to reduce heating. Sonicated antigenic extract was centrifuged at 10 000 rpm for 30 min at

4°C. The pellet was taken, treated as per the protocol given by Chakravorty *et al.* (1982). Briefly, pellet was washed thoroughly with sterile normal saline solution, then treated with 2% beta-mercaptoethanol and 2% of sodium dodecyl sulphate (SDS) and reaction was kept at 60°C for 20 min. Mixture was centrifuged at 10,000 rpm for 20 min at 4°C. Supernatant was collected, dialyzed against normal saline solution overnight, filter sterilized and kept at - 20°C. The thiomersal (0.01%) was used as preservative.

Gel filtration chromatography

The membrane associated antigen of *M. bovis* AN5 was resolved by gel filtration chromatography using Sepharyl S-200 column (1.5cm × 80cm). The void volume of the column bed was 42 ml. The column was precalibrated with standard protein molecular weight markers, viz bovine serum albumin, egg albumin, chymotrypsinogen A and cytochrome C having molecular weight of 66 kDa, 45 kDa, 23.5 kDa and 12.5 kDa respectively. The membrane associated antigen (40 mg protein in 2 ml normal saline solution) was eluted @ 18 ml/hr with tris buffer (30 mM, pH 7.5) containing 0.1 M sodium chloride. Absorbance of elutes was monitored at 280 nm. The fractions under each region according to absorbance at 280 nm were pooled, dialyze against distilled water and vacuum concentrated by vacuum concentrator filter sterilized through a membrane filter (0.22 mm), and stored at -20°C.

Anion exchange chromatography

The sephacryl S-200 fractionated regions which were intensely reacting in ELISA and DTH were subjected to anion exchange chromatography. The material (10 mg protein in 2 ml 30 mM, tris buffer, pH 8.7) was applied to DEAE-Sepharose CL-6B column (1.5 cm × 30 cm) equilibrated with 30 mM tris buffer (pH 8.7) and then eluted with 30 mM tris buffer (pH 8.7) containing 3M urea, using a linear gradient of sodium chloride from 0. to 0.4M gradient. Flow rate was 20 ml /hr and fractions were vacuum concentrated, filter sterilized (0.22 mm membrane), and stored at -20°C.

Enzyme linked immunosorbent assay (ELISA)

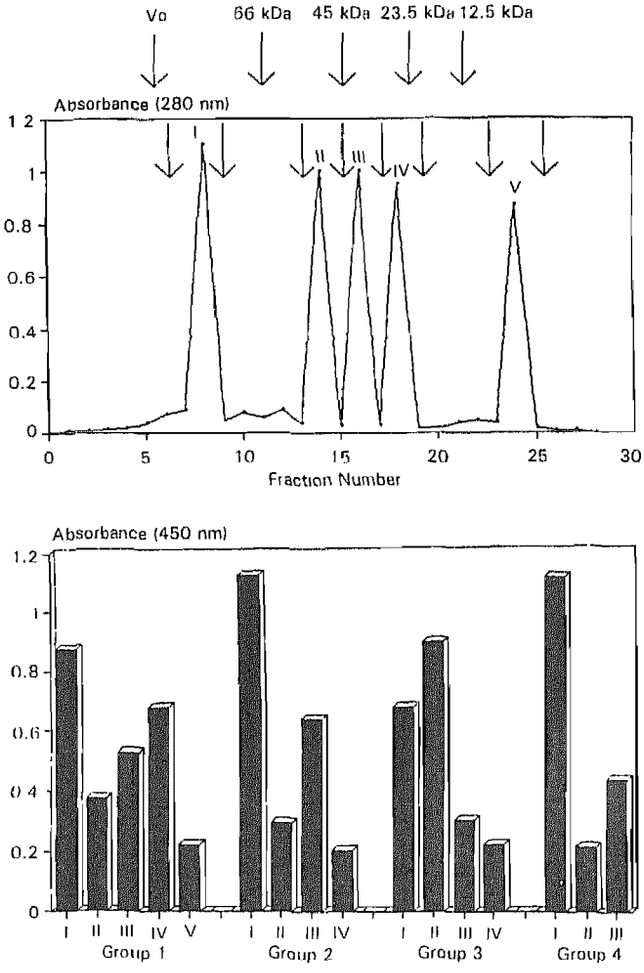
ELISA was performed using the method of Engvall and Pearlman (1971). Triplicate wells of 96 well flat bottom plates were coated with 20 mg /ml protein (crude MAA) and 10 mg/ml protein (partially purified /purified protein). Rabbit anti *M. bovis* AN5 serum was used as the source of the first antibody.Goat anti rabbit Ig HRPO conjugate was used as the second antibody. Substrate(0- phenylene diamine dihydrochloride) was added and the test was read at 450 nm using an ELISA.

Sodium dodecyle sulphate-polyacrylamide gel-electrophoresis (SDS-PAGE)

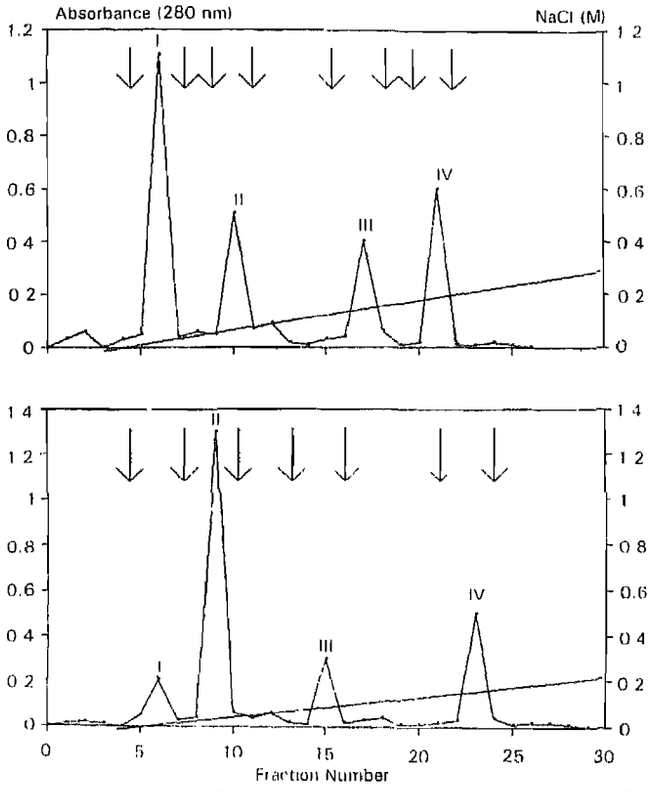
M.bovis AN5 crude and fractionated membrane associated antigens were analyzed by SDS-PAGE (Laemmeli 1970) with some modification (Hames 1981). A slab gel electrophoresis system was used with 10% polyacrylamide gels containing 0.1% SDS. The samples were diluted in loading buffer (11% v/v glycerol, 1% w/v SDS, 0.01% w/v bromophenol blue, 26% v/v 0.05 M tris-Hcl buffer, pH 6.8) to which 5% v/v beta mercaptoethanol was added so that the quantity of protein was 20 g / track for crude MAA and 10 mg / track for fractionated regions. The samples were heated at 100 °C for 3 min before loading. Gels were run at 40 volts for 18 hr and then stained for protein using Coomassie brilliant blue.The calculations for the determination of molecular weight was based on the distance the polypeptide migrated in gel compared to polypeptide markers of known molecular weight.

Western immunoblotting

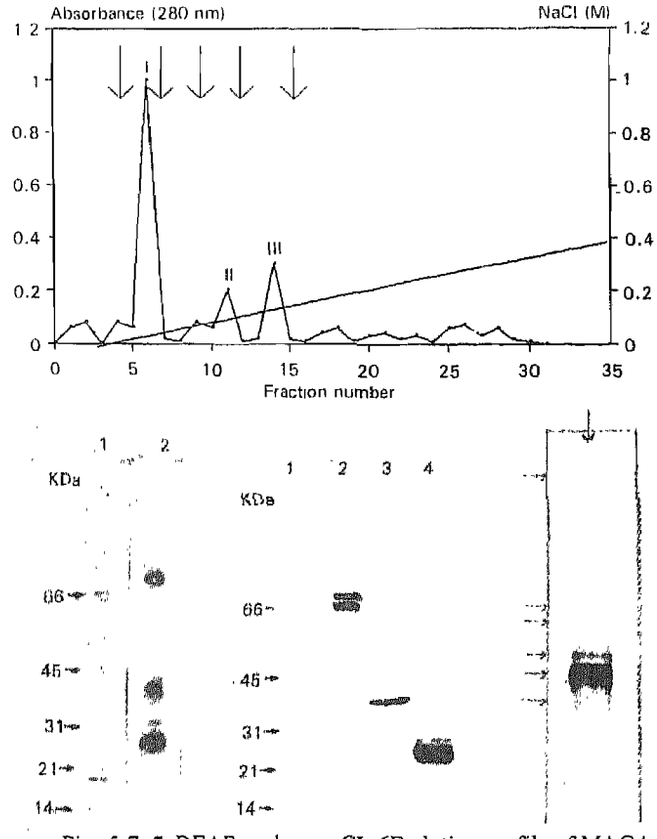
M. bovis AN5 membrane associated antigen was separated on 10% (w/v) polyacrylamide slab gel electrophoresis under



Figs 1-2. **1.** Sephacryl S-200 elution profile of *M. bovis* AN5 membrane associated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30 mM tris buffer, pH 7.5 containing 0.1M NaCl. **2.** Reactivity of different regions in ELISA (with *M. bovis* AN5 antiserum) obtained in sephacryl S-200 and DEAE-sepharose CL-6B elutions of *M. bovis* AN5 membrane associated angigens.



Figs 3-4. **3.** DEAE-sepharose CL-6B elution profile of MAG1 of *M.bovis* AN5 membrane associated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.3M. **4.** DEAE-sepharose CL-6B elution profile of MAG3 of *M. bovis* AN5 membrane associated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and a linear gradient of NaCl from 0.00 to 0.2M.



Figs 5-7. **5.** DEAE-sepharose CL-6B elution profile of MAG4 of *M. bovis* AN5 membrane associated antigens. Area between arrows indicate the fractions pooled under each region. Buffer used = 30mM tris buffer, pH 8.7, containing 3M urea and alinear gradient of NaCl from 0.00 to 0.4M. **6a.** Commasie brilliant blue stained SDS-PAGE (10% slab gel) showing polypeptides of *M. bovis* AN5 membrane associated antigens: Lane1- SDS-PAGE molecular weight markers; lane2- polypeptides of MAG1D1; lane3- polypeptides of MAG3D2; lane4- polypeptides of MAG4D1. **7.** Western immuno-blot analysis of *M. bovis* AN5 membrane associated antigens with *M. bovis* AN5 antiserum. Figures against arrows indicate mw in kDa.

reducing condition in the presence of SDS(13). The separated antigens were then transferred electrophoretically to a nitrocellulose membrane (NCP) in tris glycine methanol buffer (15) and the membrane was immersed overnight at room temperature in PBS containing 3% bovine serum albumin and 0.05% tween-20.After thorough washing in washing buffer NCM was incubated for 2 hr at 37°C in 30 ml of 1: 50 diluted rabbit anti *M. bovis* AN5 serum washed thoroughly and incubated with 30 ml of 1: 1 000 diluted goat antirabbit Ig HRPO conjugate for 2 hr at 37°C. The NCM was washed like previous step assuring complete removal of the unbound conjugate and then NCM was incubated in substrate solution (diamino benzidine in tris buffer) with gentle shaking. NCM was washed after development of desirable colour reaction avoiding excessive background colour.

Cross-reactivity of different regions

The cross-reactivity of different regions obtained in gel filtration and anion exchange chromatography was tested using ELISA (Engvel and Pearlman 1971).Rabbit anti *M. tuberculosis, M. simae, M. fortuitum, M intercellulare,M. Kansasii, M., phlei, M. smegmatis, M. xenopi* and *M. chelonae* sera were used as source of first antibody. Goat anti rabbit Ig HRPO conjugate was used in the second antibody.

RESULTS AND DISCUSSION

Gel filtration chromatography of MAA revealed 5 regions (Fig.1). All regions were given separate nomenclature (Table1). These all regions were evaluated for their immunoreactivity in ELISA (Fig.2). MAG1 showed more sero reactivity, which was followed by MAG4 and MAG3. Anion exchange chromatography of MAG1 revealed 4 were regions (Fig. 3), of these regions MAG1D1 showed more antigenic reactivity (Fig. 2). MAG3 and MAG4 resolved into 4 and 3 further regions, respectively, on anion exchange

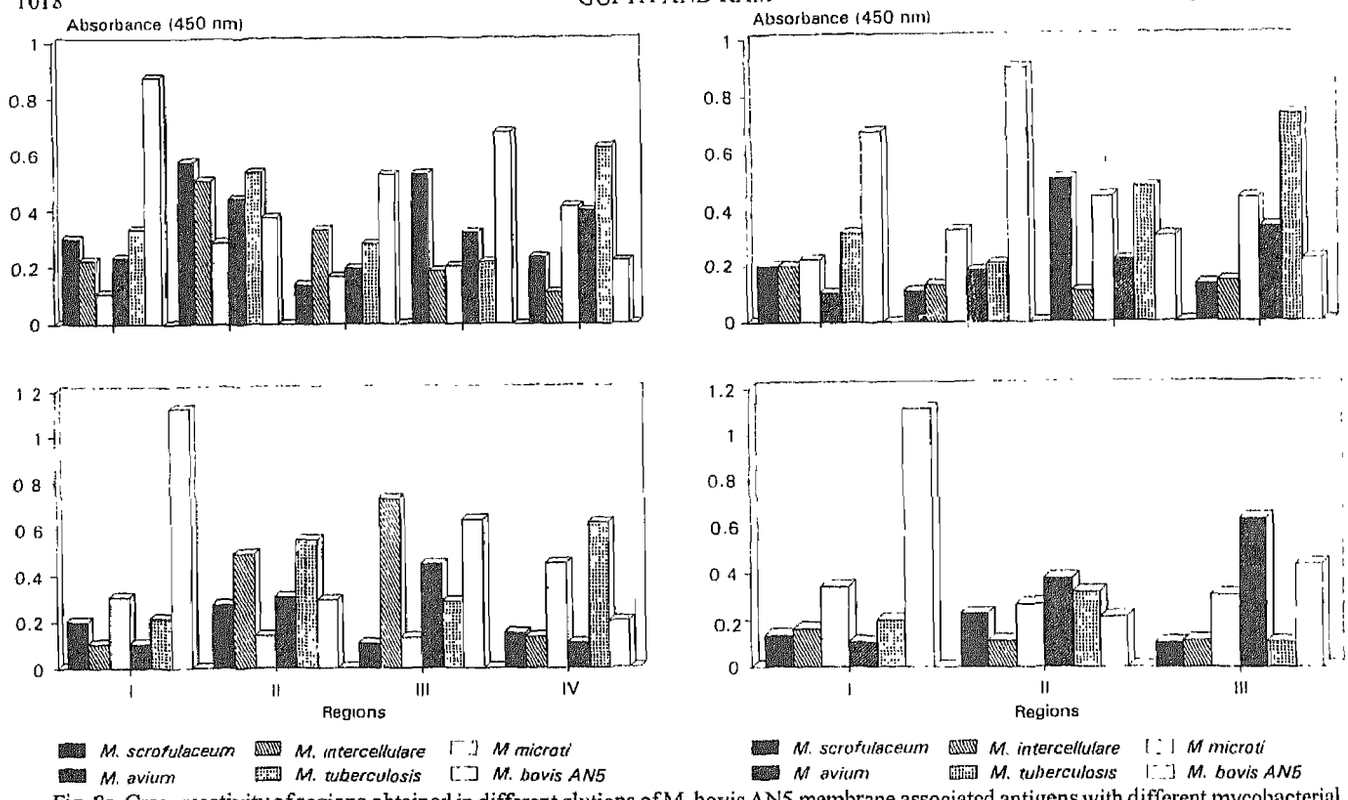


Fig. 8a. Cross reactivity of regions obtained in different elutions of M. bovis AN5 membrane associated antigens with different mycobacterial antisera by ELISA: 8a. Regions obtained in sephacryl S-200 elution; 8b. Regions obtained in DEAE-sepharose CL-6B elution of MAG1; 8c. Regions obtained in DEAE-sepharose CL-6B elution of MAG3; 8d. Regions obtained in DEAE-sepharose CL-6B elution of MAG4.

Table 1. Nomenclature of different fractionated regions of *M. bovis* AN5 membrane associated antigens

Elution	Regions	Name
Sephacryl s-200 elution	I	MAG1
	II	MAG2
	III	MAG3
	IV	MAG4
	V	MAG5
DEAE-sepharose CL-6B	I	MAG1D1
Elution of sephacryl	II	MAG1D2
s-200 eluted III region	III	MAG1D3
(MAGl)	IV	MAGlD4
DEAE-sepharose CL-6B	I	MAG3D1
Elution of sephacryl	II	MAG3D2
S-200 eluted III region II	MAG3D3	
(MAG3)	IV	MAG3D4
DEAE-sepharose CL-6B	I	MAG4D1
Elution of sephacryl	II	MAG4D2
S-200 eluted IV region	III	MAG4D3
(MAG4)		

chromatography (Figs 4,5). Of these regions MAG3D2 and MAG4D1 showed higher immunoreactivity (Fig.2). The high immunoreactivity of MAG1D1, MAG3D2 and MAG4D1 regions indicated the presence of immunodominant polypeptides.

SDS analysis of MAA of *M. bovis* AN5 revealed 3 major (68,38,32) and 7 (66,45,44.5, 32.5,32,27,14 kDa) minor structural polypeptides (Fig. 6a). On SDS-PAGE, MAG1D1 was heterogenous polypeptide and resolved in to 2 polypeptides (66 and 68 kDa), while MAG3D2 was homogenous protein of 38 kDa mw (Fig. 6b). Another region MAG4D1 resolved into 2 polypeptides (26 and 28 kDa), in which 26 kDa was the major polypeptide (Fig. 6b). Strong immunoreactivity is a condition for a component to be of value in immunological studies,particularly in the development of diagnostic reagent in relation to mycobacterial disease.

Most of the time protein present as integral part of cell membrane are missed during extraction. These proteins associated with cell membrane generate significant T-cell response (Germain 1994). These protein could be of a diagnostic or prophylactic value. In addition, proteins working as vital enzymes or virulence factors for the microbe could serve a suitable targets for new drug development. An array of antigenic proteins has been isolated and characterize from various compartments of a mycobacterial cell viz- the cytosol, cell membrane and cell wall, and from growth medium (Gupta *et al.* 1994a,b, Young *et al.* 1992). A large number of these

antigens particularly the cytosolic ones, have also been cloned and studies addressing the vaccine or diagnostic potential of some of them are well underway (Young and Cole 1993, Galbre *et al.* 1994). But the few reports are available regarding the membrane associated proteins / antigens of *M. bovis* AN5 because most of the work is from human tuberculosis.

The immunodominance of antigens isolated in present study corroborate earlier reports showing a prominant seroreactivity and T- cell activation by mycobacterial antigens even though the studied antigens were cytosolic and secretory rather than membrane associated (Anderson and Heron 1993, Ottenhoff *et al.* 1989, Boesen *et al.* 1995, Sinha *et al.* 1987).

Western immunoblot analysis of MAA showed that immune response to MAA mainly directed towards 68,45,38,32,26 and 14 kDa mw antigens (Fig. 7). These antigens might be having certain immunodominant epitopes. All regions cross reacted with different mycobacteria used in this study (Fig. 8). MAG1D1 cross reacted with *M. tuberculosis* and *M. microti* in ELISA. MAG4D1 cross-reacted with *M.microti* in ELISA. All other fractionated regions were also found to possess cross reactive epitopes with other mycobacteria used in this study. Other fractionated MAA elicited either very lower or no seroreactivity. Hydrophobicity and lipidation, the characteristics typical of membrane associated protein have been regarded as important attributes for antigen causing parent activation of immune system (Young and Garbe 1991, Chamberlain *et al.* 1989). Though comparable data on mycobacterial integral membrane antigens are not available. It was reported that the presence of 4 lipoproteins in the soluble extract of *M. tuberculosis* by way of metabolic labelling (Young and Garbe 1991).

It is presumed that the antigenic molecules isolated in the present study are antigenically important and are likely to be the constituent of integral membrane protein (IMP). The relevance of such membrane associated antigens for development of vaccine/diagnostic test for bovine tuberculosis would depend substantially on whether they can be used in conjunction of other cellular assays. Present study clearly indicated that capability to induce strong humoral response made these antigen possible conditions for inclusion in any diagnostic test which could be used for the diagnosis of bovine tuberculosis.

ACKNOWLEDGEMENTS

We thank Director, Indian Veterinary Research Institute, Izatnagar, for providing necessary research facility. This work is partly funded by USIF-IN-AES 572.

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Indian Journal of Animal Sciences **70** (10): 1021-1024, October 2000

Pharmacokinetics and urinary excretion of ciprofloxacin in crossbred cow calves

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Received· 8 October 1999: Accepted: 7 April 2000

ABSTRACT

The pharmacokinetics, urinary excretion and dosage regimen of ciprofloxacin after it's single intravenous administration of 5 mg.kg^{-1}was investigated in crossbred cow calves. At 1 min the concentration of ciprofloxacin in plasma was *10.3 ± 0.10 μg.ml which rapidly declined to 3.07 ± 0.02 μg.ml^{-1}at 10 min. The minimum therapeutic concentration (0.1* μgml^{-1}) in plasma was maintained up to 6 hr. The elimination half life and volume of distribution were 3.75 ± 0.06 hr and 6.80 ± 0.15 L.kg^{-1}· respectively. The distribution half life and AUC were 0.21 ± 0.003 hr and 3.98 ± 0.05 μg.ml^{-1} hr respectively. The total body clearance (Cl_B) and tissue/plasma(T/P) ratio were 1.24 ± 0.02 L.kg^{-1} hr and 8.01 ± 0.22 respectively. Approximately 18% of the total administered dose of ciprofloxacin was recovered in urine within 24 hr after administration. A satisfactory intravenous dosage regimen of ciprofloxacin in cow calves would be 6 mg. kg^{-1} followed by 5 mg kg^{-1} repeated at 12 hr intervals

Key words: Calves, Ciprofloxacin, Dosage regimen, Plasma levels, Pharmacokinetics, Urinary excretion

Ciprofloxacin is one of the new fluoroquinolones being extensively used in humans as well as in animals.It is a broad spectrum antibacterial agent, used to treat bacteraemia, septicaemia and mild to severe bacterial infections of respiratory, urinary and reproductive tracts, cardiovascular system, skin, soft tissue, bones and joints (Goldstein and Citron 1993, Martiarena and Llorente 1993). The disposition kinetics of ciprofloxacin have been investigated in dogs (Abadia *et al.* 1994, 1995), ponies (Dowling *et al.* 1995) and buffalo (Raina 1993). In view of marked species variations in kinetic data for antimicrobial drugs, the present study was planned to determine the pharmacokinetics, urinary excretion and appropriate dosage regimen of ciprofloxacin in crossbred cow calves after a single intravenous administration.

MATERIALS AND METHODS

Experimental animals and drug administration

The experiments were performed on 6 healthy male crossbred calves in the age group of about 1-1.5 years and weighing between 75-102 kg. The animals were maintained under uniform conditions of management for 15 days before the commencement of experiment and were provided green fodder and water *ad lib.* Ciprofloxacin as ciprofloxacin lactate (2 mg.ml^{-1} was injected into the left jugular vein of each animal at the dose rate of 5 mg.kg^{-1} body weight, while an intravenous canula was passed and fixed in the contralateral jugular vein for collection of blood samples.

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Collection of blood and urine samples

Before the start of experiment, the animals were kept in metabolic stalls of standard size. The stalls were prepared in such a way that all the urine voided by animals could be collected over the chosen time intervals without any contamination or spillage. Following the administration of ciprofloxacin, blood samples (10 ml) were collected through the canula fixed in jugular vein into heparinized glass centrifuge tubes before and at 1, 2.5, 5, 7.5, 10, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16 and 20 hr after administration of the drug. The plasma was separated after centrifugation at room temperature, and stored at -20 ° C until it was analyzed. Total volume of urine voided by animals was collected at 1, 2, 4, 6, 8, 10, 12, 18 and 24 hr following administration of the drug. Sample of 20ml urine was filtered and stored for analysis.

Analytical procedure

The concentration of ciprofloxacin in the plasma and urine were determined by microbiological bioassay technique (Arret *et al.*1971) as modified by Srivastava and Bal (1994) using *E.coli* (ATCC 25922) as test organism.

The accuracy and sensitivity of estimation of ciprofloxacin was further enhanced by developing and fabricating a new Media Punching Machine (Developed and fabricated by A K Srivastava and Karamjeet Singh, Department of Pharmacol-

Table 1. Plasma levels ($\mu g ml^{-1}$) of ciprofloxacin in calves following a single intravenous dose of 5 mg. kg^{-1}.

Time after ciprofloxacin administration	Animal Number 1	2	3	4	5	6	Mean±SE
1 min	10.3	10.2	10.5	10.7	9.90	10.32	10.3±0.10
2.5 min	6.88	7.18	7.32	7.60	6.32	7.10	7.06±0.17
5 min	4.10	4.15	4.14	4.31	3.93	4.13	4.12±0.05
7.5 min	3.49	3.34	3.50	3.54	3.50	3.47	3.47±0.02
10 min	3.04	3.03	3.07	3.20	3.01	3.07	3.07±0.02
15 min	2.88	2.74	2.74	2.86	2.60	2.73	2.74±0.03
30 min	1.96	1.92	2.05	2.05	1.67	1.92	1.93±0.05
45 min	1.29	1.35	1.33	1.26	1.20	1.22	1.27±0.02
1 hr	1.03	1.07	1.04	1.06	0.97	1.05	1.03±0.01
1.5hr	0.83	0.85	0.84	0.80	0.78	0.68	0.79±0.02
2hr	0.58	0.62	0.59	0.59	0.60	0.53	0.58±0.01
3hr	0.38	0.37	0.37	0.39	0.39	0.34	0.37±0.007
4hr	0.24	0.20	0.23	0.23	0.22	0.20	0.22±0.006
6hr	0.14	0.15	0.14	0.14	0.15	0.13	0.14±.0.003
8hr	0.08	0.08	0.08	0.07	0.09	0.08	0.08±0.002
10hr	0.04	0.04	0.03	0.04	0.04	0.04	0.038±0.001
12hr	0.03	0.03	0.03	0.03	0.03	0.03	0.03±0.00

ogy and Toxicology, Punjab Agricultural University, Ludhiana). In this improved method only seed layer (20ml) was used and wells were punched by machine.By doing so, the diffusion of sample was homogenous and without any spillage. The minimum detection level of ciprofloxacin by this modified technique was 0.02 $\mu g.ml^{-1}$ The repeatability of result was 98.6% and correlation between concentration of drug and zone of inhibition was straight between 0.02 to 1.0 $\mu g.ml^{-1}$ of ciprofloxacin.The value of regression coefficient (γ)of straight line was 0.99. Various pharmacokinetic parameters were calculated by computed least square technique (Gibaldi and Perrier 1982). The pharmacokinetic parameters were calculated for each animal and the data are presented as mean ± SEM.

RESULTS AND DISCUSSION

The mean concentrations of ciprofloxacin in the plasma at different time intervals are given in Table 1. At 1 min the drug concentration was 10.3±0.10 $\mu g.ml^{-1}$ which rapidly declined to 3.07±0.02 $\mu g.ml^{-1}$ at 10 min. Thereafter the concentration of ciprofloxacin fell gradually and were detected up to 12 hr (0.03$\mu g.ml^{-1}$).

The minimum therapeutic concentration (MTC) of fluoroquinolones for its therapeutic effect have been found to be in the range of 0.02 - 0.5 $\mu g.ml^{-1}$ (Leysen *et al.*, 1989). Keeping in view the influences of different unavoidable factors *in vivo*, in the present study, a concentration of 0.1 $\mu g.ml^{-1}$ was taken as MTC of ciprofloxacin. Following single intravenous administration of ciprofloxacin,in plasma a concentration of $\geq$ 0.1 $\mu g.ml^{-1}$ was maintained for 6 hr. The semilogarithmic plot of observed plasma concentration of ciprofloxacin against time showed distinct distribution and elimination phases and hence data was best fitted in 2 compartment open model and adequately described in terms of bi-exponential equation

$$Cp = Ae^{\alpha t} + Be^{-\beta t}$$

Where Cp is the concentration at time t, A and B are plasma concentrations at zero time intercepts of distribution and elimination phases, respectively, α and β are distribution and elimination rate constants respectively.

Various pharmacokinetic parameters of ciprofloxacin were calculated and presented in Table 2. The rapid decline of plasma concentration of ciprofloxacin is attributed to the rapid distribution of drug into the tissues. This is corroborated by high values of distribution rate constant (α) and T/P ratio.The high values of AUC (3.98±0.05 $\mu g.ml^{-1}.hr$) and AUMC reflects that most of the body area is covered with drug. Hong *et al.* (1995) have reported that in male volunteers the value of AUC of ciprofloxacin was 7.06±1.21 $mg.L^{-1}.hr$. Further the calculated value of apparent volume of distribution (6.8 ±0.15 L. kg^{-1}) indicated that there is an extensive penetration of ciprofloxacin into various body fluids and tissues. Abadia *et al.* (1994) has reported the value of $Vd_{(area)}$ of ciprofloxacin as 3 $L.kg^{-1}$ in dogs following its single intravenous administration.

Among all the pharmacokinetic parameters, the most important one is elimination half life ($t_{1/2\beta}$). In the present study, the elimination half life of ciprofloxacin in cow calves was calculated to be 0.18±0.003 hr. As compared to present findings in calves, the $t_{1/2\beta}$ of ciprofloxacin in dogs (Abadia *et al.* 1994, 1995) and pigs (Nouws *et al.* 1988) were found to

Table 2. Pharmacokinetic parameters of ciprofloxacin in crossbred calves (n=6) after a single intravenous administration (5mg.kg^{-1})

Parameter[1]	Unit	Mean±SEM
A	μg.ml^{-1}	6.24±0.08
α	hr^{-1}	3.27±0.06
B	μg.ml^{-1}	0.38±0.02
β	hr^{-1}	0.18±0.003
$t_{1/2\alpha}$	hr	0.21±0.003
$t_{1/2\beta}$	hr	3.75±0.06
K_{12}	hr^{-1}	1.46±0.07
K_{21}	hr^{-1}	0.36±0.01
Kel	hr^{-1}	1.66±0.03
Cl_B	L.kg^{-1}.hr^{-1}	1.24±0.02
AUC	μg.ml^{-1}.hr	3.98±0.05
AUMC	μg.ml^{-1}.hr^2	11.20±0.03
Vd (area)	L.kg^{-1}	6.80±0.15
Vd (B)	Lkg^{-1}	13.1±0.60
Vd(ss)	L.kg^{-1}	3.78±0.09
T/P	Ratio	8.01±0.22
MRT	hr	2.96±0.06

[1]The kinetic parameters are as described by Gibaldi and Perrier (1982). A = Zero-time plasma drug concentration intercept of regression line of distribution phase; α = Overall distribution rate constant; B = Zero-time plasma drug concentration intercept of regression line of elimination phase; β = Elimination rate constant; $t_{1/2\beta}$ = Elimination half life; K_{12} = Rate of transfer of drug from central to peripheral compartment; K_{21} = Rate of transfer of drug from peripheral to central compartment; Kel = The first order elimination rate constant from central compartment; Cl_B = The total body clearance of drug; AUC and AUMC = Total area of body which is under the plasma drug concentration-time curve and its first moment; Vd(area), Vd(B), Vd(ss) = Volume of distribution calculated on the basis of AUC, zero time plasma drug concentration intercept of elimination phase and average steady state plasma level, respectively; T/P = tissue/plasma ratio of drug concentration; MRT = Mean residential time.

be 129-180 min and 2.5 hr respectively.In the present investigation, the mean value of total body clearance of ciprofloxacin was 1.24 ±.0.017 L.kg^{-1}.hr^{-1} whereas in ponies it was 18.24±3.99 ml.kg^{-1}.hr^{-1} after its single intravenous administration (Dowling *et al.*1995)

The results on urinary excretion of ciprofloxacin are presented in Table 3. The concentration of ciprofloxacin in urine from 1-24 hr was several fold higher than the required MIC (0.1 μg.ml^{-1}).Approximately 18% of the total administered dose of ciprofloxacin was recovered in urine within 24 hr after its single intravenous administration. The results revealed that ciprofloxacin may be the drug of choice for the treatment of urinary tract infections.

The ultimate objective of the disposition kinetics study was to determine an appropriate dosage regimen of ciprofloxacin. Using a convenient dosage interval, the calculation of maintenance dose (D^1) is based on the minimum inhibitory concentration {Cp(min)$^\infty$} and may be calculated

Table 3. Urine concentration and excretion of ciprofloxacin in crossbred cow calves after its single intravenous administration of 5 mg.kg^{-1}

Excretion interval (hr)	Urine concentration (μg.ml^{-1})	Percentage of total dose excreted
0-1	57.50±8.90	2.54±0.49
1-2	59.60±10.70	4.61±1.60
2-4	45.33±7.10	4.82±1.04
4-6	43.80±5.80	2.76±0.40
6-8	25.10±4.80	1.94±0.23
8-10	14.80±0.80	0.66±0.16
10-12	8.40±0.30	0.71±0.17
12-18	5.40±0.90	0.74±0.18
18-24	6.80±3.50	1.50±0.99
0-24	Total =	17.89±2.54

Table 4. Priming (D) and maintenance (D^1) doses (mg.kg^{-1}) of ciprofloxacin in calves at various dosage intervals for microorganisms of different susceptibility.

Micro-organism Susceptibility, MTC (μg.ml^{-1})	Dosage interval					
	6		8		12	
	D^1	D	D^1	D	D^1	D
0.1	1.3	2.0	2.2	2.9	5.2	5.9
0.2	2.6	4.0	4.4	5.7	10.4	11.8
0.3	4.0	6.0	6.6	8.6	15.6	17.7
0.5	6.0	10.0	10.9	14.3	26.0	29.5

from the formula (Baggot 1997)

$$D^1=Cp\ (min)^{\infty}.Vd_{(area)}\ (e^{\beta\tau}-1)$$

Where β is the elimination rate constant and γ is the dosage interval. The priming dose is calculated by omitting the -1 from this equation.

Taking different dosage intervals, and using values of β and $Vd_{(area)}$ from Table 2, the priming and maintenance doses of ciprofloxacin were calculated for maintaining various MTCs and presented in Table 4.

On the basis of Table 4, it is suggested that the most appropriate dosage regimen for ciprofloxacin in calves would be 6 mg.kg^{-1} (as loading/primary dose) followed by 5 mg. kg^{-1} (as maintenance dose) at 12 hr intervals.

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Indian Journal of Animal Sciences **70** (10): 1025-1027, October 2000

Angiography of renal artery with special reference to the blood supply of the kidney in camel

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Received: 8 September 1999; Accepted: 23 March 2000

ABSTRACT

The vascular system of kidney of the camel is strikingly different from that of other domestic animals. The right and left renal arteries emanated independently from the ventrolateral aspect of abdominal aorta behind cranial mesenteric artery at the level of cranial border of third and fourth lumbar vertebra respectively. The renal artery after short lateral course divided into medial and lateral lobar arteries before reaching the hilus. The lobar artery after entering into the hilus gave 9-11 interlobar arteries. These interlobar arteries at the corticomedullary junction formed 2 - 7 arcuate arteries. The arcuate arteries detached numerous straight branches from their convex surfaces and then ended by dividing into 2 - 4 terminal branches. The straight branches and the terminal branches were present in a radiating manner as interlobular arteries.

Key words: Angiography, Camel, Kidney, Renal artery

The amount of blood that flows continuously through kidney is very large. Therefore, it becomes necessary to carry out a full investigation of blood vascular system of the kidneys in camel. The normal topographic anatomy of blood vessels of kidney is prerequisite for any surgical procedure on the area involved. Therefore, a treatise on the normal topographic anatomy of blood vessels of kidney is essential and important for veterinarians.

MATERIALS AND METHODS

Apparently healthy adult camels (4) of either sex were embalmed with 10% formalin solution (Grossman 1959). The abdominal aorta was exposed and bilateral origin, course and distribution of the renal artery were recorded *in situ*. Then kidneys along with blood vessels at the level of abdominal aorta were segregated from the carcass. The renal artery was cannulated and arterial system was flushed by injecting lukewarm heparinized saline solution (100 IU/100 ml). A radio-opaque suspension (20% lead oxide in liquid soap) was injected by steady and constant digital pressure. After satisfactory filling of the vessels, the organ was radiographed at 8 Mas, 50 KVP and 900mm FFD in different profiles (M/L, A/P and O/O) to obtain the arteriographs depicting the course and branching pattern of the vessels. The specimens were placed in deep freeze at 4°C for 24 to 48 hr and sectioned longitudinally and approximately 5 mm thick sections were cut. These sections were radiographed to study the intraparenchymal ramifications of the renal artery.

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RESULTS AND DISCUSSION

The vascular system of the camel kidney was strikingly different from that of other domestic animals. The right and left renal arteries emanated independently from ventrolateral aspect of abdominal aorta behind cranial mesenteric artery (Fig 1). The vessels arose at the level of the cranial border of third and fourth lumbar vertebrae respectively. Smuts and Bezuidenhout (1987) reported that the renal artery of camel arises from abdominal aorta at the level of fourth lumbar vertebra, while Beniwal (1995) demonstrated the origin of right and left renal arteries at the level of second and third lumbar vertebra respectively. In bovine calves, right and left renal arteries emanate close together from lateral aspect of abdominal aorta on the respective sides at the level of second lumbar vertebra (Jain and Singh 1987b), while Ghosh and Datta (1990) reported that the right renal artery arises slightly cranial to the left one in cow. The renal arteries in donkey arise from lateral aspect of abdominal aorta close to anterior mesenteric artery at the level of second lumbar vertebra (Jain and Dhingra 1986). Nickel *et al.* (1981) reported that renal

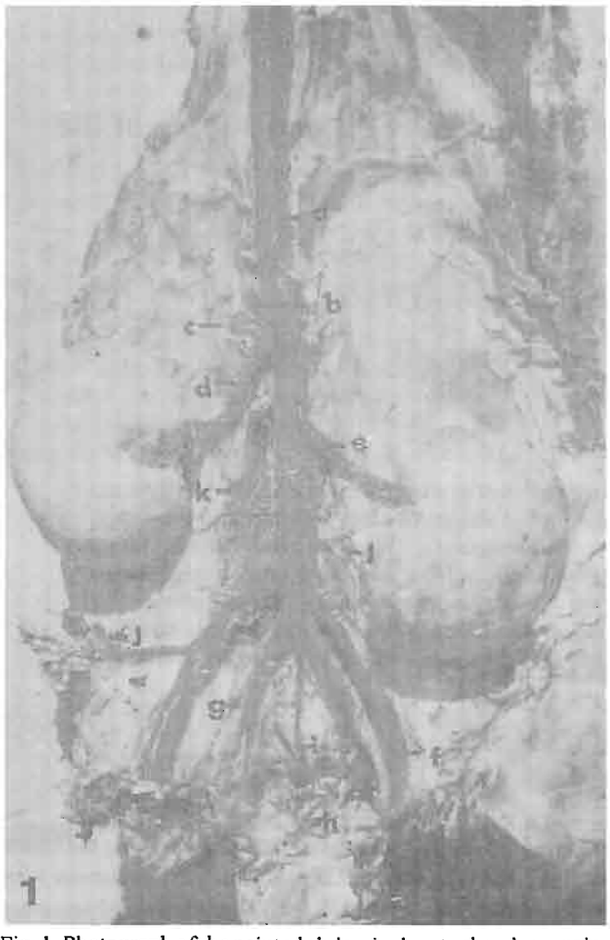


Fig. 1. Photograph of the painted abdominal aorta showing origin of renal arteries.a, abdominal aorta; b, coeliac artery; c, cranial mesenteric artery; d, right renal artery; e, left renal artery; f, external iliac artery; g, internal iliac artery; h, caudal mesenteric artery; i, middle sacrale; j, deep circumflex iliac; k, right testicular artery; l, left testicular artery.

arteries arise at the level of first lumbar vertebra in horse, whereas Ghoshal (1977) described their origin just near cranial mesenteric artery in equines.

The right renal artery arose 5.5±0.7 cm cranial to left one (Fig.1). The right and left renal arteries arose about 0.5±0.02 cm and 6.0±0.32 cm caudal to the cranial mesenteric artery respectively. Similarly, average distance of right and left renal arteries from coeliac artery was 1.03±0.17 cm and 6.5±0.58 cm respectively. The right and left renal arteries arose 9.6±0.87 cm and 5.0±0.25 cm cranial to testicular arteries repectively. The distance of former from caudal mesenteric and external iliac arteries measured 18.1±1.67 cm and 17.8±1.20 cm while that of the latter was 12.5±0.82 cm and 12.2±1.28 cm respectively. The right renal artery (7.0±1.07cm) was longer than that of left one (4.7±0.43cm).

Each renal artery after a short lateral course divides into 2 lobar arteries before reaching renal hilus. The average diameter of the right and left renal arteries was 1.01±0.17 cm and 0.70±0.04 cm respectively. The average length of the lobar arteries of right and left sides was 5.0±0.3 cm and 4.5±0.04 cm respectively. Jain and Singh (1987a, b) described that each renal artery on reaching the renal hilus divides into cranial and caudal lobar arteries in bovine calf. In donkey, each renal artery divides into 5 to 8 lobar branches before entering into the hilus (Jain and Dhingra 1986).

Each lobar artery after entering into renal hilus formed a shallow curve and finally tapered down and ramified 5 into the kidney (Figs 2,3). It gave off 9 - 11 branches of various caliber in renal medulla at different levels from hilus (Figs 2,3). These are referred to as the interlobar arteries. These interlobar arteries issued several small tapering branches of various caliber which were responsible for the medullary circulation (Figs 2, 3). Occasionally, intra-arterial anastomoses were also observed between these branches. Jain and Singh (1987a, b) described that each lobar artery divides into 3 to 6 interlobar arteries in bovine calves and 4 to 8 interlobar arteries in goat. Motwani and Harneja (1982) named these branches as segmental arteries. Jain and Dhingra (1986) described that each lobar artery divides into 2-4 interlobar arteries in donkey.

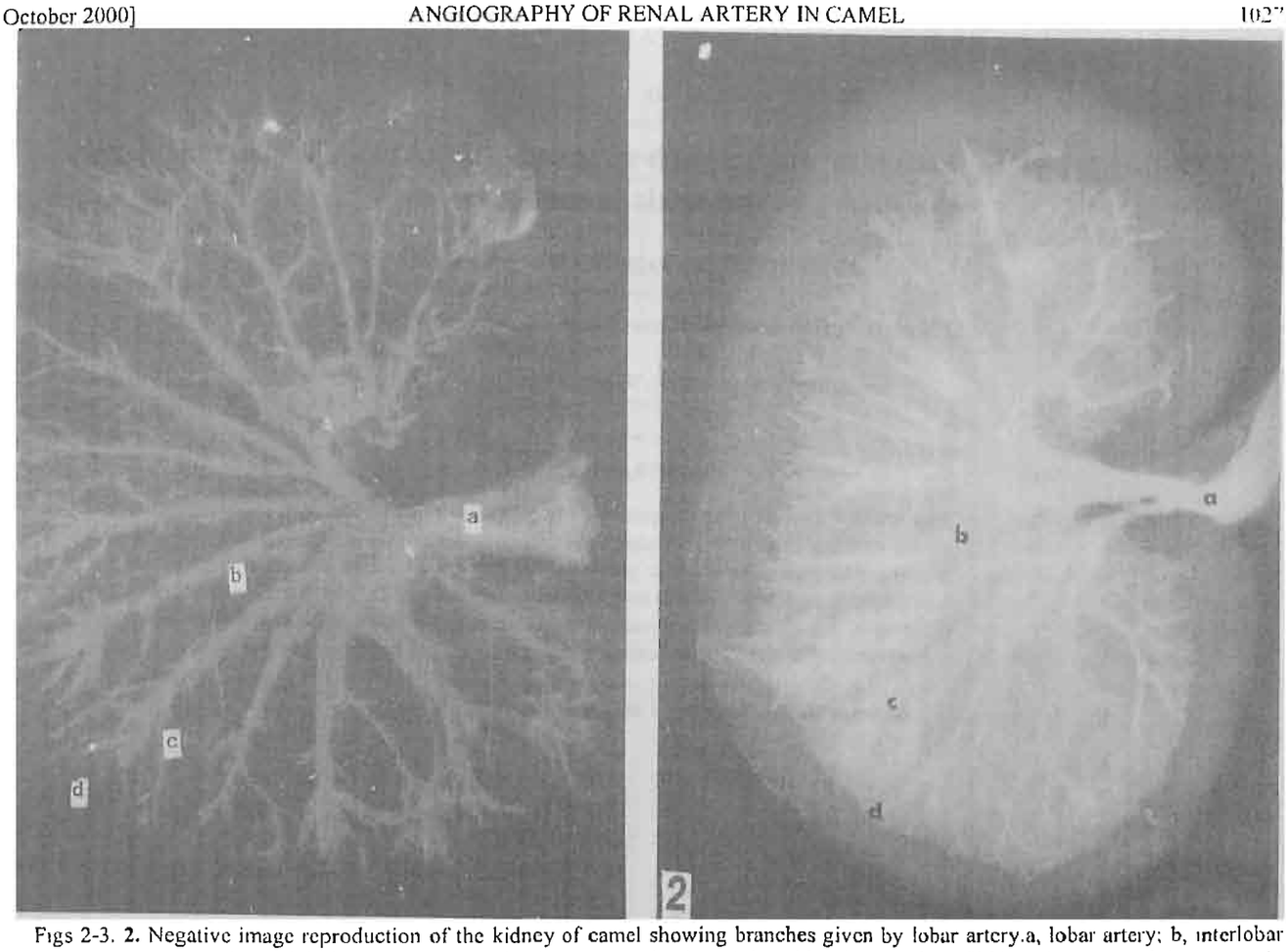
The interlobar arteries then coursed towards the periphery of the kidney and at the corticomedullary junction formed several arched branches (2-7) called arcuate arteries (Figs 2, 3). These vessels curved in various directions at different angles to their parent vessels (acute to right angles). The arcuate arteries followed the curvature of base of the medullary pyramids. Jain and Singh (1987b) observed similar findings in bovine calf. Trautmann and Fiebiger (1952) named them as subcortical arteries. Similarly Jain and Dhingra (1986) stated that interlobar arteries form several arched branches referred to as arcuate arteries in donkey.

The arcuate arteries detached numerous straight branches from its convex surfaces and then ended by dividing into 2-4 terminal branches. These straight branches and terminal branches were present in a radiating manner described as the interlobular arteries. In addition to these, arcuate arteries also gave off 2-5 minute long branches to the renal medulla. At few places the intra-arterial anastomoses were also observed between adjoining arcuate arteries (Figs 2, 3). El-Shaieb *et al.* (1981) observed similar findings in camel, while Jain and Singh (1987b) stated that the cortical surface of arcuate arteries in bovine calves gives numerous interlobular arteries which supply cortex and capsule of kidney, and there is no intra-arterial anastomosis between these vessels.

The interlobular arteries were fine, straight and nearly lay at a right angle to the arcuate arteries (Figs 2, 3). These interlobular arteries supplied the renal cortical parenchyma and capsule of the kidney.

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Figs 2-3. **2.** Negative image reproduction of the kidney of camel showing branches given by lobar artery.a, lobar artery; b, interlobar artery; c, arcuate artery; d, interlobular artery. **3.** Negative image reproduction of the midsagittal section of kidney in camel.a, lobar artery; b, interlobar artery; c, arcuate artery; d, interlobular artery.

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Indian Journal of Animal Sciences **70** (10): 1028-1031, October 2000

Angiography of testicular artery with special reference to the blood supply of the testis in camel

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Received: 27 August 1999; Accepted: 23 March 2000

ABSTRACT

The right and left testicular arteries in camel arose independently from the ventrolateral aspect of abdominal aorta at the level of fourth or fifth lumbar vertebra. Both arteries although had different relations and course inside the abdominal cavity had similar inguinal and post inguinal course. The artery described a great number of convolutions in close apposition to each other, thus formed an elongated vascular cone with its apex at the superficial inguinal ring and the base on the caputal extremity of the testis. As testicular artery reached caputal extremity of testis it showed 2 patterns in its course. The intratesticular arborization of testicular artery consisted of centripetal and centrifugal arteries.

Key words: Angiography, Camel, Testicular artery, Testis

The normal topographic anatomy of blood vessels of testis is a prerequisite for any surgical procedure on area involved. Any abnormality or obstruction of arterial supply not only affect physical efficiency of the animal but also tells upon its productive and reproductive capacity. Therefore, a treatise on the normal topographic anatomy of arterial vessels of this organ is very essential and important for veterinarians. Hence the present study was undertaken.

MATERIALS AND METHODS

Apparently healthy adult male camels (4) were embalmed with 10% formalin solution (Grossman 1959). The abdominal aorta was exposed to the vessels. The organ was radiographed at 8 Mas, 50 KVP and 900 mm FFD in different profiles (M/L, A/P and O/O) to obtain arteriographs depicting the course and branching pattern of the vessel.

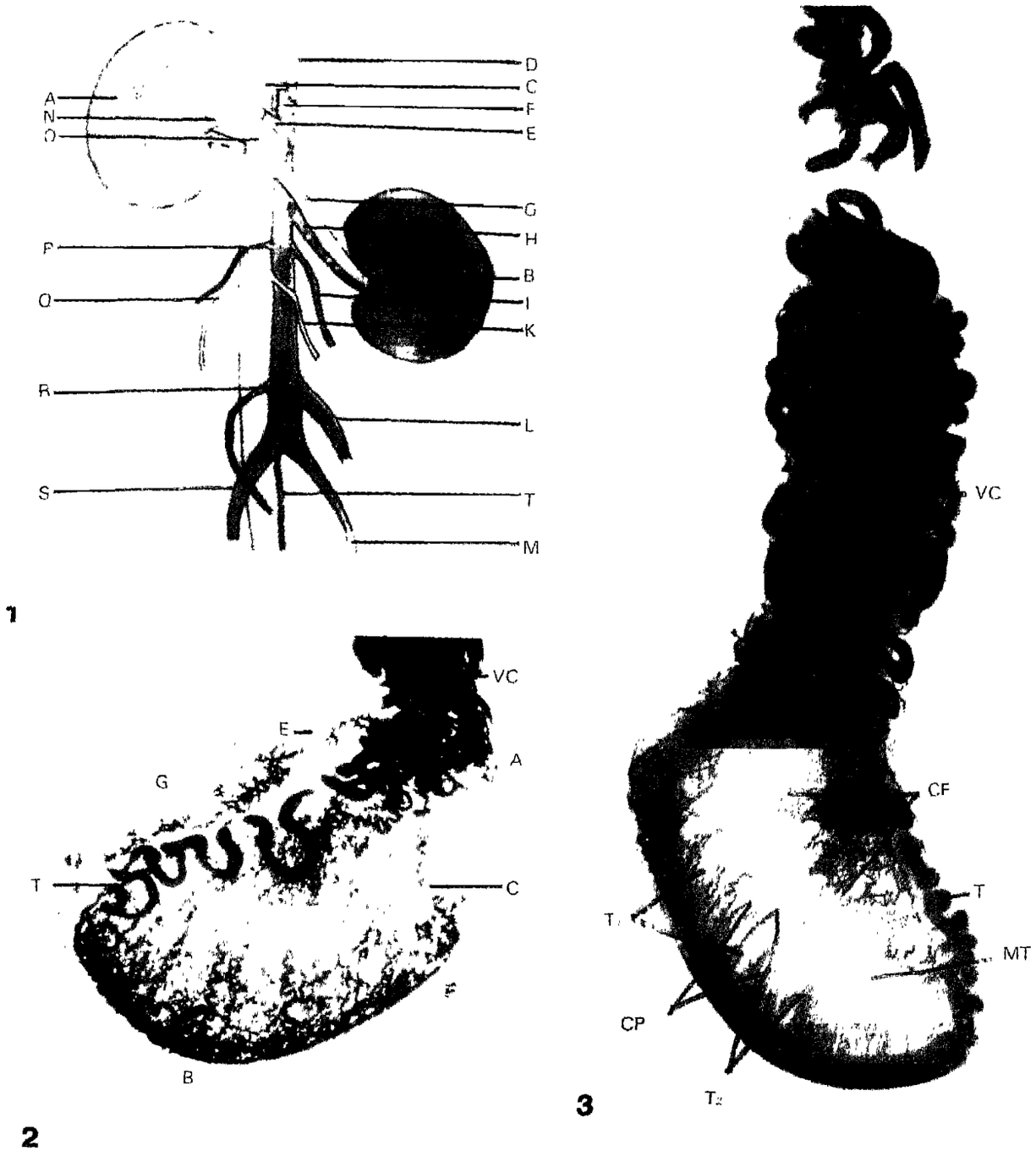
The specimens were placed in deep freezer at 4°C for 48 hr. The testes were sectioned longitudinally, and approximately 5 mm thick sections were cut. These sections were radiographed to study the intraparenchymal ramifications of testicular artery.

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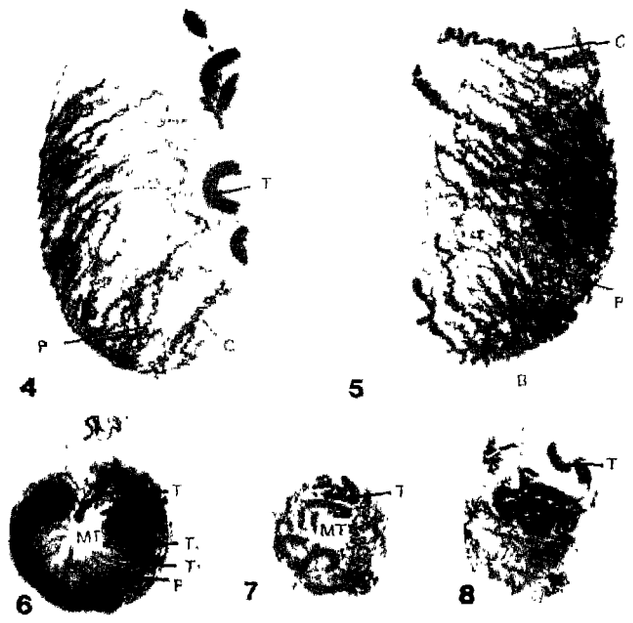
RESULTS AND DISCUSSION

The testicular artery of camel is a long slender vessel which supplied testis and epididymis of the corresponding sides. The arteries of both sides arose independently from ventrolateral aspect of abdominal aorta at the level of fourth or fifth lumbar vertebra. Generally right testicular artery arose at the level of fourth lumbar vertebra while the left one arose at the level of fifth lumbar vertebra. On an average, right testicular artery arose 1.5±0.2 cm cranial to contralateral vessel of left side (Fig.1). However, in animal both right and left testicular arteries emanated at the same level ventral to cranial half of fifth lumbar vertebra. Osman *et al.* (1979) reported that testicular artery of camel arises from abdominal aorta just cranial to its bifurcation, while Smuts and Bezuidenhout (1987) described that left and right testicular arteries arise at the level of fifth lumbar vertebra from ventral aspect of abdominal aorta. Ghoshal (1977) stated that in ox the right testicular artery originates slightly cranial to left artery approximately mid-way between renal and caudal mesenteric arteries. On the contrary, Kock (1970) reported that the left testicular artery in ox usually originates cranial to the right one. Sisson and Grossman (1953) reported variations in origin of testicular 4 artery in ox. They stated that the testicular artery may arise from caudal mesenteric or renal artery or as a common trunk with contralateral vessel from ventral face of abdominal aorta close to caudal mesenteric artery. However, such variations were not observed in camel during present investigation.



Figs 1-3 **1.** Schematic representation showing the origin of testicular arteries in camel. A, right kidney; B, left kidney; C, posterior vena cava: D, abdominal aorta; E, cranial mesenteric artery; F, coeliac artery; G, left renal vein, H, left renal artery; I, left testicular artery; K, left testicular vein; L, left external iliac artery; N, right renal vein, O, right renal artery; P, right testicular artery; Q, right testicular vein, R, caudal mesenteric artery; M, right internal iliac artery; S, left internal iliac artery; T, middle sacral artery. **2.** Negative image reproduction of the arteriograph of testis in camel.A, caputal extremity; B, caudal extremity; C, capsular arteries; E, epididymal artery, F, free border of testis; G, epididymal border of testis; T, testicular artery; VC, vascular cone. **3.** Negative image reproduction of the arteriographs of mid sagittal.

Although the right and left testicular arteries had different relations and course inside abdominal cavity, they had similar inguinal and post inguinal course. The arteries of both sides ran a straight abdominal course up to the level of deep inguinal



Figs 4-8. Parasagittal. (4, 5) and cross sections (6, 7, 8) of the testis in camel CP, centripetal arterics; CF, centrifugal arteries; T_1, type I centripetal arteries, T_2, type II centripetal arteries; T, testicular artery; P, parenchymal branches; MT, mediastinum testis; VC, vascular cone.C, capsular artery.

ring, where it became embedded in vascular fold of the tunica vaginalis.

The right testicular artery after its origin diverged caudo-ventrally and crossed caudal vena cava and right ureter. The artery passed over psoas major muscle in company with satellite vein enclosed in a superficial fold of peritoneum. It traversed downward and backward in relation with external iliac artery and vein and then after a short course crossed deep circumflex iliac artery to reach deep inguinal ring through which it entered inguinal canal. Inside canal, the artery pursued a convoluted course and incorporated in spermatic cord. The left testicular artery after its origin from aorta traversed caudolaterally for 5.1 cm and crossed ventral face of the left ureter. Enclosed in a fold of peritoneum, it descended along a groove between the psoas major and quadratus lumborum muscles up to the level of the external iliac artery. The artery during this course was related to the caudodorsal blind sac of the rumen, duodenum and descending part of the colon in its intra- abdominal course. It crossed the external iliac artery and then deviated laterally to reach the deep inguinal ring. It then entered the inguinal canal to follow its inguinal course through the deep inguinal ring. Similar findings were observed by Osman *et al.* (1979)in camel. In ox, the artery passes over the ureter and circumflex iliac vessels to reach the inguinal canal of its side and is accompanied by the satellite veins, lymph vessels and nerves in spermatic cord (Sisson and Grossman 1953). However, Ghoshal (1977) reported that both the testicular arteries in their abdominal course cross the ventral surface of corresponding sublumbar muscles and ureter to become one of the constituent of spermatic cord at the inguinal ring.

The course of the right and left testicular artery was similar inside the inguinal canal. It pursued slightly convoluted course and was incorporated in the spermatic cord. It passed down in the inguinal canal in company with testicular vein, lymphatics, nerve and external cremaster muscle. As the testicular artery emerged out of the superficial inguinal ring, it exhibited a remarkable highly tortuous disposition. It described a great number of convolutions in close apposition to each other, thus forming an elongated vascular cone with its apex at the superficial inguinal ring and the base on the caputal extremity of the testis (extremitas capitata) (Figs 2, 3). The testicular artery at the level of apex of vascular cone gave rise to several convoluted branches of variable length and size. These vessels supplied coverings of spermatic cord, epididymis and vas deferens (Figs 2, 3). The vascular cone was surrounded by numerous veins of different diameter which formed pampiniform plexus. Kirby (1953) stated that testicular artery in bulls rarely divides into 2 branches soon after leaving superficial inguinal ring.

In general, the testicular artery reached caputal extremity of testis, it enlarged and became more compactly coiled and less tortuous. It coursed along epididymal border of testis to become sub albugineal and partly covered under body of the epididymis. It reached caudal extremity of testis and turned around it. Here, it divided into 3 - 4 branches which ascended along free border of testis (Figs 2, 3). Each branch issued 5-8 convoluted branches for tunica albuginea referred to as capsular arteries (Figs 4, 5).

In 1 animal testicular artery became straight as it reached the caputal extremity of testis. It passed along epididymal border of testis covered under body of epididymis and reached caudal extremity. It turned around it and ascended along the free border by dividing into 3 - 4 branches. Borthakur and Dhingra (1979) reported in buffalo that the testicular artery divides dichotomously at caudal extremity of testis.

The capsular arteries gave off numerous fine branches which penetrated and arborized through testicular septa. The intratesticular arborization of these vessels consisted of centripetal and centrifugal arteries (Figs 3, 6, 7). The centripetal arteries arose from capsular arteries and ran towards mediastinum. These branches also emanated further branches during their course for testicular parenchyma (Figs 4, 5). The centripetal arteries, based on differences in their intraparenchymal course were further classified into 2 types.

Type I: These arteries were short and did not reach up to mediastinum testis, rather they terminated as peripheral vessels invading outer three-fourth part of testis (Figs 3, 6, 7).

Type II: These arteries were comparatively larger in size and reached mediastinum to arborize there (Figs 3,6,7). The

centrifugal arteries resulted by reflection of centripetal arteries from mediastinum towards periphery. These branches during their course arborized with the branches given by the centripetal arteries and thus resulted into a few arterial clusters, somewhere between capsule and mediastinum testis (Figs 3,6). Dhingra (1979) reported that centripetal arteries of buffalo form a cluster of spirally coiled vessels along the longitudinal axis of mediastinum testis before turning as centrifugal arteries. However, Kirby (1953) did not find such an arrangement in testis of bull. Setchell (1970), in bull, ram and pig described non-branching centripetal arteries and branching centrifugal arteries in testis. Dhingra (1979) described angle bending of the centripetal arteries at mediastinum testis in bull.

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Indian Journal of Animal Sciences 70 (10): 1032-1034, October 2000

Semen production performance of Frieswal bulls

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Received: 10 August 1999; Accepted: 27 March 2000

ABSTRACT

Semen production performance of Frieswal bulls revealed that out of 186 bulls, 173 (93.01%) ejaculated semen in artificial vagina and only 78 (45.09%) of these 173 bulls produced freezable semen. Rest of the 95 (54.91%) bulls had either very poor initial quality semen or semen with poor freezability. Thin to very thin, azoospermic and less than +3 initial motility semen producing bulls were 41.05, 7.36 and 47.36% respectively. Four (4.21%) bulls though had satisfactory initial semen quality but had poor freezability. The overall age was 628.91 21.26 days and weight at first semen donation 399.3±8.13 kg in Frieswal bulls (Holstein-Friesian × Sahiwal).

Key words: Crossbred bulls, Reproductive wastage

Introduction of exotic breeds for crossbreeding our indigenous cattle was done to increase the milk production. Accordingly the use of crossbred bulls in breeding programme became important. Although a large number of male calves of high genetic merit are selected but only a few actually reaches the breeding stage (Sethi *et al.* 1989). The information on semen characteristics of crossbred bulls is very meagre. It was realized that beside genetic selection earlier in life rigorous and meticulous culling of inferior males at regular intervals and earliest elimination of poor performers are of immense importance for achieving desired goals in milk production and development of new breed. The present investigation was carried out to study the reproductive performance specially the ability to donate freezable quality semen in Frieswal bulls.

MATERIALS AND METHODS

Frieswal bulls (200; HF × Sahiwal, 62.5% exotic inheritance) born between 1989 to 1995 at different Military Farms, located in diverse agroclimatic conditions and maintained under standard feeding and management conditions of bull rearing unit of Project Directorate on Cattle, Meerut, were introduced to training for semen collection at the age of 15 to 18 months. Weekly collections were made from young bulls after their first ejaculation whereas collections were obtained twice in 1 week after the bulls attained the age of 24 months. Semen samples were analyzed for volume, colour, motility, sperm concentration and per cent live sperm. Ejaculates with more than 60% initial progressive motility were diluted in egg yolk tris diluent with 7% glycerol and were equilibrated for 4 hr before horizontal vapour freezing in 0.25ml french straws for 10 min. Semen was evaluated 24hr after freezing for post-thaw revival of sperm motility. Data obtained on various parameters of age and weight for first ejaculation and freezing were analyzed using student t test (Snedecor and Cochran 1971).

RESULTS AND DISCUSSION

Out of 186, 13 (6.99%) bulls maintained up to 2 years or more did not ejaculate the semen in artificial vagina. Libido in these bulls was poor. Percentage of such bulls in Brown Swiss crosses was reported 11.5% at 50% exotic inheritance and 1.9% in purebreds (Mathew *et al.* 1982). Our results of poor libido are in agreement to Rao and Rao (1991). However, higher values of poor libido up to 30% were observed in indigenous breeds (Surya prakasam and Narasimha Rao 1993).

In this study certain minimum standards like initial motility (≥60%), sperm concentration (≥600×106/ml) and post-thaw motility (≥45%) were strictly adhered and semen samples below these standards were rejected. Out of 173, 54.91% bulls produced poor quality semen and semen from 45.09% (78) bulls could only be frozen. The poor quality and freezability of crossbred bull semen are well documented although definite reasons for this have not been ascertained. Rao and Rao (1991) observed that higher percentage of crossbred bulls had questionable and unsatisfactory breeding soundness. Similar observations were also made by Deka *et al.* (1982). The rejection rate in bulls was even higher than the present study

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Table 1. Yea wise number of bulls with poor initial semen quality

Attributes	Year								Total percentage	
	1989-90	1991	1992	1993	1994	1995	1996	1997		
Bull donating semen	22	13	32	23	07	34	24	21	173	93.01
Bulls with poor semen quality	07	05	21	15	04	16	13	14	95	54.91
Thin to very thin semen	02	02	08	03	01	09	07	07	39	41.05
Watery semen (azoospermia)	-	-	-	02	-	02	02	01	07	7.36
Mass activity <+3 (0-5 scale)	05	03	11	08	03	05	04	06	45	47.36
Poor freezability (mass activity ≥+3 but poor freezabiltiy)	-	-	02	02	-	-	-	-	04	4.21

Out of 39 bulls which produced thin to very thin semen, 11 bulls had initially dead semen.

Table 2. Average age and weight at first ejaculation and semen freezing of Frieswal bulls

Character	During the period		Overall
	1989-94	1995-97	
Age of introduction to training (days)	540	450	
Age at first ejaculation (days)	726.1±34.49** (31)	534.9±9.12 (32)	628.9±21.26 (63)
Weight at first ejaculation (kg)	400.1±11.62 (31)	398.6±11.56 (32)	399.3±8.13 (63)
Age at first semen freezing (days)	931.8±31.06** (31)	668.3±22.14 (32)	788.0±25.18 (63)
Weight at first semen freezing (kg)	500.6±13.56 (31)	479.5±17.60 (32)	489.9±11.15 (63)

** (P<0.01), values in parenthesis indicate number of animals.

(Mathew *et al.* 1982). They opined that the semen production is best with the purebred exotic animals and declines as the percentage of exotic blood in crossbreds decreases. The genotype of individual bull may also effect the semen production profile of crossbred bulls. Patel *et al.* (1997) observed that crossbreeding of Holstein-Friesian (HF) with Kankrej and Gir is well suited in terms of semen quality parameters, however, the performance is poor with crossbred developed with Sahiwal. Similarly semen quality parameters in Brown Swiss and Jersey crosses were good compared to crossbred HF (Rao and Rao 1978, Sagdeo *et al.* 1990). The present study revealed that 41.05% of the culled bulls (Table 1) had thin to very thin semen having less than 600 million sperm/ml; 7.36% bulls produced azoospermic ejaculates with watery consistency; 47.36% bulls had apparently normal semen, however, the sperm motility in these bulls ranged from 20% to just below 60%. The initial semen quality in 4.21% bulls although satisfied the prefreezing standards but semen could not be frozen due to poor post-thaw revival (<45%). The poor semen picture in the majority of culled bulls in the present study is indicative of some testicular anomaly.

The average age of first semen donation in Frieswal bulls was 628.9± 2.26 days which is earlier than the reported values of 705 days by Sethi *et al.* (1989) in crossbred bulls. However, Singh *et al.* (1996) reported age at first donation at an early age of 62.0 ± 1.29 and 64.0 ± 1.91 weeks from Jersy ×Kankrej (J × K) and J × K interse animals. The earliest semen donation at the age of 415 days in the present study, is in agreement with them.

Although age at first semen donation and freezing differed significantly, the body weight did not differ between groups (1989-94 vs 1995-97) in this study. Frieswal bulls ejaculated first time on attaining an average 399.3±8.13 kg body weight and produced freezable semen on further putting up about 90 kg. In crossbred animals, Sethi *et al.* (1989) and Singh *et al.* (1996) could get the first semen donation on attaining 363 and 267 kg body weights respectively. The differences in body weight at first semen donation may be due to differences in the breeds involved, plane of nutrition and other environmental factors which influences the onset of puberty.

The percentage of Frieswal bulls producing freezable semen are comparable with the results of Sethi *et al.* (1989) and Rao and Rao (1991) but higher to that reported by Sagdeo *et al.* (1992). About 30-50% of crossbred bulls produce semen which is not suitable for cryopreservation (Bhavsar 1993, Sahni and Mohan 1998). Variation in spermatozoal resistance

to hypertonic solutions (Maule 1962) may contribute between bull differences in freezability. Pangawkar and Sharma (1989) did not found significant differences in physical characteristics (volume, initial motility, live sperm% and sperm concentrate/ ejaculate) between normal, medium and poor freezable HF bull semen. On the contrary, significant differences in semen volume, mass activity and parity of dam between bulls giving freezable and non-freezable semen were observed by Sethi *et al.* (1989). Present study thus indicates that a large proportion of crossbred bulls produces poor quality semen which is not suitable for freezing. Therefore, for the success of such programmes large number of crossbred male calves should be reared to meet the requirements.

ACKNOWLEDGEMENTS

Authors are thankful to the Director, Project Directorate on Cattle and Frieswal Project for providing necessary facilities.

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Indian Journal of Animal Sciences **70** (10): 1035-1046, October 2000

Follicular atresia in goat: A review

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Received: 8 November 1999; Accepted: 25 April 2000

ABSTRACT

Follicular atresia causes extensive loss of germ cells during development, and reproductive life in female mammals. The primordial follicles are most affected during embryonic life while antral follicles out number in atresia during the active life. Atresia is a fundamental process that modulates the success of folliculogenesis and the number of ovulations. Both morphological and functional events of follicular atresia have been discussed. The morphological (including ultra structure) and histochemical characteristics of atresia include alteration of extracellular matrix, loosening of cell to cell interactions, pycnosis, chromolysis, hyalinization, appearance of crystalloid bodies, chromophilic granules, pseudocleavage, mitochondrial aggregations, shrinking of plasma membranes, hyalinization of zona pellucida etc. These morphological features are associated with a decline in the sensitivity of granulosa cells to gonadotrophins, degeneration by apoptosis, loss of cell to cell communication, loss of proliferative and steroidogenic activities. Endocrinologically, the estradiol production declines, progesterone titre changes, FSH-binding receptors decrease, FSH-binding inhibitors increase, androgen production increases, and LS/LI ratio is altered. These changes activates some cytoplasmic or membrane bound factors that activates apoptotic genes like P53, C-myc, bcr-ab, ras, bcl-2, bcl-x, bax, and anti-oxidant family of genes causing an elevation in calpains, caspases, p53 suppressor protein, IL-2, BCL-2 and BAX that activates endonucleases leading to apoptosis. Most of the cellular components are endocytoised by the theca interna cells, which hypertrophy and transmutate into steroidogenically active interstitial gland tissue. The information available on atresia in goats and sheep is discussed in relation to other mammalian species to explain the intricacies of this process and to point out the gaps for further studies.

Key words: Atresia, Follicles, Goat, Ovary

In mammals, a large population of ovarian follicles is lost through a wide spread phenomenon of follicular atresia that limits the number of ovulation thus restricting the full reproductive potential of a species. The modulation of frequency of follicular atresia can regulate the fertility in the animal. Actually, many infertility problems are associated with atresia and the fundamental interest of animal scientists lies in boosting the reproductive potential and production of farm animals. Therefore, a thorough understanding of structure, function and control involving morphology, including ultrastructure, cell biology, physiology, endocrinology, biochemistry, immunology, pharmacology, and neurobiology of follicle atresia had been studied by various workers in different mammalian species. Goat occupies an important position in the economics of medium and small farmers (India 1999). Follicle atresia in the ovaries of ruminants is a wide spread degenerative phenomenon by which follicles lose their structural integrity and oocytes are lost from the ovaries other than the process of ovulation (Guraya 1997). Follicular atresia results in extensive loss of germ cells during development, prenatal, neonatal, prepubertal, pubertal, estrous cycle, pregnancy, lactation and post reproductive life of mammals (Guraya 1997, 1998). Follicles at various stages of development and maturation are affected by atresia. However, during early development primordial follicle population is most affected (Guraya 1998) where as in the active reproductive phase the frequency of antral follicles is maximum (Rajaoksi 1960, Guraya 1968a, 1979a, Daniel 1987, Sharma *et al.* 1992, Guraya *et al.* 1994). In pig about 55% of antral follicles undergo atresia during 100-300 days of age and an additional loss of antral follicles occurs during the estrous cycle (Foxcroft and Hunter 1985, Guthrie *et al.*1994, Guthrie and Cooper 1996). Only 25% of the total antral follicles of 3 to 6 mm diameter in mature ovary ovulate and remaining 75% of this population are lost by atresia (Clark *et al.*1975, 1982, Guthrie *et al.* 1993, 1994, 1995, Guthrie and Cooper 1996). In bovine ovaries 85% of the follicles greater than 1 mm in diameter undergo atresia (Blondin *et al.* 1996). Such an informat on on goat ovary is not available. Studies in our laboratory have indicated that it is approximately 80% of the antral follicles (> 1mm) in goat

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that undergo atresia (Sharma RK unpublished observations).

In bovine ovaries the macroscopic and microscopic correlative studies provided an excellent method for selection of follicles for *in vitro* maturation (Choudary *et al.* 1968, Scarmuzzi *et al.* 1980, Kruip *et al.* 1982, Daniel 1987, Driancourt *et al.* 1993, Guraya 1997, 1998).The application of this technique for ascertaining whether the follicle is atretic or not in goat ovaries has provided encouraging results (Sharma RK unpublished observations). Follicular atresia not only decreases the number of ovulations but also the transformed follicle components act as a steroidogenically functional interstitial gland tissue (Sharma and Guraya 1992), thus helps in the regulation of ovarian physiology. The teleological rationale of the phenomenon of atresia is largely unknown and the factors directing most follicles towards this degenerative path are poorly understood (Guthrie *et al.* 1994). Keeping in view the impact of follicular atresia on reproductive efficiency and the challenges in understanding its morphology and physiology, a lot of information in the form of research papers and reports is generated on diverse parameters of atresia in Bovidae (Guraya 1997, 1998, Guthrie *et al.*1993, 1994, 1996). An attempt is made in this review to integrate the results of recent findings on mammalian ovary with special emphasis on goat.

Morphology and histochemistry

A distinct set of morphological and histochemical changes are observed in different cellular and noncellular components of the follicles undergoing the of atresia. These changes are highly variable and do not occur in a specific sequence. The set of alterations varies in follicles of different developmental status and the stage of atresia (Sharma *et al.*1992, Guraya *et al.* 1994). It is still controversial whether atretogenic changes first appear in the membrana granulosa or oocyte (Guraya 1985, 1997, 1998, Greenwald and Terranova 1988, Driancourt, 1993 Greenwald and Roy 1994). In foetal goat ovary, the atresia of primordial follicles is characterized by condensed, hyperchromophilic ooplasm which is generally seen adhering to one side of the oocytic membrane. The nucleus of some of the primordial cells also reveal elliptical to elongated shape and with dense nucleoplasm, and a few hyperchromatic granules (Sharma unpublished observations). The granulosa cells encircling such oocytes were characterized by darkly staining nucleus and reduced size. Histochemically reactivity to PAS, alcian blue, toluidine blue, methyl green pyroniny was enhanced in such chromophilic cells. The opaque cloudy cytoplasm of the oocyte was granular in nature in condensed zone and hyaline in rest of the portions (Sharma RK unpublished observations). Such atretic primordial oocytes were also observed in adult ovaries (Monga 1987).

Atresia is a progressive degenerative process and is distinguishable into 2 degrees of atresia in buffalo and 3 in cattle on the histomorphological basis of pycnosis of granulosa or total disappearance of granulosa cells (Rajakoski 1960). Guraya *et al.* (1994) described atretic changes in 4 categories, initially they have followed stage 1 and stage 2 as in cattle and rat (Rajakoski 1960, Osman 1985), which are further subdivided into 1a,1b, 2a, 2b. The same classification is used to explain the details of follicle atresia.

Stage 1.	Atretic changes restricted only to granulosa cells
Stage 1a.	Pycnosis of granulosa cells appear only at a few regions and such pycnotic cells are lodged in membrana granulosa.
Stage 1b.	Chromophilic nuclear particles resulting from the pycnosis lie scattered at the periphery of the antrum
Stage 2.	Both granulosa cells and oocyte show atretic changes
Stage 2a.	Intact cumulus-oocyte complex (atretic) lies in the antrum
Stage 2b.	Naked oocyte (atretic) lies in the antral fluid

In prepubertal and mature goat ovaries the preantral atretic follicles, the atretic oocyte is characterized by shrinked ooplasmic contents surrounded by a peripheral zone of lipoidal components which is strongly sudanophillic. This zone in a few atretic oocytes is so dense that it occuludens the central mass (Sharma *et al.* 1992, Guraya *et al.* 1994). The ooplasm gave negative test for 3-β-HSDH (Guraya *et al.*1994). Pycnosis in granulosa cells was initially restricted only to specific area which subsequently expanded and large zone of atretic pycnotic granulosa cells was formed resulting in loosening of membrana granulosa (Sharma *et al.* 1992). These pycnotic granulosa cells give positive test for lipids and 3β-HSDH, thereby indicating their active role in steroidogenesis (Guraya *et al.* 1994). The mural layer of granulosa cells gave strong reaction as compared to cumulus cells (Guraya *et al.* 1994). The histochemical characteristics observed in goat are similar to those observed in other mammalian species (Cran *et al.* 1983, Kim *et al.* 1987). Pycnosis of granulosa cells as the first sign of atresia is suggestive of the general plan and path of atretogenic changes in mammals (Brand and de Jong 1973, Hay *et al.* 1976, Guraya *et al.* 1987). Electron microscopic investigations on atretic granulosa cells in rodents (Peluso *et al.* 1980, Sharma and Guraya 1997) have suggested that morphological and biochemical characteristics similar to the phenomenon of phagocytosis was observed during atresia.

Histomorphological characteristics of atresia granulosa cells

1. Appearance of pycnotic granulosa cells in intact membrana granulosa
2. Free-floating large-sized granulosa cells with pycnotic nuclei
3. Hyalinization of granulosa cells

4. Chromolysis of the granulosa cell nuclei and their enucleation
5. Loosening of the intercellular matrix
6. Delamination of intercellular matrix
7. Colloidal, opaque and cloudy follicular fluid
8. Detachment of cumulus-oophorous complex from mural granulosa cells
9. Appearance of RBCs in the follicle
10. Invasion of connective tissue fibres within the follicle
11. Huge accumulation of follicular fluid resulting in the cyst formation

Oocyte

1. Condensation of germinal vesicle
2. Pseudocleavage
3. Breakdown of the oocyte nucleus
4. Fragmentation of the oocyte nucleus
5. Mitochondrial aggregation
6. Appearance of crystalloid bodies
7. Appearance of large-sized chromophillic globules/ granules
8. Hyalinization of zona pellucida
9. Shrinkage of ooplasmic contents leaving a clear space with in zona pellucida
10. Hyalinazation of the ooplasmic contents

In atretic preantral follicles, the various nuclear and ooplasmic contents are drastically affected. The number of organelles showing abnormal morphology and distribution increase and proceed towards disintegration. These changes lead to an accumulation of lipids in the oocyte and degenerating granulosa cells possibly because of liberation of membrane bound lipids (Byskov 1978). The thecal vascularity and sudanophilia is on rise (Prashad and Guraya 1983, Guraya 1985, 1997, 1998). The granulosa cells in the atretic preantral follicles develop histochemical characteristics of steroidogenic cells as evidenced by weak 3β-HSDH activity. Hypertrophy and invasion of theca interna is closely associated with atresia. Accumulation of lipid droplets and 3β-HSDH activity are suggestive of increased steroidogenic potential (Monga 1990).

In antral follicles atresia is characterized by the presence of pycnotic nuclei in the membrana granulosa, appearance of spaces and release of fragments of pycnotic nuclei in the peripheral zone of antrum in type 1β (Sharma *et al.* 1992, Guraya *et al.* 1994). Sharma and Guraya (1997) using scanning electron microscopy have reported the sequential course of atresia in granulosa cells in the house rat (*Rattus rattus*) ovaries treated with atropine and testosterone propionate. Granulosa cells undergoing atresia showed degenerative changes in the following chronology. (1) Loosening of intercellular matrix; (2) changed morphology and texture of secreting granules; (3) destabilization of granulosa cell membranes; (4) erosion of cell membrane; (5) formation of specific degenerative belts; (6) pycnosis; and (7) ghost cell formation and their subsequent mixing in hazy follicular fluid of cyst.

The granulosa cell glyco-conjugates are changed during follicular atresia in the pig and rat ovaries as shown with lectins (Sharma and Guraya 1992, Kimura *et al.* 1998). Within the wall of granulosa cells levels of fibronectin, laminin, type IV collagen, hepran sulfate, proteoglycans, insulin like growth factor II/ mannose 6 phosphate (IGF-II/ M6P) receptors, and matrix metaloproteinases 2 and -9 increased whereas 450 arom and connexin 43 decreased during follicular atresia in sheep (Huet *et al.*1997, 1998, Sharma and Guraya 1998a, b).

In type 2 antral atretic follicles, the degenerative signs also appear in cumulus cells (Guraya *et al.*1994). In type 1b atresia proceeds gradually, membrana granulosa thins off and theca hypertrophy enhances (Sharma *et al.*1992, Guraya *et al* 1994). Sudanophilia in the peripheral granulosa cells intensifies and so does the 3β-HSDH. Similar trend was also revealed in the theca type hypertrophied interstitial gland cells (Guraya *et al.* 1994, Sharma and Guraya 1998b, c). Oocyte reactivity was negative for lipids and 3β-HSDH.

In goat, the appearance of pycnotic granulosa cells as the first sign of atresia strongly advocate the concept that phenomenon of atresia in bovine species is similar (Zimmerman *et al.* 1987). The chromophilic pycnotic granules observed in goat atretic follicles were similar to that of DNA - positive masses observed in sheep (Hay *et al.* 1976, Hay and Cran 1978). However, in cow such granular material is negligible (Guraya 1997). The delamination or detachment of mural granulosa cells from the basal lamina leads to disruption thus causing a decline in oxygen carrying blood transudate that decreases the metabolic pace and results in onset of atresia (Guraya 1985, 1998 Sharma and Guraya 1998b, c). However, Hay *et al.* (1976) suggested that it is because of decline in estrogen synthesis and release.

There is a tendency towards accumulation and storage of sudanophilic lipids in the atretic oocytes as they advance in the stage of atresia (Guraya 1973). A weak 3β-HSDH activity is due to luteinization of granulosa cells invading and phagocytosing the oocyte was seen. Comparatively granulosa cells acquired more lipids (Byskov1979, Parshad and Guraya 1983). The mural layers acquire more lipids as compared to cumulus cells or peripheral granulosa cells. Similar trends were reported in hamster and rabbit (Guraya and Greenwald 1964), thus indicating granulosa heterogeneity in morphology and physiology (Parshad and Guraya 1983, Sharma and Guraya 1990). The NADH and NADPH-dependent tetrazolium reductase activity in goat further endorse this concept.

The theca interna of atretic antral follicles undergo hypertrophy and develop lipid droplets largely comprised of phospholipids which are associated with steroidogenesis in mammals (Guraya 1973, 1974a, b, 1977, 1978, Nicosia 1980). This hypertrophied theca interna in atretic follicles of goat finally constitute conspicuous masses of interstitial gland

tissue which show strong 3β-HSDH activity (Guraya 1971, 1973, Guraya *et al.* 1994). These lipid droplets are largely phospholipids and triglycerides which change to cholesterol, its esters and phospholipids, and remain associated with theca type interstitial gland tissue. Various carbohydrates, proteins and enzyme histochemistry were used as an index to indicate whether atresia has initiated or not (Guraya 1984, 1985, Sharma and Guraya 1992,1997). But such specific studies have not been made so for on goat. A decline in cAMP receptors was reported in cattle (Guraya 1997). The follicles undergoing the process of atresia finally disintegrate oocytes and granulosa cells are reabsorbed while theca interna hypertrophy and acquire morphological and histochemical characteristics of a steroidogenic tissue.

Physiology and biochemistry

Intrafollicular paracrine steroid interactions are dependent on FSH and LH which regulate follicular development and oestrogen synthesis and release (Guraya 1985, 1997, 1998, Ireland 1987, Richards *et al.*1993, Gore-Langton and Armstrong 1994, Hillier 1994, Adashi 1994, Guthrie and Cooper 1996). Alterations in the production of steroid i.e oestrogen, testosterone anderostenedione etc. in the antral fluid are the first biochemical manifestations of atresia (Guraya 1985, 1998, Hillier 1985, Greenwald and Terranova 1988, Westhof *et al.* 1991, Gore-Langton and Armstrong 1994, Greenwald and Roy 1994). Variations in normal and atretic follicular fluid concentration of androgens and that of progesterone are not significantly different (Hillier1985, Westhof *et al.* 1991, Moor 1977, Gore-Langton and Armstrong 1994). Hormonal profile of normal and atretic follicles depends largely on the stage of advancement of atresia. Normally it is oestrogen to progesterone ratio which is an indicator of atresia (Moor *et al.* 1978, Ireland and Roche 1982, 1983 a, b, Guraya 1997). In cattle large atretic follicles show a diminished LH-stimulated anderostenedione production *in vitro* (Henderson *et al.* 1984, Mc Natty *et al* 1985b). It is the balance between the oestrogens and androgens that determines whether a follicle becomes atretic or not (Richards 1980, Guraya1985, 1994, 1998; Terranova and Greenwald 1988, Driancourt *et al.* 1993, Adashi 1994, Gore-Langton and Armstrong 1994, Sharma and Guraya 1997). The oestrogen is highest in the preovulatory healthy follicles of pig, sheep, and cow, as compared to atretic ones (Eiler and Nalbandov 1977, Moor *et al.*1978, Carson *et al.* 1981, Fortune *et al.* 1988). But such studies on goat atretic follicles are still lacking although normal profiles are on records (Sharma *et al.* 2000).

Atretic granulosa cells synthesize and secrete estrogen 17-β in lower quantity compared to normal granulosa cells *in vitro* (Henderson *et al.* 1987, Westhof *et al.* 1991). Dibutyryl cyclic AMP stimulates significantly the progesterone production in atretic granulosa cells but not in the non-atretic follicles (Guraya 1968, 1979a, 1998).The fine structural details and histochemistry endorse these findings. On morphometric grounds, mean cell volume, mean plasma and nuclear membrane and granular endoplasmic reticulum is more in granulosa cells of atretic follicles (Sharma RK unpublished observations). The changes reveal hypertrophy and incipient luteinization of granulosa cells also evidenced by low estrogen production and increased output of progesterone (Bellin and Ax 1984). In cattle, other intrafollicular co-relates include chondroitin sulphate, albumin, and lactate dehydrogenase as an index of atresia (Bellin and Ax 1984). In cattle FSH-binding inhibitor (FSH-BI) suppresses the responsiveness of the granulosa cells to FSH and induces atresia (Sluss *et al.* 1983). The presence of FSH-suppressing protein m-RNAs in follicles of cattle are suggestive of their possible involvement in the regulation of atresia (Guraya 1997).

Detailed studies have suggested that ^{125}I hCG binding to theca interna of healthy and atretic follicles of cattle remain unchanged and binding goes on increasing with the advancement in the stage of follicle development to a maxima in the preovulatory follicles (Henderson *et al.* 1984, Ireland and Roche 1982, 1983a, b), thereby suggesting that failure of theca from atretic follicles to response is not due to the absence of LH-receptors (Henderson *et al.* 1984), but can be attributed to some other factors. Mc Natty *et al.* (1985a, b) divided theca interna from large follicles (>8mm) of cattle into 3 types. Type I theca when perfused with LH *in vitro* respond by increased production of cAMP, anderostenedione and testosterone. Type II produce cAMP and progesterone (early atretic), and type III no response to LH, although all the 3 types possessed LH-receptors (Guraya 1985, Greenwald and Terranova 1988, Hillier 1994, Greenwald and Roy 1994, Magoffin and Erickon 1994, Adashi 1994). Response to FSH, directly linked to estrogen production, also depicts similar decline as also evidenced by histochemical studies (discussed earlier) thus suggesting that production of progesterone and estrogen by granulosa cells from the atretic follicles decline, while hypertrophied theca is the site of increased output of mainly progesterone and some other steroids (Westhof *et al.* 1991). Although such histochemical results are on records (Guraya *et al.* 1994), but biochemical studies and *in vitro* evidences in this regard are still remain to be investigated in goat.

Apoptosis

Palumbo and Yeh (1994) demonstrated that molecular mechanism of atresia can be best explained on the basis of apoptosis. Apoptosis is a type of physiological cell death that affects single scattered cells in the midst of normal living tissues and is regarded as antithesis of mitosis (Kerr *et al.* 1994). Apoptosis does not require an inflammatory reaction but is elicited by the withdrawal of growth factors and hormones. It is a widespread phenomenon associated with remodeling of tissue during development and in regulation

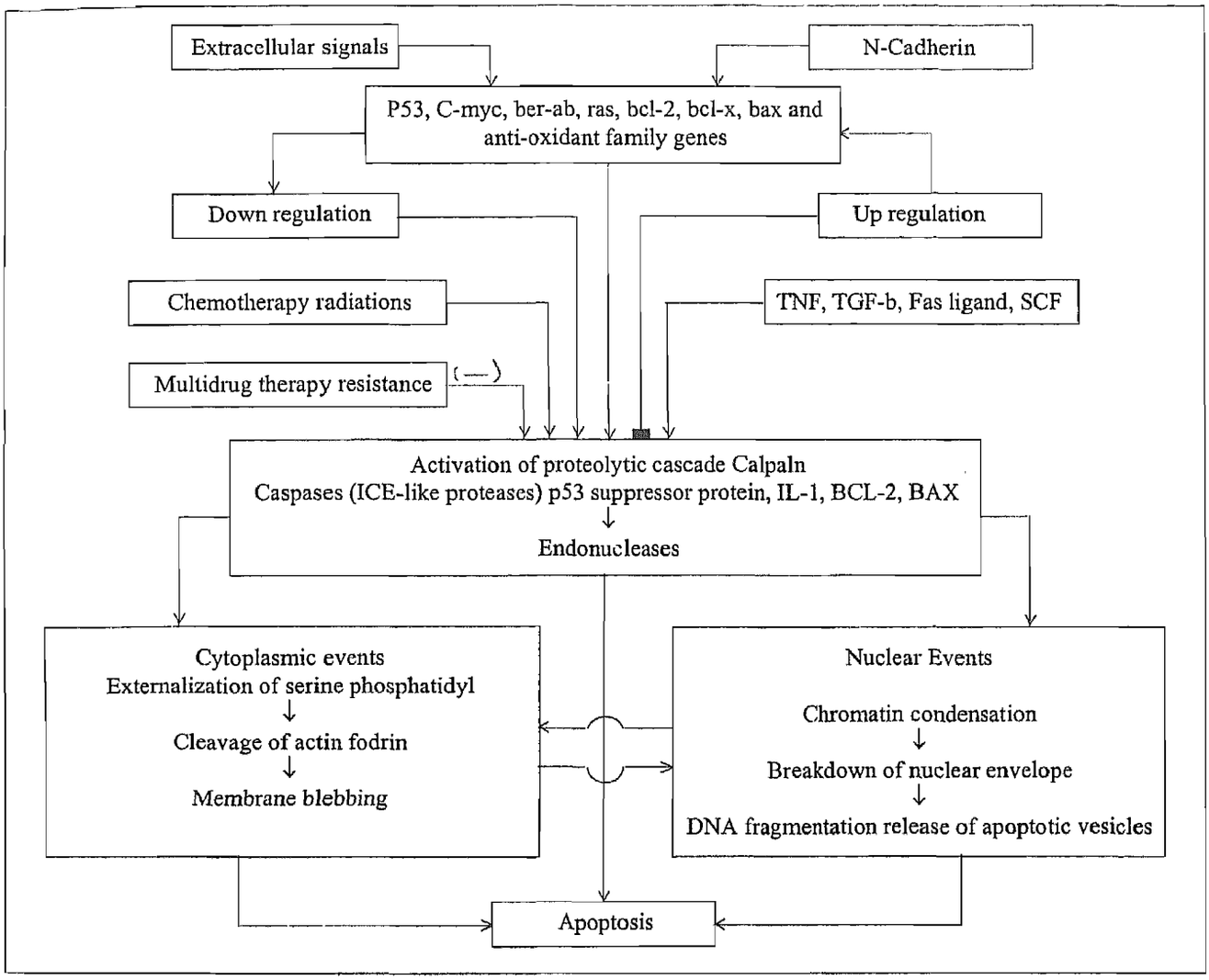


Fig. 1. Molecular Mechanism of apoptosis.

of tissue homeostasis in adult (Vaux 1993, Stellar 1995).

Hughes and Gorospe (1991), have demonstrated apoptotic DNA fragmentation in eCG-induced atresia in rat characterized by oligonuleosomes of 180 to 200 base pair and a ladder-pattern was reported in avian and porcine atretic follicles (Tilly *et al.* 1991). Subsequent studies of Tilly *et al.* (1991) documented that it can be prevented by adding specific growth factor in *in vitro* culture. Bellig *et al.* (1994) suggested that estrogen (diethylstilbestrol) withdrawal in hypophysectomized rats induce apoptosis, which can be reversed by replacement therapy, and restoration was achieved by androgen administration. The systemic analysis of apoptosis in rat ovary at different physiological stages strongly support the hypothesis that apoptosis is involved at the basis of follicular atresia (Palumbo and Yeh 1994).

Oogonia and oocytes which degenerate by the process of atresia *in vivo* or *in vitro* show a number of apoptosis specific characteristics like chromatin margination and fragmentation leading to pycnosis, cellular condensation and induction of cell death markers such as tissue transglutaminase (Coucouvanis *et al.* 1993). Somatic cell-derived growth factors like Steel gene (stem cell growth factor /SCF or kit ligand) are required for the maintenance of germ cells. Mutation in SCF and c-kit genes lead to gonadal dysgenesis and sterility (Eppig 1994). SCF mediates germ cell survival via enhanced expression of bcl-2 (Ratts *et al.* 1995, Tilly 1993). The ability of FSH and LH to suppress apoptosis and atresia involves enhanced signaling through intrafollicular growth factors (Tilly *et al.* 1992, Chun *et al.* 1994) which may involve final autocrine mediator like progesterone (Luciano *et al.* 1994). The "knock out" of the bax gene (BAX protein) renders granulosa cells resistant to normal induction of apoptosis in the follicles destined for atresia (Knudson *et al.* 1995). Antioxidant genes (superoxide dismutase, catalase)

expression decrease apoptosis (Tilly and Tilly 1995). Since gonadotrophins enhance their expression and the rescue in apoptosis may be due to increased production of protective enzymes (Tilly and Tilly 1995). The p 53 tumor suppressor protein accumulates in nucleus of rat granulosa cells destined for apoptosis (Tilly *et al.* 1995). The available data suggested that the bax death gene may in fact be a target for p53 transactivation in ovary (Miyashita *et al.* 1994).

Cytoplasmic proteases, especially members of the ICE gene family (Flaws *et al.* 1995), the primary products of ICE activity such as interleukin-I increases basal rate of apoptosis in granulosa cells. Enhanced intra-follicular activity of other members of ICE protease family like ICH-I and CPP32 may be responsible for such endpoints as endonuclease activation and disrupted messenger RNA processing in granulosa cells during apoptosis (Flaws *et al.* 1995, Trobovich and Tilly 1995).

In pig flow cytometric determination of degraded DNA in granulosa cells was used to identify atretic follicles during preovulatory maturation (Guthrie *et al.* 1994). The increased incidence of granulosa cells with low DNA florescence is a new biochemical marker for atretic follicles in pig. Increased percentage of granulosa cells undergoing DNA degradation and a loss of estradiol 17-β production *in vivo* between days 5 and 7 post estrous shows high incidence of atresia of 3-6 mm follicles that have started to grow after ovulation signaling the end of their growth period (Guthrie *et al.* 1995). Such studies in goat ovary are lacking and shall be of great value in the regulation of ovarian functions.

Follicular atresia is a process of apoptosis in granulosa cells of these follicles, which may be induced by extra or intraovarian factors (Nandedkar 1998). The signaling mechanism may involve genes such as c-mys, Fas, FasL, belx-causing apoptosis. In addition to granulosa cells, oocyte can also show apoptotic changes prior to or post ovulation. Growth factors such as IGF, TGF, and cytokines e.g. TNF-α and IL-1 may regulate luteal cell apoptosis (Nandedkar 1998). In PMSG and 4kDa peptide injected model were used to induce atresia and apoptosis in granulosa cells was demonstrated by i) acridine orange staining ii) DNA low cytometric analysis, iii) DNA low molecular weight ladder on agarose electrophoresis iv) *in situ* 3' end TUNEL technique. Expression of c-myc in the theca cells of atretic follicles indicated hypertrophy during apoptosis (Nandedkar 1998).

The apoptotic cell death is involved in the degeneration of granulosa cells and atresia during 2 different stages of follicular development in pigs (Guthrie *et al.* 1995a,b). Stimulation of granulosa cells primary by high levels of cAMP catalyzes programmed cell death, while stimulation of the cells by gonadotrophins, which result in a moderate, cAMP response, followed by desensitization to further stimulation, can prolong the life span of the luteinized granulosa cells (Jolly *et al.*1997). Tilly *et al.* (1995) have observed that the ability of gonadotrophins to prevent apoptosis and atresia in ovarian follicles may be linked to a shift in the ratio of death repressor (bcL-2, bci-X-long) to death inducer (bax, bci-X-long) gene expression through alterations in bax.

The cross-talk between cAMP and P53-generated signals in the induction of differentiation and apoptosis in steroidogenic granulosa cells. Recent studies demonstrate that apoptosis, an actively physiologically regulated form of cell death at the molecular level is the underlying mechanism of atresia (Tilly 1996; Nandedkar 1998, Jolly *et al.* 1997). The hormonal regulation of follicle apoptosis using *in vitro* system has been documented (Tilly 1996, Jolly *et al.* 1997). Preovulatory follicles dissected form ovaries of immature PM5G primed rats were cultured for 24 hr in serum free media results in spontaneous onset of apoptosis. The increase in apoptosis can be prevented by treatment with LH/hCG, FSH, and GH. The growth factors EGF, TGFβ, bFGF, IGF-I and the cytosine IL also suppress apoptosis. Unlike preovulatory follicles in which all hormones suppress apoptosis to the some degree, apoptosis in early antral follicles is suppressed maximally by FSH while LH, EGF, bFGF, IGF-I and IL-I suppress apoptosis to a lesser extent and GH has no effect (Guraya 2000). IGFBP modulation of IGF and gonadotrophin action is believed to play an important role during follicular atresia as supported by three fold higher levels of IGFBP-2 in the follicular fluid of atretic follicles than in non-atretic follicles. The quantity of IGFBP-2 is positively correlated with index of atresia,% apoptotic granulosa cells under different experimental situation in porcine follicles (Guthrie *et al.* 1995a,b). Ovine (Monget *et al.* 1993) and bovine (Echternkamp *et al.* 1994) follicles response in a similar manner. The accumulation of low molecular mass IGF binding protein by a reduction in IGFs may be the cause of atresia in dominant follicle during the oestrous cycle in bovine ovary. During early atresia apoptosis occurs randomly and is not restricted to specific areas within follicles (Jolly 1997b).

Angiotonsin II (A II) can induce locally an increase in follicular fluid androgen / oestrogen ratio and possibly participate in causing atresia and AI-II might modulate the onset and progression of atresia involving apoptosis of granulosa cells (Guraya 2000). Huet *et al.* (1998) have proposed that (1) TGF-α, βFGF TNF-α and IL-1β may be atretogenic ligands, and (2) the autocrine/paracrine secretion of these growth factors may play a role in apoptosis of granulosa and atresia by virtue of their ability to neutralize intrinsic IGF-I activity (Guraya 2000). Tilly (1996) has suggested that homophobic binding of N-cadherin molecules on the surface membranes of adjacent granulosa cells initiates a signal transduction cascade that inhibits apoptosis. DNA from all atretic follicles showed fragmentation typical of apoptosis (Jolly *et al.* 1997).

Regulatory factors, physiology and significance

The development of primordial follicles to preovulatory stage is influenced by a number of intrinsic and extrinsic

factors. Any variation in these factors, deviates the follicle from normal development path to atresia (Guraya 1985). The mechanisms, which augment and regulate atresia, are still controversial (Sharma and Guraya 1997). The factors affecting the frequency of atresia include age, stage of reproductive cycle, pregnancy, lactation, hypophysectomy, ovariectomy (unilateral), hormones of intra-and extra-ovarian sources, ischemia, nutrition, radiations, genetics etc. (Ingram 1962, Richards 1980, Nicosia 1980, Ryan 1981, Guraya 1985, 1997). Recent advances in *in vitro* culturing of follicles have suggested the role of a number of factors (peptides) on follicular atresia (Ireland *et al.* 1994, Guthrie *et al.* 1994, 1995a,b, Guthrie and Cooper 1996, Blondin *et al.* 1996). Today some information is available as what determines the follicle to grow and ovulate or undergo atresia which is an inevitable outcome of follicle development and ageing, unless rescued at a critical stage of development by hormones (Hirshfield 1989). The cyclic appearance of atresia of dominant follicle in particular and follicles of variable sizes in general show a correlation between trophic hormones, and ovarian steroids (Guraya 1997). Extensive loss of follicles due to atresia was recorded during anestrous in buffalo and goat (Guraya 1979, Danell 1987, Sharma *et al.*1992). The exact endocrine profile during this phase is still lacking. The various causes that can induce anestrous include a persistent corpus luteum due to mummified foetus, cystic corpus luteum or inadequate hypophyseal hormones or ovarian insensitivity to hormones (Madan 1988). This phase can also be due to environmental stress (temperature), lactation, or due to deficiency in nutrients (protein, phosphorus, copper, iodine, calcium, manganese, etc. (Madan 1988, Sharma and Sharma 1997). The role of calcium and phosphorus in cattle feed was discussed in details (Hussian *et al.* 1981). The frequency follicular atresia during anestrous is maximum in buffalo followed by cattle (Rajakosi 1960). The status of sheep and goat in this regard need to be investigated further as to endorse emphatically the genetic basis of atresia.

Despite variable modes and mechanisms of atresia depending upon the stage of the follicle, the hormonal treatment alters the pace of atresia to a variable extent in mammals (Guraya 1985, Greenwald and Terranova 1988, Hirshfield 1989, Greenwald and Roy 1994). PMSG or eCG (equine chronic gonadotrophin) administration *in vivo* significantly increased the number of preantral and antral follicles in bovines (Monniaux *et al.* 1984, Pierson and Ginther 1988). This increase is due to increased recruitment (Driancourt 1991, Driancourt *et al.* 1993, Jablonka-Shariff 1994). In sheep and cattle PMSG increases the rate of entry into > 2mm follicle category and prevention of atresia (Dott *et al.* 1979). Extensive studies on sheep revealed that rescue of atretic follicles does not significantly contribute to ovulatory response in PMSG treated animals (Driancourt 1987). The active follicular turnover (growth and atresia) in < 2mm follicles can occur in the absence of gonadotrophins as evidenced in hypophysectomized ewes (Driancourt 1991, Driancourt *et al.* 1993). The increased number of large follicles in PMSG treated ewes are therefore due to the number of follicles prevented from undergoing atresia (Driancourt *et al.* 1987, 1993, Driancourt 1991), which add to the number of ovulations. Similar observation of Monniaux *et al.* (1984) on cattle suggest that PMSG either prevents some follicles from undergoing atresia or it rescues a few atretic follicles and put them on normal development path. However, in sheep, rescue of atretic follicles by PMSG has been denied (Driancourt *et al.* 1987). It is therefore deduced that PMSG prevents follicles from undergoing atresia. Such studies in goat are still lacking.

Recent studies have put forth a hypothetical model highlighting the role of the ratio of LS/LI in altering the follicle response to changing titre of gonadotrophins (Tsafriri and Adashi 1994). In this model the follicle with higher LS would grow and ovulate while those with elevated LI undergo atresia (Guraya 2000).

Ovarian GnRH-like peptide stimulatory action appears to be atretogenic in function as they enhance progesterone production and induce precocious maturation of oocytes in preantral follicles (Tsafriri and Eikastein 1986, Tsafriri and Braw 1984, Erikson *et al.* 1983). The antagonistic observations suggest that ovarian GnRH-like peptide contrary to hypothalmic GnRH and pituitary FSH are involved in follicle development, selection and atresia (Birnbaumer *et al.* 1985). The adenosine increases LH-dependent cAMP in greater proportion than FSH-stimulated cAMP thereby indicating its role as a promoter of follicular atresia (Ohkawa *et al.*1985). Findlay and Clarke (1987) have demonstrated a negative feedback by follicular fluid inhibin that lowers FSH leading to atresia.

The quantity of intrafollicular inhibin decreases during the growth of dominant ovulatory follicle but the reverse is true in the non-ovulatory antral follicle (Martin *et al.* 1981). Studies on unilateral ovariectomy in different mammalian species have pointed out that FSH is needed either to enhance follicular recruitment or to decrease follicular atresia (Greenwald and Terranova 1988, Greenwald and Roy 1994). During anestrous it is the progesterone, prolactin, estrogen and LH titre and their interactions induce atresia in buffalo in summer (Singh *et al.* 1991). However, such studies on goat are totally lacking. It is the perfect balance of the hormones i.e. FSH, estrogen, LH, and progesterone that are required for normal follicular development (Greenwald and Roy 1994, Guraya *et al.* 1994). Any factor that inhibits the estrogen production from androgens lead to follicular atresia in absence of FSH (Tsafriri and Adashi 1994, Sharma and Guraya 1997). In atretic follicles there is a progressive accumulation of androgens possibly due to interruption in estrogen synthesis at some step (Westhof *et al.* 1991). The role of innervation in atresia still remains to be investigated (Dominguez *et al.* 1989, Ojeda *et al.* 1993, Guraya *et al.* 1991). The involvement of

innervation in atresia is possibly through regulation of blood vascularity of the follicle (Guraya *et al.* 1992).

The influence of endocrine regulating general metabolism needs to be investigated with special reference to atresia. In this regard studies on hormones of the adrenals, thyroid, pancreas, parathyroid, and their effect on follicular growth and atresia shall be highly rewarding. It would describe the role of intermediary metabolites and their alterations in normal development or deviation to atresia.

The most important significance of atresia is to limit the number of ovulations, either through selection of the dominant follicle or by decreasing the number of normal follicles (Westhof *et al.* 1991, Driancourt *et al.* 1993). The second important contribution of follicular atresia is the formation of theca-type interstitial gland tissue that produces progesterone and other peptides, which regulate ovarian functions in diverse endocrine situations (Guraya 1997, 1998, Sharma and Guraya 1998c).

With the present state of art, follicular atresia can be regulated to a greater extent by hormone therapy at an early stage. The rescue of the early atretic follicles would add to the number of ovulations in threatened species. The chronological events of atresia are now better understood and the screening of such follicles would enhance the success rate *in vivo* and *in vitro* fertilization and embryo transfer technology. The studies on pointing out specific markers of atresia especially on peptides are going on and in near future they will become a handy tool in fertility regulation programme in mammals.

ACKNOWLEDGEMENT

The author is thankful to the University Grants Commission, New Delhi for the financial assistance.

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Indian Journal of Animal Sciences **70** (10): 1047-1048, October 2000

Comparison of different serological tests for detection of bovine herpes virus 1 antibodies in sheep*

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Received: 8 November 1999; Accepted: 14 April 2000

Key words: BHVI, Indirect ELISA, Dot-ELISA, PHA, AGID

Infectious bovine rhinotracheitis (IBR) caused by bovine herpes virus 1 (BHVI) is causing considerable conncern for livestock industry in various parts of the world. BHV1 infection occurs not only in cattle but also has been reported to cause infection in sheep (Trueblood *et al.* 1978, Buonavoglia *et al.* 1996). Evaluation of ELISA, PHA and VN test for detection of antibodies to BHV1 in vaccinated and experimentally infected cattle was done by Edwards *et al.* (1986). They found highly singnificant correlation between ELISA, PHA and VN tests. ELISA was carried out by Florent and Demarneffe (1986) for detection of antibodies against IBR in cattle and they concluded that ELISA detected low levels of antibodies and it appeared to be more sensitive than the VN or PHA tests. Suresh (1992) in cattle, compared AGID and PHA tests with ELISA and observed that ELISA was more sensitive than PHA test and PHA test was more sensitive than AGID test. He recommended PHA test as a suitable test for screening animals for BHVI antibodies. Singh *et al.* (1994) compared dot-ELISA and indirect ELISA for detection of BHV1 antibodies in cattle. They found that dot-ELISA to be more sensitive, economical as well as easy to use in the field level. With this background, a study was planned to evaluate the efficacy of dot-ELISA, indirect ELISA, passive haemagglutination and agar gel immunodiffusion tests for detection of BHV1 antibodies in sheep.

The BHV1 (IBR) strain BFA-OCS-W was passaged in Madin Darby Bovine Kidney cells. The method of Shimizu *et al.* (1972) was follwed for antigen preparation. Positive bovine hyperimmune serum and negative serum were obtained from Central University Laboratory, Centre for Animal Health Studies, Madhavram, Chennai. Dot-ELISA positive sheep serum was taken as positive sera. The negative sera were collected from sheep which proved negative by dot-ELISA test and from rabbit before raising hyperimmune serum. A total of 50 serum samples from sheep were collected from salughter house, Bangalore. Animal Food Corporation, inactivated at 56°C for 30 min and stored in sterile vials at-20°C after adding merthiloate to a final concentration of 1:10,000. Serum samples were screened by dot-ELISA, indirect ELISA, PHA and AGID tests.

Agar gel immunodiffusion test was conducted as per Le-Jeune *et al.* (1977). The test conducted was a macro method. PHA test was carried out as described by Shimizu *et al.* (1972). Indirect ELISA was performed as per the procedure of Florent and Demarneffe (1986). Dot-ELISA was performed as per the procedure of Mishra and Mallick (1997).

The results of screening 50 sheep sera sample for BHV1 antibodies by dot-ELISA, indirect ELISA, PHA and AGID tests are shown in Table 1. Twenty eight (56%) samples were positive by dot-ELISA, 26 (52%) samples were positive by indirect ELISA, 25 (50%) samples were positive by PHA test and 11 (22%) samples were positive by AGID test. The number of positive samples was significantly ($P \leq 0.05$) less in AGID test as compared to other 3 tests. However, there was no significant ($P \leq 0.05$) difference in the result of dot-ELISA, indirect ELISA and PHA tests. Sensitivity of indirect ELISA, PHA and AGID tests were calculated by taking dot-ELISA as standard. The results of analysis by indirect ELISA, PHA and AGID tests taking know to dot-ELISA positive and negative sera sample are given in Table 2. The sensitivity of

Table1. Comparison of different tests for detection of BHVI antibodies is sheep

	Dot-ELISA	Indirect ELISA	PHA	AGID
No. tested	50	50	50	50
No. positive	28	26	25	11
No. negative	22	24	25	39
Per cent positive	56	52	50	22

AGID is significantly ($P \leq 0.05$) different from other 3 tests.

*Part of the M.V. Sc. Thesis submitted by the first author to the University of Agricultural Sciences, Karnataka 560 204.

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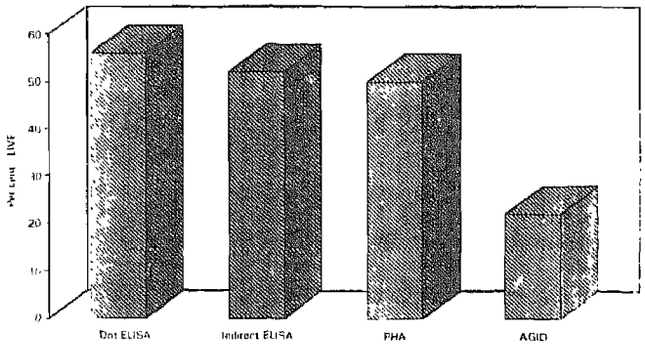


Fig. 1. Comparison of different tests for detection of BHV1 antibodies in sheep.

these tests are given in Table 3. The sensitivity of indirect ELISA,PHA and AGID test were 89.28%, 89.28% and 39.28%. The results of evaluation of differnt serogical tests in sheep indicated that the number of samples detected positive by PHA test was comparable to that of indirect ELISA and dot-ELISA.The sentivity of indirect ELISA and PHA test as compared to dot-ELISA were same in sheep. AGID test detected significantly ($P \leq 0.05$) less number of samples as positive and it was least sensitive.

No literature is avaibable regarding the evaluation of different tests for detection of BHV 1 antibodies in sheep and this work seems to be first of its kind in India. Perusal of available literature could not reveal any work in India regarding conduction of indirect ELISA for detection of BHV1 antibodies in sheep. However, similar studies have been conducted to compare various serological tests for detection of BHV1 antibodies in cattle. Edwards *et al.* (1986) in surrey observed a singificant correlation between ELISA and PHA titres for detection of BHV 1 antibodies in cattle. Solsona *et al.* (1980), Florent and Demarneffe (1986) and Suresh (1992) were of the opinion that ELISA was more sensitive than PHA test in detection of BHV 1 antibodies in cattle which corroborates the findings of the present study in sheep.

Further, it can be concluded that dot-ELISA, indirect ELISA and PHA test are equally good tests for serosurvey of BHV 1 in sheep. However, PHA test was more ecomomical,

Table 2. Comparison of indirect ELISA,PHA and AGID tests with dot- ELISA for detection of BHVI antibodies in sheep

	Dot-ELISA (+ve) n= 28	DOT-ELISA (-ve) n = 22	Total N = 50
Indirect ELISA (+VE)	25	1	26
Indirect ELISA (-Ve)	3	21	24
PHA (+Ve)	25	0	25
PHA (-Ve)	3	22	25
AGID (+ve)	11	0	11
AGID (-ve)	17	22	39

Table 3. Sensitvity of different tests in comparison to dot-ELISA in sheep

	Percent sensitivity
Indirect ELISA	89.28
PHA	89.28
AGID	39.28

suitable and reproducible test as compared to other tests.

ACKNOWLEDGEMENTS

The authors wish to thank Dr R Manickam, Centre for Animal Health Studies (CAHS) Madhavram, and the Scientists of CAHS for the reagents and facilities provided for the study.

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Indian Journal of Animal Sciences **70** (10): 1049-1051, October 2000

Morphogenesis of aorta and pulmonary trunk in pre- and post- natal goats

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Received: 27 September 1999; Accepted: 25 March 2000

Key words: Aorta, Cross sectional area, Diameter, Growth spurt, Histomorphogenesis, Pulmonary trunk

Nagpal *et al.* (1976a) studied the age associated changes in segments of aorta in goat. Such changes on aorta and pulmonary trunk in prenatal and postnatal goat appears meagre. Hence, the present experiment was undertaken.

Thirty foetuses / goats were procured from small animal slaughter house / local market, Jabalpur. The animals were divided into 5 groups with 6 animals in each group comprising prenatal, neonatal and adult stages. The prenatal stage comprised group 1, up to 10 cm; group 2, 10+ to 20 cm, group 3, above 20 cm CR length; group 4, comprised newly born kids and group 5, adult goats.

The diameter and cross - sectional (CS) area of cranial aorta, caudal aorta and pulmonary trunk were taken 1-2 cm from their origin. The diameter was measured with fine pointed compass. The cross - sectional area was calculated by the formula πr^2 (where $\pi = 3.14$ and r =½ diameter). For histological studies the samples of these vessels were processed in acetone - benzene and alcohol - cedarwood oil sequence. The sections were stained with H & E, Van Gieson, Gomoris reticulin, Verhoeff's, Periodic acid schiff's, Toluidine blue and Gomori's calcium phosphate stain for normal histological structure, collagen, reticulin, elastin, PAS - positive material, metachromasia and alkaline phosphatase activity respectively. The thickness of total wall, tunica intima, tunica media and tunica adventitia were measured with standardized ocular micrometer. The number of concentric smooth muscle cell layers in tunica media were counted. The data were analyzed as per Snedecor and Cochran (1967). Over all mean percentage increase represents the mean of percentage increase in different age - groups.

The diameter and CS area showed increase of 460 and 3407%, respectively, from groups 1-3 (Table 1). Greater increase of diameter and CS area of larger vessels in the early stage of gestation (groups 1-2) observed in the present investigation is in support of Shumway and Adamstone (1954) and Arey (1965). They reported maximum multiplication, differentiation and growth of cells, tissues and organs in early foetal life which decline during later developmental stages on account of structural differentiation and varying physiological responses of the organs, systems and body as a whole to establish its respective normal physiological task at congenial stage of time.

Among groups maximum increase observed in caudal aorta diameter and CS area (Table 1) in groups 1-2 and minimum

Table 1. Mean and SE ± of diameter (mm) and CS area (mm^2) of arteries in thorax of prenatal, neonatal and adult goat

	Cranial aorta		Caudal aorta		Pulmonary trunk	
Group	Diameter	CS area	Diameter	CS area	Diameter	CS area
1. Mean	0.60	0.28	0.59	0.27	0.58	0.25
SE±	0.01	0.01	0.00	0.00	0.00	0.00
2. Mean	2.26	4.16	2.89	6.68	2.05	3.15
SE±	0.20	0.79	0.17	0.87	0.10	0.28
3. Mean	3.36	9.82	3.84	12.67	4.49	16.16
SE±	0.49	2.80	0.25	1.03	0.29	1.84
4. Mean	3.70	10.78	4.80	18.79	6.51	35.53
SE±	0.03	0.19	0.43	3.47	0.75	8.70
5. Mean	7.63	47.15	9.68	74.39	11.08	97.05
SE±	0.60	7.24	0.45	6.90	0.41	7.20

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Table 2. Mean and SE of thickness (μm) of total wall and different tunics of arteries in thorax of prenatal, neonatal and adult goat

Artery		Groups									
		1		2		3		4		5	
	Thickness	Mean	±SE	Mean	± SE	Mean	±SE	Mean	±SE	Mean	±SE
Cranial aorta	Intima	23.73	1.28	8.37	0.38	8.58	1.21	15.65	0.45	17.00	1.33
	Media	109.08	5.54	577.45	13.81	579.15	30.78	732.40	22.52	987.62	16.12
	Adventitia	27.99	4.42	187.02	4.25	316.93	22.24	91.28	6.77	174.36	2.60
	Total	170.00	8.68	764.17	12.75	868.33	28.09	882.19	24.09	1177.30	30.32
Caudal aorta	Intima	17.10	1.39	12.49	1.11	12.96	0.72	14.38	0.57	17.03	1.30
	Media	131.09	2.83	693.21	18.24	752.51	18.24	967.60	32.96	1782.08	68.04
	Adventitia	34.88	4.15	252.33	7.33	256.90	12.80	297.84	5.79	111.21	7.40
	Total	165.42	1.49	957.21	17.39	1015.82	20.12	1277.02	34.19	1910.82	57.82
Pulmonary Trunk	Intima	20.24	0.92	7.99	0.40	12.96	0.48	13.50	1.31	12.96	0.54
	Media	29.72	1.80	616.93	2.69	860.12	19.62	938.15	29.95	873.00	35.89
	Adventitia	7.29	0.51	163.91	9.04	131.74	7.00	160.41	3.56	133.71	7.56
	Total	162.14	8.95	854.36	26.26	926.92	40.66	1112.24	26.00	993.35	63.31

in groups 3-4. However, diameter and CS area showed increase of 551% and 4592%, respectively, from groups 1-3. The equal diameter of the cranial and caudal aorta in group 1 and then relative increase in the size of caudal aorta in subsequent groups is suggestive of the more blood flow to the anterior part of body in the early stage of gestation. In groups 1 and 2 pulmonary trunk was smallest. However, it was largest and cranial aorta smallest among the arteries in group 3 onward. Pulmonary trunk increased maximum in groups 1-2 and minimum in groups 3-4.

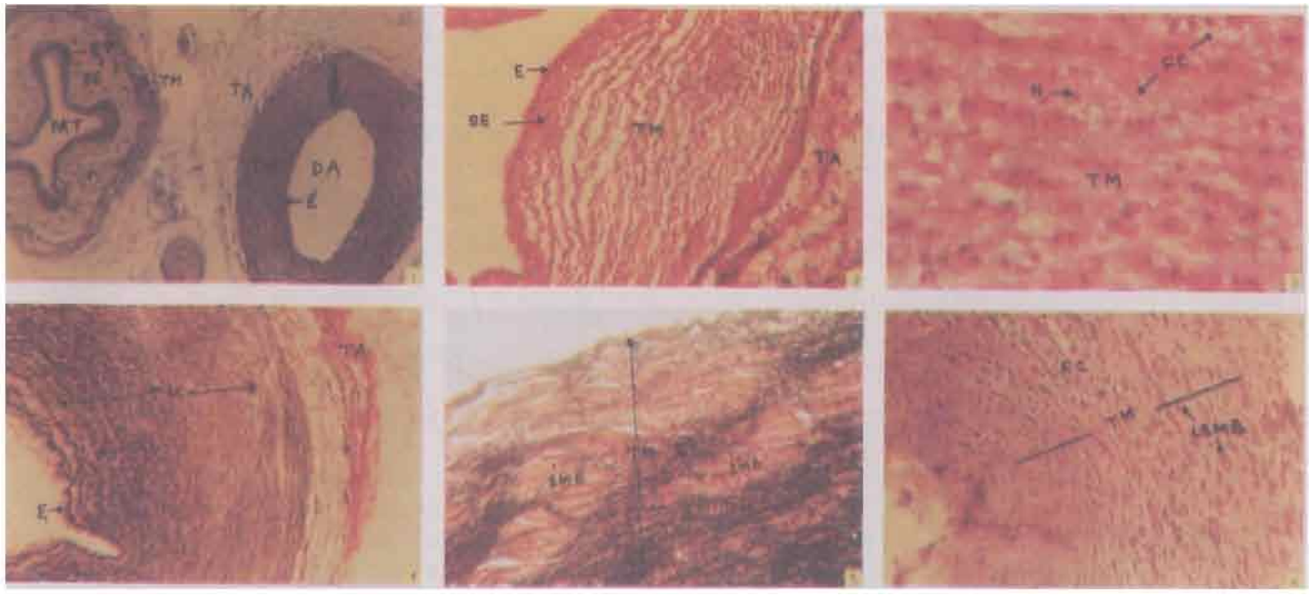
Histology

In group 1, the tunica intima was more folded in pulmonary trunk and cranial aorta than in caudal aorta (Fig. 1). The endothelial lining of cranial aorta and pulmonary trunk comprised 1-3 layers of undifferentiated cells while in caudal aorta it was made up of single layer of flattened endothelial cells in group 1. From group 2 onward the endothelium of all the 3 arteries of the region was lined by single layer of cells and their nuclei protruded into the lumen. However, it was more folded in cranial aorta than in caudal aorta and pulmonary trunk.

The subendothelium of pulmonary trunk and cranial aorta was thickest and predominantly comprised stellate mesenchymal cells and amorphous ground substance in group 1 (Fig. 1). However, it was inconspicuous in caudal aorta. In groups 2, 3 and 4 subendothelium was not well defined in all the arteries. In adult the subendothelium was in form of narrow fibrocellular strip in cranial aorta and pulmonary trunk while it was distinct in caudal aorta.

Tunica media of cranial aorta in group 1 was divisible into an outer thick and inner thin layers separated by loose connective tissue. In pulmonary trunk it comprised 2-3 irregular concentric layers of smooth muscle cells (SMC), separated by loose connective tissue. However, in caudal aorta it was uniform throughout and consisted of 10-15 concentric layers of fibromuscular tissue (Fig. 6). In group 2, the concentric laminae were more convoluted in inner half of the media, while in pulmonary trunk and caudal aorta it was uniform throughout. Concentric layers in group 2 varied between 58-76, 45-50, 55-66 in cranial aorta, pulmonary trunk and caudal aorta respectively. In group 3, the inner half of the tunica media of cranial aorta was comprised widely separated wavy elastic laminae in comparison to outer half (Fig. 2) and their number ranged between 50-55. The tunica media of pulmonary trunk consisted of 55-65 concentric layers of SMC, which were more densely packed in its inner half than outer half. The concentric laminae of caudal aorta were separated by loose fibrous connective tissue and varied between 48-61. In neonatal goat, the tunica media of cranial aorta and pulmonary trunk was dense in its inner one-third and were thicker than in group 3. The number of SMC ranged between 45-51 in cranial aorta and 34-38 in pulmonary trunk. The thickness of tunica media of caudal aorta decreased from inside to outside and the number of concentric layers varied between 50-55. In adult goat the SMC / bundles increased mediolaterally and were separated by fibroelastic tissue. The outer two-third of media showed islands of SMC with prominant dark nuclei (Fig. 3). The islands were distinct and diffusely scattered in the tunica media of pulmonary trunk in comparison to other 2 arteries of this region (Fig. 6). Tunica adventitia in group 1 was loose and fibrocellular in all the 3 arteries. It was one-third of tunica media in group 2 from group 3 onward it comprised loose fibrous tissue which contained vasa vasorum and nervi vasorum.

The internal elastic lamina was not conspicuous in any of the 3 arterial trunks in group 2, was distinguishable in pulmonary trunk and caudal aorta in group 3 (Fig. 4). It was



Figs 1-6. Photomicrograph - cross section of; **1.** Caudal aorta (DA) and pulmonary trunk (PT) (group 1). H&E. × 100 showing endothelium (E), subendothelium (SE), tunica media (TM) and tunica adventitia (TA); **2.** Cranial aorta (group 3) H&E. ×100 showing endothelium (E), subendothelium (SE), tunica media (TM) and tunica adventitia (TA).; **3.** Cranial aorta (group 5). H&E. ×400 showing nuclei (N) of the smooth muscle cells in the tunica media (TM) and fibrocartilage (FC); **4.** Caudal aorta (group 3). Verhoeff's elastic stain -VG. × 100 showing endothelium (E), concentric elastic laminae (CEL) in inner part of tunica media (TM) and collagen fibres in tunica adventitia (TA); **5.** Caudal aorta (group 5). Verhoeff's elastic stain -VG. × 100 showing smooth muscle bundles (SMB) and elastic fibres (EF) in patches in the tunica media (TM); **6.** Pulmonary trunk (group 5). H&E. × 100 showing patches of longitudinal smooth bundles (LSMB) in outer part of tunica media and fibrocartilage (FC) type structure in the inner part of the tunica media (TM).

not conspicuous in any of the arterial trunk of this region in neonatal and adult goat. Coarse elastic fibres were more in the subendothelium of cranial aorta than pulmonary trunk, and were inconspicuous in caudal aorta of the adult goat. The elastic lamina in the tunica media were dense in the inner half than outer half and were widely separated by SMC and loose collagen fibres in all the 3 arterial trunks from group 2-4 (Fig. 5). Longitudinal SMC were observed in outer half of the tunica media in all the 3 arteries in group 5. Increase in the thickness of elastic laminae with advancement of age in this study is in line with the study of Mac Callum (1969) in rat. The tunica adventitia comprised predominantly of collagen fibres with a mixture of few elastic fibres.

The total wall thickness of all the arteries of this region in this study increased gradually in prenatal, neonatal and adult goat except the pulmonary trunk where total wall thickness was maximum in neonatal goat (Table 2). The decrease in the wall thickness of the pulmonary trunk in postnatal life is probably for greater physiological activity and consequent increase in the luminal area because in the foetus the work of the artery appeared to be shared partly by ductus arteriosus.

In group 1 the cranial aorta showed maximum mean thickness of tunica intima and total wall in contrast to tunica media and adventitia which were thickest in caudal aorta. From group 2 onward thickness of intima, media and adventitia were greatest in caudal aorta (Table 2). The thickness of tunica intima decreased significantly in group 2, while remaining 2 tunics increased gradually with the advancement of age except the tunica adventitia of caudal aorta which decreased to 91.28 ± 6.77 μm in neonatal goat and 131.74 ± 7.00 μm in pulmonary trunk in group 3.

Growth spurt

The thickness of the total wall and its tunics except tunica intima of all the arteries revealed maximum increase in groups 1-2. The increase in prenatal period (groups 1-3) was maximum for tunica media in pulmonary trunk. The tunica adventitia was next in order. However, the tunica intima revealed maximum increase (64%) in cranial aorta and decrease in remaining 2 vessels. The thickness of tunica adventitia of cranial aorta decreased in groups 3-4 and that of pulmonary trunk in groups 2-3.

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Indian Journal of Animal Sciences **70** (10): 1052-1053, October 2000

Induction of acute renal failure by uninephrectomy along with subtotal nephrectomy in goats

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Received: 1 February 1999; Accepted: 21 March 2000

Key words: Creatinine, Nephrectomy, Renal failure, Uremia

The present study deals with methodology employing right total nephrectomy along with left partial nephrectomy for inducing acute renal failure (ARF) in goats.Clinically healthy nondescript adult goats (12), 1½ to 2 years old, randomly divided into groups A(2), B(5) and C(5) were utilized, for the study. The animals of groups A and B were used for inducing ARF as follows.

Group A: Both goats were subjected to right total nephrectomy (OPI) as per Kshama *et al.* (1996). This was followed 10 days later by partial left nephrectomy (OPII). The animals were kept under close observation for 30 days.

Group B: The surgical technique applied in all the 5 goats of group B was similar to group A animals except for removal of comparatively more tissues form the left kidney. This was achieved by placing through interrupted sutures on 3 sides of the left renal pelvis i.e. dorsal and both lateral sides and disecting out the entire renal parenchyma just above the suture line. The total renal mass dissected out in each of the 5 animals were weighed. Subsequently all the animals were kept under close observation until their death.

All the 5 goats of group C, which served as control also underwent similar surgical manaoeuvring in 2 stages at the interval of 10 days followed by post operative care as in groups A and B animals except for the removal of right kidney and resection of left kidney. Both the kidneys were simply handled and put back in original position and the abdomen was closed. These animals, while under observation for 30 days, received feeding and management similar to the animals of groups A and B.

Samples of blood, urine and CSF from the animals of the 3 groups were collected at different intervals and processed for analysis of urea nitrogen (UN) by DAM method (Wybenga *et al.* 1971) and creatinine (Cr) by alkaline picrate method (Bonses and Taussky 1945). The special renal function tests viz. the glomerular filteration rate (GFR) and plasma phenolsulfonphthalein clearance (PSP) were determined by undertaking endogenous creatinine clearance test (Osborne *et al.* 1972) and estimating the biological half life i.e. plasma t1/2 (Brobst *et al.* 1967) respectively. The data obtained was subjected to statistical analysis by the test of significance (Snedecor and Cochran 1967).

Group A To standardize the technique of sub total nephrectomy for inducing desired level of renal failure a preliminary study was first undertaken in 2 goats of group A. The technique was suitable depending on removal of desired amount of renal tissue. Both the animals developed moderate uremia with significant rise in serum Cr (3.32±0.25; day 0-1.08±0.27 mg/dl) and Un (64.4±10.12; day 0-21.39±4.12 mg/dl) along with significant reduction in GFR (0.53±0.12; day 0-1.34±0.18 ml/min/kg) and plasma PSP clearance (t1/2 21.38±4.38; day 0-7.13±0.38 min) by day 14 following OPII before the death of 1 of them 6 days later. The other goat survived until day 30 following OPII when the study was terminated. The clinical illness comprised of anorexia and depression. In this group on an average 11.5 g of left renal parenchyma was removed out of the 37 g average weight of the whole left kidney signifying moderate (65-70%) ARF. The survival of 1 goat could be attributed to the adaptive changes following reduction in nephron numbers in healthy mammalian kidney (Kaufman *et al.* 1974) because of increase in the functional capacity of residual renal tissue.

Group B The induction of fatal (80-85%) ARF in the 5 goats of group B was successfully achieved by making suitable surgical modification to dissect out 20-22 g (2/3rd) of left renal parenchyma out of the 37 g average weight of whole organ. The model thus prepared, was able to achieve the desired level of renal failure along with severe degree of uremia (Cr 7.61±1.29; day 0-1.20±0.17 mg/dl; UN 157.02±42.63; day 0-19.63±2.34 mg/dl) along with marked reduction in renal filteration (GFR 0.24±0.09, day 0-1.86±0.04 ml/min/kg) and excretory capacity (PSP t1/2

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23.13±2.13; day 0-9.50±1.38 min) of the animals by day 3 following OPII. The induced ARF proved fatal with the death of all the 5 goats by day 7 following OPII (2 males each on days 5 and 6, and 1 on day 7) after showing severe clinical illness comprising of profound depression along with complete anorexia. Though the technique of subtotal nephrectomy has been used in dogs (Vaneerdeweg *et al.* 1992), its application in goats was undertaken for the first time. The success of the model methodology in inducing varied degrees of ARF will encourage taking up future studies on different aspects of renal problems in farm ruminants.

The appreciable clinical illness as observed in the animals of both groups was the reflection of toxemia due to moderate (group A) to severe (group B) degrees of uremia. As a consequence of the massive reduction in the total nephron population, the adaptive renal mechanism could function only to a certain limit resulting in significantly reduced filteration and excretory capability of the remnant kidney mass. Both serum Cr and Un qualifies for reliably assessing the degree of renal failure, however, the possibility of non-renal causes i.e. shock, dehydration, congestive heart failure, urinary tract obstruction etc. (Stogdale 1981) needs to be ruled out.

As regards changes in CSF, Cr significantly increased (1.97±0.34; day 0-0.81±0.16 mg/dl) only in cases of severe (80-85%) renal failure in group B animals, whereas the Un had a significant increase in both moderate (group A) and severe renal failure. Creatinine being a larger molecule than Un, is reported to be less easily diffusible into the CSF with the result that its CSF value is not the true reflection of its blood concentration, whereas the concentration of urea in CSF is almost identical to that of blood due to its easy diffusibility across CSF barrier (Harrison 1957).

There was significant decrease in the concentration of Cr (Group B 57.14±16.13, day 0-89.20±11.90 mg/dl) and Un (Group B 534.85±20.92, day 0-1042.53±22.21 mg/dl) in urine with the progressive rise in their blood values because of reduction in total nephron population which includes both glomeruli and tubules. Such marked reduction in their urinary excretion was a sure indication of a considerable degree of renal failure (Hamburger *et al.* 1968). Therefore a significant decline in urinary output of urea and creatinine seems to have a promising diagnostic value for identifying marked renal failure.

As regards the utilities of special renal function tests, viz. GFR and plasma PSP clearance, both are significantly dependable in assessing the degree of renal failure. Significantly delayed plasma PSP clearance and reduced GFR values were observed by Kumar and Pandey (1994 a,b) in mercuric chloride induced nephrotoxicity in goats. GFR and plasma PSP clearance therefore have substantially proven efficiency in diagnosing renal problem associated with glomerular and tubular damage respectively.

All the 5 control goats of group C remained clinically normal until 30 days observation period following OPII without any significant variation in different renal function tests.

ACKNOWLEDGMENTS

We thank the Director of the Institute, and the Head of the Division for the facility provided for the study.

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Indian Journal of Animal Sciences **70** (10): 1054-1056, October 2000

Histological changes during various augmentation cystoplasty procedures in canines*

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Received 25 November 1999; Accepted: 25 April 2000

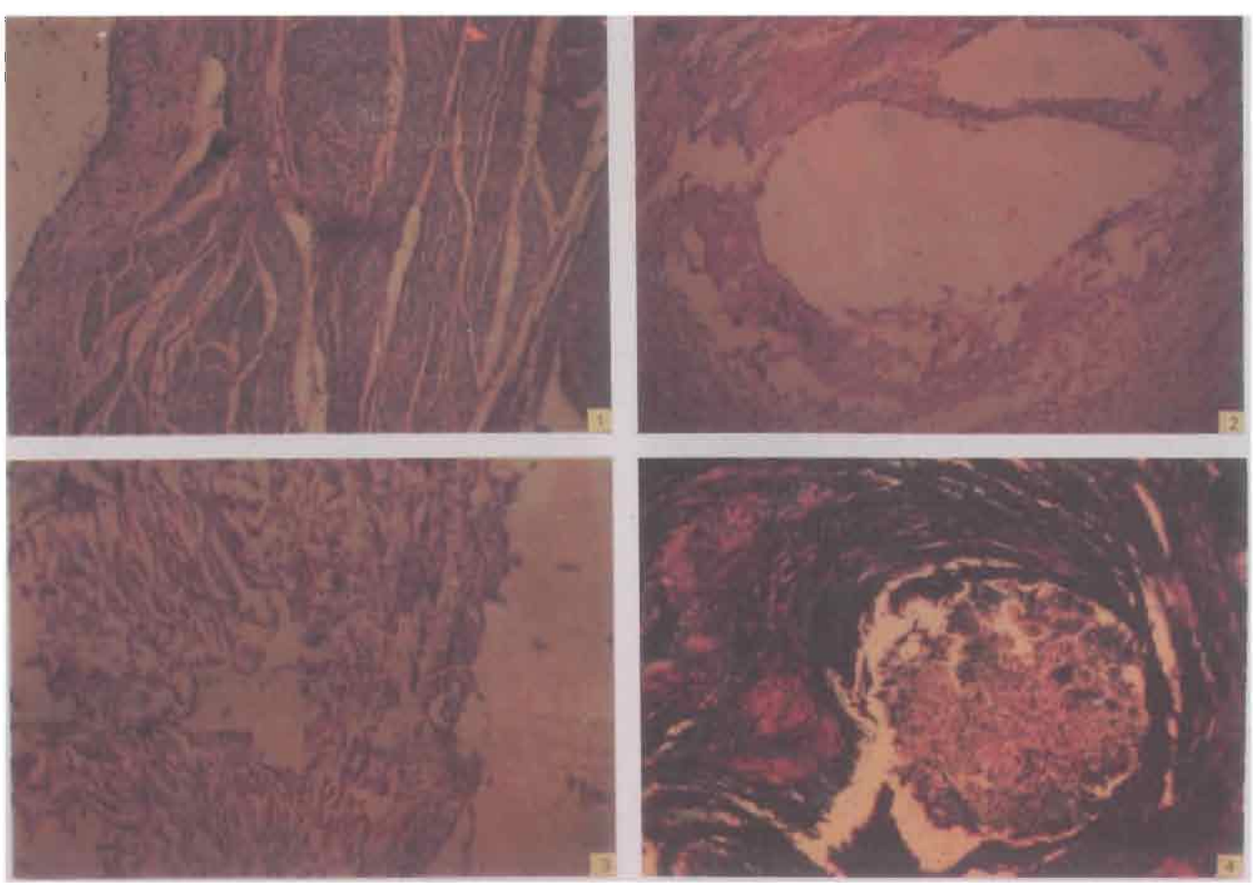
Keywords: Augmentation- Cystoplasty- Auto graft- Homologous graft

Augmentation cystoplasty is essential to restore normal bladder capacity and function in animals. In the present study augmentation cystoplasty was performed with intact ileal segment with blood supply, autologous fascial graft and homologous preserved bladder grafts in comparison to control animals.

Dogs (24) of either sex divided into 4 groups of 6 animals each were perpared for surgery by taking all aseptic precautions, and maintained in identical managemental conditions. Following midventral incision in females and parallel incision to penis in males the urinary bladder was

*Part of the Ph.D Thesis of the first author approved by Andhra Pradesh Agricultural University, Hyderabad.

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Figs 1-4. **1.** Bladder mucosa showed cystic dilatation and degeneration. **2.** Perfect union between host tissue and graft. **3.** More collagen tissue metaplasia changes by formation of body spicules. **4.** The mucous membrane appeared shrunken with atropy of living epithelium.

Table 1. Sequence of histological events following various augmentation cystoplasty procedures in different groups in canines

Post operative period (days)	Group1	Group 2	Group3	Group 4
7	Inflammatory reaction severe with more neutrophils around suture line. Mucosal lining showed hypertrophy, hyperplasia and vacuolar degeneration	Haemonhage, edema, cellular infiltration is superficial layers at anastomotic site.	Abundant granulation tissue fibroblastic proliferation, heavy cellular infiltration, heavy cellular infiltration at anastomotic site inflammatory reaction extended upto muscle layer of bladder.	Congestion, hemorrhage, edema, heavy cellular infiltration in serosa. Inflammatory reaction extended to all the layers of preserved bladder graft
15	Inflammatory reaction less. Evidence of early granulation and fibroblast.	Early granulation tissue in muscular layers. Ileal mucous methbrane showed desquamation and fibrosis with little proliferation of connective tissue. Hyperplasia of villus structure fibrosis around glandular structure.	More fibroblastic proliferation, dilated blood vassels, collagen within fibrous tissue.	At the site granulation tissue with little inflammatory reaction in both host and donor tissues.
30	More granulation tissue with little inflammatory reaction	Evidence of granulation tissue and proliferation of connective tissue at augmented site attempts of transformation of ileal mucous membrane to uroepithelium	More collagen tissue metaplasia changes by formation of body spicules (Fig.3.)	Several large foci of calcium deposits were found in granulation tissue surrounded by connective tissue. The host bladder muscle bundles showed changes of hyalinization resulting in scar formation.
60	Encapsulation of suture material by fibrous tissue. Collagen tissue could be detected by Massons trichrome stain. Low inflammatory reaction. Bladder mucosa showed cystic dilatation and degeneration. (Fig.1)	Transformation of ileal mucous membrane into uroepithelium is clear. No inflammatory reaction perfect union between host tissue and graft (Fig.2.)	Luminal surface of graft started showing signs of covering by stratified transitional epithelium.	Normal bladder was noticed as a result of regeneration of host bladder. The mucous membrane appeared shruken with atropy of living epithelium (Fig.4.)

Group I: Radical cystectomy and cystorrhaphy
II: Augmentation cystoplasty with isolated ileal segment
III: Augment cystoplasty with free facia lata graft
IV: Augumentation cystoplasty with preserved homologous bladder graft.

exteriorized under sedation and intraval sodium general anaesthesia cranial portion of bladder was resected and it was not augmented in control (group 1) animals but closed by suturing. In 2, 3 and 4 groups the cystectomised bladders were augmented with isolated segment of ileum with intact blood supply, fascia rata of the same animals ore preserved homologous baldder grafts respectively. The dogs were euthanatized at 7, 15, 30 and 60 days post-operatively, bladder specimens were collected and histopathological studies were conducted.

Sequential order of histological changes are given in Table 1. Persistent inflammatory reaction throughout the observation in group 1 could be attributed to the suture material (Hasting *et al.* 1975). In group 2 mild inflammation process was due to bacteria harbouring mucosa and muscle layers (Prasad *et al.* 1973). The excess production of mucus in the early post operative days might be due to goblet cell hyperplasia. Flattening of intestinal villi and union between corresponding layers of bladder and graft was completed 30 post operative day. Similar observations were also reported by Prasad *et al.* (1973), and Sharma and Khan (1978). Attempts of transformation of ileal mucosa to bladder mucosa were evident from 30th postoperative day and were more clear on 60th post operative day. Imperfect union between host bladder and facia lata graft with inflammatory cells and capillary invasion into the granulation tissue at 15th day was

observed in group 3 suggesting that still the graft is in the process of taking'. These changes decreased with replacement by new connective tissue which was evident at 30th post operative day. This is in agreement with findings of T suji *et al.* (1963). Intense inflammatory process noticed in group 4 in early phase could be attributed to surgical trauma and traces of formalin left in the preserved bladdar graft. It showed signs of loosening and of detachment. The results are in agreement with Prasad and Tyagi (1981). Epithelial regeneration was complete by eighth week in this study.

Based on the observations it can be concluded that if the graft is viable and possess epithelium like ileal segment they will retain the epithelium regardless of the type. If the grafts do not have epithelium like free facial grafts and preserved bladder grafts will acquire epithelium from edges of host tissue.The grafts were well taken up except preserved bladder graft which showed signs of detachment. No graft rejection was observated in any of the animals.

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Indian Journal of Animal Sciences **70** (10): 1057-1058, October 2000

Effect of methanol extract of *Stylosanthes scabra* on ixodid ticks of animals

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Received: 10 August 1998; Accepted: 13 April 2000

Key words: Ixodid ticks, Methanol extract, *Stylosanthes scabra*

There has been a growing interest in biological control of vectors including ticks. Very few biocontrol agents to date, have become popular. Therefore an attempt was made to control commonly occurring ticks on animals using *Stylosanthes scabra* which is a nutritionally utilizable and easily cultivable legume. The secretions of this plant possess acaricidal effect on ticks (Sutherst *et al.* 1982).

Cultivation of *Stylosanthes scabra,* collection and maintenance of different stages of *Boophilus microplus, Haemaphysalis intermedia* and *Rhipicephalus sanguineus* was as per the method described by Khudrathulla and Jagannath (1998a).

Methanol extraction of S. scabra *leaves*

Fresh leaves of the plant were triturated in methanol. The resultant mixture was sieved and the filtrate was kept at room temperature for methanol evaporation. The residue thus obtained constituted the plant extract. This extract was mixed with ethanol containing triton X-100 and different dilutions of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6% were prepared with distilled water. The experiment was conducted as per the description of Roulston *et al.* (1966).

In vitro trials were conducted by tea bag method (Gladney *et al.* 1972). Tea bags containing known number of larvae or nymphs were dipped in different concentrations of the extract for 4 min. Their mortality was recorded after 24 hr of the trial. The control group was dipped in distilled water.

Significant differences ($P<0.01$) were seen in the mortality of *B. microplus* larvae in methanol extract when exposed to different concentrations. A high percentage mortality of *B. microplus* larvae of 34.50 ± 1.50 was observed in 0.6% (Tables 1, 2) followed by 29.50 ± 1.50 in 0.5% and lowest (10.50 ± 0.50) in 0.1%. *H. intermedia* larval percentage mortality of 19.50 ± 2.50 was observed in 0.6% followed by 19.00±1.00 in 0.5 and lowest (8.50 ± 0.50) in 0.1% concentration. *R. sanguineus* larval percentage mortality of 40.50 ± 0.50 was observed in 0.6% followed by 38.00 ± 1.00 in 0.5 and the lowest (2.00 ± 2.00) in 0.1% concentration (Table 1). No significant differences were observed in mortality of all species of ticks when exposed to extract of leaves of plant of different ages.

The mortality of nymphs of *B. microplus* was significantly different ($P\leq0.05$) when exposed to different concentrations. A high mortality of 16.50 ± 1.50 was seen in 0.6% followed by 13.50 ± 0.50 in 0.5% and the low (4.00 ± 2.00) in 1.0% concentration (Table 2). No significant differences were observed when exposed to various ages of the plant. No nymphal mortality in *H. intermedia* was observed in different concentrations and similar results were observed when tested

Table 1. Percentage mean larval mortality in methanol extract of *S. scabra* leaves

Extract concentration (per cent)	*B.microplus* Mean±SE	*H. intermedia* Mean±SE	*R. sanguineus* Mean±SE
0.1	10.50^{a} ± 0.50	8.50^{a} ± 0.50	2.00^{a} ± 2.00
0.2	12.50^{ab}± 0.50	11.00^{a}± 1.00	22.50^{b}± 2.50
0.3	15.50^{ab}± 1.50	10.50^{a}± 0.50	24.50^{b}± 1.00
0.4	18.00^{b}± 1.00	14.50^{ab}± 0.50	35.50^{c}± 0.50
0.5	29.50^{c}± 1.00	19.00^{b}± 1.00	38.00^{c}± 1.00
0.6	34.50^{c}± 1.50	19.50^{b}± 2.50	40.50^{c}± 0.50

Table 2. Percentage mean nymphal mortality in methanol extract of *S. scabra* leaves

Extract concentration (per cent)	*B. microplus* Mean ± SE	*R. sanguineus* Mean ± SE
0.1	4.00^{a}± 2.00	9.50^{a}± 1.50
0.2	8.00^{ab}± 1.00	13.00^{ab}± 1.00
0.3	10.50^{b} ± 2.50	21.00^{bc}± 2.00
0.4	11.50^{bc} ± 0.50	24.00^{bcd} ± 2.00
0.5	13.50^{bc} ± 0.50	28.00^{cd} ± 1.00
0.6	16.50^{c}± 1.50	31.50^{d} ± 1.50

Means bearing any one common superscript in column do not differ with each other.

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against the extracts of plants of different ages. Significant difference ($P \leq 0.01$) was seen in *R. sanguineus* nymphal mortality when exposed to various concentration. The mortality of nymphs when tested against different age groups of the plant was nonsignificant. A mortality of 31.50 ± 1.50 was seen in 0.6% followed by 28.00 ± 1.00 in 0.5 and lowest (9.50 ± 1.50) mortality in 0.1% concentration (Table 2).

The chemical control of ticks is expensive and also leads to wide spread development of resistance besides causing environment pollution. The larvae of ticks ascend plants to transfer to a passing host. The larval ticks must wait for several weeks to come in contact with a host. Control of ticks in different countries using grasses (Barros and Evans 1989) and miscellaneous plants by (Dobrotvorskii *et al.* 1989) have been reported.

Stylosanthes scabra which is used as grass/legume mixture in cattle feed and it has an acaricidal property. *S. scabra* produces a sticky secretion which immediately immobilizes the larvae of ticks which are poisoned within 24 hr by an unidentified vapour from the secretion (Sutherst *et al.* 1982). The secretion appears to have the potential to substantially reduce the populations of all species of ticks because of the acaricidal property and therefore, in this study effect of its methanol extract was studied.

In methanol extract at 0.6% concentration a high mortality of 34.50 ± 2.50, 19.50 ± 2.50 and 40.50 ± 0.50 was noticed in larvae of *B. microplus, H. intermedia* and *R. sanguineus* respectively. Khudrathulla and Jagannath (1999) reported that the aqueous extract showed 100% mortality of *B. microplus,* larvae at 3.0% and above and same in *R. sanguineus* at 6.0%. Maximum mortality of 56.00 ± 2.00 was seen in *H. intermedia* larval stages at 6.0% concentration. This indicates better acaricidal effect of aqueous extract over methanol extract. It may also be concluded from these studies that acaricidal components are more soluble in water than in methanol.

In this study mortality of 16.50 ± 1.50 was observed in *B. microplus* nymphs at 0.6% and low (4.00 ± 2.00) at 0.1%. In *R. sanguineus* a high mortality (31.50 ± 1.50) at 0.6% and lowest (9.50 ±1.50) at 0.1% concentration was observed. There was no mortality in *H. intermedia* nymphs, however, Khudrathulla and Jagannath (1999) reported that the aqueous extract produced a highest mortality of 29.50 ± 2.50 in *H. intermedia.*

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Indian Journal of Animal Sciences **70** (10): 1059-1061, October 2000

Pharmacokinetics and dosage regimen of sodium salicylate in crossbred calves following a single intravenous injection

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Received: 3 May 1999; Accepted: 17 April 2000

Key words: Cow calves, Dosage regimen, Plasma levels, Salicylate

Sodium salicylate, a non-narcotic analgesic-antipyretic drug (Multz *et al.* 1974) is routinely and effectively used in veterinary practice (Bagdan *et al.* 1981) to relieve pain such as cephalgia, myalgia and other pains arising from integumental structures (Woodbury and Fingal 1975). The disposition kinetics of sodium salicylate in different species of animals revealed marked species variation (Davis and Westfall 1972, Rampal *et al.* 1994) and age difference (Davis *et al.* 1973). The disposition kinetics studies of a drug are essential in the animal species and the environment in which the drug is to be used clinically. There is no information on detailed pharmacokinetics of salicylate in cattle in tropical climate. Keeping this in view, the present investigation was undertaken to study the detailed pharmacokinetics of sodium salicylate in crossbred calves. Based on this, a suitable dosage regimen was also computed.

Clinically healthy crossbred calves (6), weighing 85-166 kg, procured from the local market were maintained in the departmental animal shed under standard conditions of management. Sodium salicylate was administered as 20% freshly prepared solution in sterilized normal saline by single intravenous injection into the jugular vein @ 50 mg.kg^{-1} body weight. Blood samples were collected from contralateral jugular vein into heparinized glass test tubes at 1, 2.5, 5, 7.5, 10, 15, 30, 45 min and at 1, 1.5, 2, 2.5, 3 and 4 hr after drug administration. Plasma was separated after centrifugation at 3 000 rpm for 15 min at room temperature and stored at —20°C till analysis, usually within 24 hr of collection. The concentration of salicylate in plasma was estimated spectrophotometrically (Trinder 1954).

Pharmacokinetics of sodium salicylate was determined by

Table 1. Plasma levels of sodium salicylate (μg.ml^{-1}) after its single intravenous administration @ 50 mg.kg^{-1} in crossbred calves

	Amimal number						
Time interval (min)	1	2	3	4	5	6	Mean± SE
1	402.8	396.5	406.9	434.7	395.3	396.5	405.5±6.13
2.5	262.0	304.9	307.8	285.2	299.1	320.0	296.5±8.32
5	201.7	219.1	234.2	220.3	229.0	241.1	224.2±5.65
7.5	169.3	168.7	199.4	204.6	NE	221.4	192.7±10.3
10	164.6	160.0	164.6	204.0	178.5	205.8	179.6±8.40
15	151.3	142.0	150.7	185.5	147.8	168.7	157.7±6.65
30	129.8	126.9	140.9	131.6	121.7	124.0	129.2±2.78
45	126.9	121.1	123.3	116.5	112.5	118.8	119.9±2.08
60	91.6	82.9	82.3	84.1	78.8	81.7	83.6±1.76
90	56.8	53.3	55.6	54.5	56.2	52.2	54.8±0.72
120	33.0	46.4	48.7	40.0	49.3	45.2	43.8±2.54
150	18.0	33.6	23.2	33.6	33.0	28.4	28.3±2.65
180	14.5	16.2	10.4	16.2	15.1	17.4	15.0±1.00
240	4.64	8.69	2.32	5.22	4.64	5.78	5.22±0.85

NE, Not estimated.

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Table 2. Pharmacokinetic parameters of sodium salicylate in cross bred calves (n=6) after single intravenous administration of sodium salicyate @ 50 mg.kg^{-1} body weight

Parameter	Unit	Mean± SE	Range
Cp^o	μg.ml^{-1}	406.0±19.2	361.1-490.6
A	μg.ml^{-1}	200.0±26.8	133.3-298.8
α	h^{-1}	17.2±2.66	12.0-27.0
t½α	h	0.045±0.006	0.026-0.058
B	μg.ml^{-1}	206.0±11.3	173.0-252.0
β	h^{-1}	0.852±0.036	0.753-0.992
t½β	h	0.82±0.003	0.699-0.92
K_{12}	h^{-1}	7.21±1.59	3.85-14.8
K_{21}	h^{-1}	9.25±1.54	5.29-16.0
Kel	h^{-1}	1.6±0.082	1.43-1.96
cI_B	L.kg^{-1}.h^{-1}	0.197±0.001	0.192-0.2
AUC	μg.ml^{-1}.h	253.7±1.54	250.5-259.6
AUMC	μg.ml^{-1}.h^2	285.5±8.23	256.3-306.9
Vc	L.kg^{-1}	0.125±0.005	0.102-0.139
Vd (area)	L.kg^{-1}	0.233±0.01	0.194-0.264
Vd (B)	L.kg^{-1}	0.246±0.013	0.198-0.289
Vd (ss)	L.kg^{-1}	0.222±0.009	0.19-0.244
MRT	h	1.13±0.038	0.987-1.22
fc	ratio	0.542±0.043	0.409-0.67
P/C	ratio	0.908±0.158	0.49-1.45
td	h	0.604±0.024	0.514-0.678

A and B are Y intercepts; α and β are distribution and elimination rate constants; $t_{½\alpha}$ and $t_{½\beta}$ are distribution and elimination half lives. K_{el} is elimination rate constant from central compartment; K_{12} and K_{21} are micro rate constants, AUC is area under the plasma concentration-time curve; AUMC is area under the first moment of plasma concentration-time curve; C_{IB} is total body clearance; Vc is volume of central compartment; $Vd_{(area)}$, Vd(B) and $V_{d(ss)}$ are the volumes of distribution; P/C is ratio of drug present in peripheral to central compartment, MRT is the mean residential time of drug in body; td is duration of therapeutic effect; Cp^o is expected plasma drug concentration at zero time; fc is the fraction of administered dose present in central compartment.

analyzing the data on concentration-time profile of each experimental animal by the method of Gibaldi and Perrier (1982) as detailed by Srivastava and Bal (1994). The dosage regimen was computed as per Baggot (1977).

The plasma concentration of sodium salicylate at various time intervals after its single iv injection are presented in Table 1. After intravenous injection, at 1 min the peak plasma, concentration of salicylate was 405.5 ± 6.13 μg.ml^{-1}. The drug was detected up to 4 hr of dosing. The pattern of decline of plasma levels showed that salicylate followed 2-compartment open model, which is similar to the findings in swines, goats, cats and buffalo (Davis and Westfall 1972, Rampal *et al.* 1994). Salicylate followed one-compartment open model in dogs, cows and horses (Davis *et al.* 1973, Gingerich *et al.* 1975).

The high values of distribution rate constant and elimination rate constant (Table 2) indicated that salicylate is

Table 3. Priming (D) and maintenance (D') doses (mg.kg^{-1}) of sodium salicylate in crossbred calves at various dosage intervals for different therapeutic concentration

	Dosage interval (h)		
Plasma therapeutic concentration (μg.ml^{-1})	2	2.5	3
20	D = 25.6	39.3	60.4
	D' = 20.9	34.6	55.7
30	D = 38.3	58.9	90.6
	D' = 31.3	51.9	83.6
40	D = 51.1	78.6	120.8
	D' = 41.8	69.2	111.5

Values given are mean of the results obtained from 6 animals.

rapidly distributed and eliminated in cow calves. The elimination half life ($t_{½\beta}$) of salicylate was similar to the $t_{½\beta}$ of salicylate in cows (Gingerich *et al.* 1975), buffalo calves (Rampal *et al.* 1994) and goats (Boulos *et al.* 1972). The $t_{½\beta}$ of salicylate in cows, buffalo calves and goats were 0.5, 0.94 and 0.7 hr respectively. However, the $t_{½\beta}$ of salicylate established in this study was lower than that reported in swines, dogs and cats (Davis and Westfall 1972). The elimination half-life of salicylate in swines, dogs and cats were 5.9, 8.6 and 37.6 hr respectively. The value of Cl in this study (0.197±0.001 Litre.kg^{-1}.hr^{-1}) was comparable to that in buffalo calves (Rampal *et al.* 1994).

The values of apparant volume of distribution of salicylate in cow calves (0.233 ±0.01 L.kg^{-1}) revealed a limited extravascular distribution which is in accordance to the findings in buffalo calves (Rampal *et al.* 1994).

The ultimate objective of the disposition kinetic study of a drug is to calculate its suitable dosage regimen so that the minimum effective concentration remain maintained for longer duration. For salicylate the minimum therapeutic concentration of > 30 μg.ml^{-1} has been reported (Gingerich *et al.* 1975). By taking the pharmacokinetic parameters of sodium salicylate in crossbred calves, various dosage schedule for different plasma therapeutic concentrations were calculated (Table 3). On the basis of present study, it is concluded that to maintain the minimum effective plasma concentration of 30 μg.ml^{-1}, sodium salicylate should be administered @ 58.9 mg.kg^{-1}followed by 51.9 mg.kg^{-1} at 2.5 hr interval in calves.

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Indian Journal of Animal Sciences 70 (10): 1062-1063, October 2000

Comparative efficacy of a herbal anthelmintic and piperazine citrate against natural infection of *Toxocara vitulorum* (Goeze 1982) in cow and buffalo calves*

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Received: 8 September 1999; Accepted: 12 April 2000

Key words: Herbal anthelmintic, Piperazine, *Toxocara vitulorum*

From ancient times, herbal anthelmintics enjoy good reputation and their efficacies are well documented. Out of the commonly available herbal plants, *Butea frondosa* and *Embelia ribes,* have been reported for promising anthelmintic efficacy against poultry ascariasis and other intestinal worms of animals including those of human beings (Sharma and Sisodia 1976, Satyanarayanana Rao and Krishnaiah 1982, Kirtikar and Basu 1975). Recent trend in veterinary medicine is the intensive search for and use of herbal products, as the modern anthelmintics are much costlier.

The present study was, therefore, undertaken to evaluate and compare the efficacy of a proprietary herbal anthelmintic with piperazine citrate against economically important *Toxocara vitulorum* in cow and buffalo calves.

Locally available 30 calves (18 buffalo and 12 cow) below 2 months of age and naturally infected with *Toxocara vitulorum*, were randomly divided into 3 groups, each comprising 10 calves (6 buffalo and 4 cow). Faecal egg counts (EPG) were done by Stoll's dilution technique on day 0, 7 and 21 post-infection (PI). Each calf of group 1 was treated orally with 2 doses of 50 ml proprietary herbal anthelmintic on day 0 and 15. Each calf of group 2 was treated twice with 200 mg kg^{-1} body weight piperazine citrate. The calves of group 3 served as infected-untreated control. Parasite free calves (6 buffalo and 4 cow calves) served as healthy control. Blood and serum samples were collected from all the calves on day 0, 7 and 21 PT for evaluation of haematological and biochemical parameters. Percentage efficacy of the drug was calculated as follows:

$$\text{Per cent efficacy} = \frac{\text{Pretreatment mean EPG} - \text{Posttreatment mean EPG}}{\text{Pretreatment mean EPG}} \times 100$$

Data on efficacies of the drugs were analyzed by paired 't' test between days of treatment and Fisher 't' test between the drugs (Snedecor and Cochran 1968).

Each 10 ml of the herbal anthelmintic contains: *Embelia ribes* 0.5; *Punica granatum* 0.55; *Caesalpinia crista* 0.5; *Artemisia maritima* 0.45 ; *Carum copticum* 0.5; *Gardenia gummifera* 0.4; *Centratherum anthelminticum* 0.4; *Butea monosperma* 0.8, *Cassia angustifolia* 0.5 and *Acorus calamus* 0.4 (all in g).

The herbal anthelmintic (Table 1) showed on an average 52.88% efficacy on day 7 and 77.60% on day 21 PT. The worms started to be expelled out in faeces after 48 hr of treatment and continued for 4-6 days. Piperazine citrate was 79.84% effective on day 7 and 94.50% on day 21 PT. The worms started passing out within 24 hr and continued for 3-4 days. Both the drugs were well tolerated and no clinical signs of side effects were seen. Statistical analysis showed that efficacy of each drug between days 7 and 21 of treatment was significant ('t' values = 6.09*–9.89** and 15.49**–17.30** respectively), but when the efficacies of these drugs were compared with each other the differences were found nonsignificant ('t' values = 0.130^{NS} and 0.027^{NS} respectively). An explanation for statistical failure to prove significance between 77.60% and 94.50% efficacies might possibly be because of rather large variations in EPG counts. In addition to significant decrease in PT EPG counts, moderate to marked increase in Hb, PCV, TEC, TSP and A/G ratio were recorded in calves treated with these drugs which were significantly decreased prior to treatment. The increase was more marked in calves treated with piperazine citrate, the values reaching almost to normal level, as compared to the herbal anthelmintic treated calves.

Butea frondosa and *Embelia ribes* of the herbal present anthelmintic were found quite effective anthelmintics against *Ascaridia galli* of poultry (Sharma and Sisodia 1976,

*Part of M.V.Sc. Thesis submitted by the first author to Birsa Agricultural University, Ranchi, Bihar.

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Table 1. Anthelmintic efficacy of kriminth and piperazine citrate against *T. vitulorum* infection in treated and untreated cows and buffalo calves

Groups	Species	No. of	Drug	Dose	Av.pretreatment	Average eggs per gram of faeces			
calves	used				EPG 0 days	7 days	Efficacy(%)	21 days	Efficay (%)
1	Cow	4	Kriminth	50ml/calf	20250±1205.82	9725±1704.16	51.97	4600±194.93	77.28
	Buffalo	6	Kriminth	50ml/calf	19225±59.17	8883.3±121.09	53.79	4241.6±53.22	77.93
2	Cow	4	Piperazine citrate	200mg kg^{-1} b.w.	24800±1784.7	5200±948.68	79.03	1312.5±723.09	94.70
	Buffalo	6	Piperazine citrate	200mg kg^{-1} b.w.	23708.3±89.26	4708.3±54.54	80.14	1350±116.66	94.30
3	Cow	4	-	Infected untreated	11375±214.58	16250±107.37	-	11700±400.17	-
	Buffalo	6	-	Infected untreated	14325±45.99	40766±301.39	-	40766±301.39	

Gr. 1, Infected treated with harbal anthlmintic; Gr. 2, infected treated with piperazine citrate, Gr. 3, infected untreated.

Satyanarayanana Rao and Krishnaiah 1982) and Oxyurids of mice (Mehta and Parasar 1966). Misra (1977) reported that Wopel was 96.13% effective against monieziasis in calves. In this study the herbal drug was moderately effective in two doses, but presumably, its efficacy could be increased with repeated doses at weekly intervals, as the drug contains most effective ingredients stated above, besides being apparently safe and comparatively cheaper. However, further study is needed to confirm the present finding and before the drug may be fully patronized for animal health package. The reference drug, piperazine citrate has a high, though not cent per cent efficacy. Our finding are in close agreement with the reports of Baruah *et al.* (1980) and Joshi *et al.* (1997).

Based on the findings of the present study it is concluded that efficacy of piperazine citrate is superior to the herbal anthelmintic.It is recommended that the herbal drug may be used as an alternative next to piperazine citrate.

ACKNOWLEDGEMENTS

We thank the Vice-Chancellor, Birsa Agricultural University, and the Dean, Ranchi Veterinary College, for providing facilities for work. The supply of herbal anthelmintic by M/s Bodesa pharmaceutical, Saharanpur, (UP), for conducting the therapeutic trials, is gratefully acknowledged.

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Indian Journal of Animal Sciences **70** (10): 1064-1066, October 2000

Embryo recoveries to different superovulatory regimes and live births subsequent to nonsurgical embryo transfer in Rathi and its crossbred cows

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Received: 29 July 1999; Accepted: 23 March 2000

Key words: Embryo, eCG, FSH, Superovulation

Superovulation is an important and integral part of the fast developing MOET programmes. The response to superovulation is known to vary between breeds (Misra *et al.* 1992, Chauhan *et al.* 1994), besides many other factors influencing it. Superovulatory responses of Rathi cattle to PMSG and FSH and the subsequent embryo recoveries were described by Bhojwani (1994), Chaudhary *et al.* (1997), Sharma and Purohit (1998, 1999) and Purohit *et al.* (1999). This paper describes the embryo recoveries to different superovulatory regimes, live births and pregnancy rates from nonsurgical embryo transfer in Rathi and its crossbred cows.

Rathi and its crossbred cows (63) were superovulated under 2 groups. Cows (26) superovulated using 1 500-3 000 IU, IM of eCG, injected on days 7-12 of the natural or progestagen implant treatment formed the group A; 37 cows were superovulated using porcine administered on days 7-10 of the natural or CIDR-B treated cycle in equal (n=11) or descending (n=26) doses (group B).The prostaglandin injection (injection lutalyse, 5.0 ml or injection Illireu, 3.5ml IV) was administered 12 and 24 hr before the last dose of FSH and 48 hr after the eCG injection. Mating was done with a proven bull or 2 inseminations were done 10 hr after the estrual onset with a gap of 12 hr. Embryos were recovered by standard nonsurgical techniques (Purohit and Sharma 1997) and the number of ovulation's recorded by recto-genital palpation. Recovered embryos were kept in holding medium containing 0.4% BSA, antibiotics, pyruvate and sucrose, washed twice, filled in straws and transferred the same day to synchronized recipients. Pregnancy diagnosis was done at 53 day post transfer by rectogenital palpation.

Superovulatory responses and embryo recovery

The mean superovulatory responses and embryo recoveries with eCG (Group A) using different dose schedules and day of initiation of superovulation were 7.92±0.66 and 3.96±0.49 respectively. The detailed results are presented in Table 1. The number of good quality embryos (transferable grade) include morula and blastocysts.

Table 1. eCG and embryo recovery

No .of animals	Days of SOV induction	Dose	Mean SOV response	Un-ovulated follicles	No. of good quality embryos	UFO recovered	Overall mean embryo recovery
7	7	3000IU	9.28±2.08	3.0	11/7	-	1.57±0.33
17	8-10	2000IU	8.06±1.78	8.0	61/17	31	5.40±1.08
2	12	1500IU	2.0±1.19	4.0	0/2	-	0.0
Total 26			7.92±0.66		72/26	31	3.96±0.49

Days 8-10 with 2000 IU as the dose is the best regime for recovering good quality embryos, however, with this dose and day of initiation of SOV treatment more number of unovulated follicles remain leading to recovery of more number of unfertilized ova. This appears to be more related with the prolonged half life of eCG, and similar findings have been reported earlier (Siedel *et al.* 1978, Crister *et al.* 1980, Zeitoun *et al.* 1991). The superovulatory response and embryo recoveries were best when superovulation was initiated in mid-luteal phase as compared to early or late luteal stage. This finding is similar to that observed in buffaloes by Beg *et al.* (1997) and supported by previous findings of Moor *et al.* (1983) in cattle that during day 8-10 maximum number of antral follicles are present on the bovine ovary which respond favourably to exogenous gonadotropins.

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Table 2. Porcine FSH and embryo recovery

No .of animals	Days of estrus	Schedule	Mean SOV response	Un-ovulated response	Embryo recovery (good grade)	UFO	Overall mean embryo recovery
11	7	Eq. dose	8.28±1.37	-	44/10	37	7.36±0.89
26	10	Des. dose	6.80±0.55	-	130/23	10	5.38±0.45
Total 37	-	-	7.24±0.52	-	174/33	47	5.97±0.56

The mean superovulatory response and embryo recovery in group B were 7.24±0.52 and 5.97±0.56 respectively. The detailed findings in the FSH group are presented in Table 2 which shows that initiation of superovulation during early luteal phase with equal dose schedule evokes a better superovulatory response but, yields more number of unfertilized ova. Since the purest forms of FSH have some contaminating LH hence a decreasing dose schedule has been reported to be better (Mapletoft *et al.* 1990). 4 animals in the group B did not respond to the superovulation treatment.

The superovulatory response was nonsignificantly different between groups A and B when SOV was induced on day 7 of the oestrus cycle, however, the embryo recoveries were significantly higher in group B ($P<0.05$ $t=5.03$). Contrary to this the superovulatory response and embryo recoveries were nonsignificantly different between groups A and B, when the SOV was initiated on day 10 of the oestrus cycle.

The overall superovulatory response and embryo recoveries in both groups A and B are nearly similar, however, the overall embryo recoveries were significantly higher in group B ($P<0.01$, $t=2.57$) This finding is similar to that of Pawshe *et al.* (1992), Crister *et al.*(1980), however, these workers have recorded slightly higher response compared to our studies. The SOV response and embryo recoveries are also similar to previous reports of Subramaniam *et al.* (1990), Khanna *et al.* (1994), but slightly lower than those reported by Arora *et al.* (1996).

After effects of SOV

The after effects of SOV seen were mild. Only 4 animals superovulated with eCG developed cystic ovaries which resolved spontaneously in 2-3 months. No animal superovulated with FSH developed cystic ovary. The future fertility of superovulated animals was not adversely affected as animals continued to cycle and conceive normally subsequently. This finding is similar to that of Chaudhary *et al.* (1997).

Embryo transfer to recipients and live births

Embryos recovered from donors were transferred to synchronized recipients. The technique is still far from perfect. To 31 donors 61 recipients were synchronized but embryos could be transferred to 46 recipients only. This was due to poor response by the donors to SOV treatment, non-response by recipients to prostaglandin treatment,non-recovery of transferable grade embryos and synchrony between the number of embryos recovered and the number of recipients synchronized. Only 5 pregnancies could be established leading to a pregnancy per cent of mere 10.86%. Two live births have resulted so far from 5 of these pregnancies. The pregnancy per cent is lower than that reported by Subramaniam *et al.* (1990), Totey *et al.* (1992), Misra *et al.* (1992). Lower pregnancy rates in indigenous cows have been previously reported (Totey *et al.* 1991).

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Indian Journal of Animal Sciences **70** (10): 1067-1068, October 2000

Effect of protein concentration and ferning pattern of bovine cervical mucus on sperm penetration

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Racived: 30 October 1998; Accepted: 25 April 2000

Key words: Bovine, Cervical mucus, Protein fern pattern, sperm penetration

Cervical mucus of good quality may act as a medium for selecting morphologically normal and abnormal sperm (Pretorius *et al.* 1984). The quality of bovine cervical mucus is assessed on the basis of its constituents and crystallization pattern. Assessment of spermatozoal movement within cervical mucus through its penetration is being used to determine the fertilizing ability of spermatozoa. The *in-vitro* bovine cervical mucus penetration test (BCMPT) is increasingly being used world over for fertility assessment of spermatozoa. Hence in the present experiment an attempt was made to study the effect of BCMPT in relation to protein concentration and crystallization pattern of cervical mucus.

Cervical mucus were collected from estrous cow at AI centre of the institute under aseptic condition. All mucus samples were selected on the basis of its clarity and elasticity. A white side test using 5% sodium hydroxide (NaOH) was performed to evaluate the mucus for infection (Pateria and Rawal 1990). Further, sperm receptivity test was conducted in the laboratory (Guard 1960) before the final selection of the cervical mucus. The selected cervical mucus samples were subjected to protein estimation (diagnostic kits) and study of crystallization pattern. The cervical mucus penetration test was performed as per Kremer (1965) and Matousek (1989).

Swimming movement of spermatozoa was observed under microscope and sperm penetration distance (mm 60 min) was measured. The data were analyzed as per Snedecor and Cochran (1989).

Cervical mucus samples which showed nil fern pattern (Fig. 3) had significantly higher concentration of protein than those having typical (Fig. 1) and atypical (Fig. 2) fern pattern (Table 1). The distance travel was more in samples with a typical fern pattern as compared to those in typical fern pattern. A negative correlation was observed between protein concentration and mucus penetration distance in cervical mucus.

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Ferning patterns, crystallization at right angle, thought to be associated with sodium and chloride and are a typical measurement of the adequacy of mucus, In this study the type of crystallization and protein concentration of mucus had a significant effect on penetration of spermatozoa. The atypical crystallization pattern showed the lowest protein concentra-

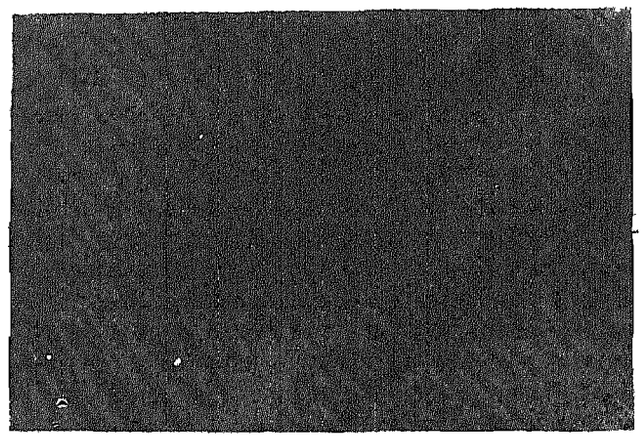


Fig. 1. Micro photographs of bovine cervical mucus showing typical fern pattern.

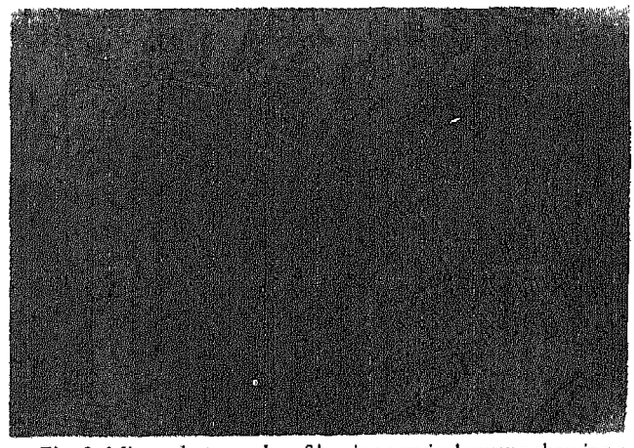


Fig. 2. Micro photographs of bovine cervical mucus showing a typical fern pattern.

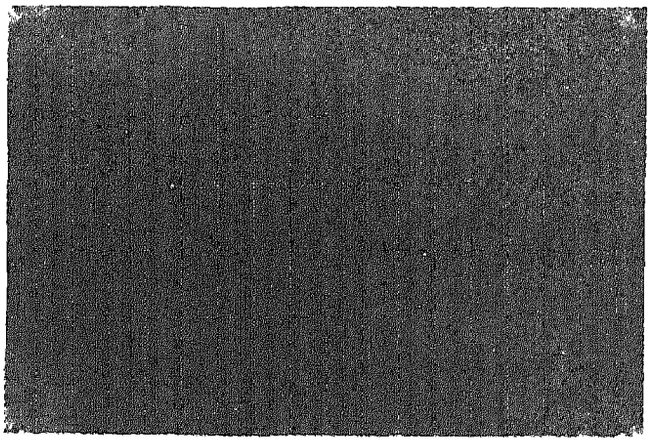


Fig. 3. Micro photographs of bovine cervical mucus showing nil fern pattern.

Table 1. Relationship between protein concentration and fern pattern of cervical mucus with sperm penetration distance (n=40)

Type of crystalization	Protein concentration (mg/ml)	Sperm penetration distance (mm/60min)	Correlation coefficient
Typical fern pattern	2.46±0.88[a]	24.85±0.71[a]	-0.056
Atypical fern pattern	01.40±0.15[c]	28.92±0.86[a]	-0.368
No crystalization	22.34±0.99[a]	04.61±1.17[c]	-0.376

tion and higher penetration distance, whereas, samples without ferning pattern had significantly higher total protein concentration and lower penetration distance indicating resistance offered to spermatozoa in the presence of high concentration of protein in the cervical mucus.

ACKNOWLEDGEMENTS

The authors thank the Director, Indian Veterinary Research Institute. Izatnagar, and the Head, Division of Animal Reproduction IVRI, Izatnagar for providing facilities.

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Indian Journal of Animal Sciences **70** (10): 1069-1070, October 2000

Comparative oestrus behaviour of Muzaffarnagri sheep and its crosses with Dorset

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Received: 20 October 1998; Accepted: 21 March 2000

Key words: Oestrus behaviour, Sheep

The sheep exhibits oestrus throughout the year in the tropics showing distinct peak in certain months (Sahni and Roy 1967, Mittal and Ghosh 1980). The present study was undertaken to study the normal pattern of oestrus profile and oestrus cycle in Muzaffarnagri sheep and its crosses with Dorset maintained under semi-intensive system of management.

The present study was conducted on 557 ewes (442 dry and 115 lactating) which included muzaffarnagri (387) and Dorset crossbred (170). Dry ewes were provided 250 g concentrate/pelleted feed throughout the year, whereas lactating were given 500 g per day for 3 months besides 5-6 hr grazing and supplementary feeding of green and dry fodder. Ewes were detected in heat 3 times a day i.e. morning (8.0 hr), evening (16.0 hr) and night (24.0 hr) during the 2 breeding seasons to know the oestrus activity and 6 hourly for estimating duration of oestrus. For determining oestrus cycle pattern in Muzaffarnagri ewes, 15 cyclic ewes of 2-3 years old age were randomly earmarked and were hand teased for heat detection using vasectomized rams for 12 months and were not bred. The data were analyzed statistically to examine the effects of breed, time of onset of oestrus on oestrus duration in dry and lactating ewes and different age groups.

Statistical analysis revealed that time of onset of oestrus exhibition differed significantly among the 3 timings in dry group indicating that higher percentage of ewes (43.14% Muzaffarnagri and 49.65% crossbred) exhibited oestrus first time in the evening as compared to morning (38.13 and 38.46%) and night (18.73 and 11.89%). Hulet *et al.* (1962) reported almost similar results. However, Sahni and Roy (1967) recorded higher incidence in morning hours in some Indian breeds. In lactating ewes also the trend was almost similar to dry ewes and significant difference was observed only in crossbreds. The reason for high incidence in the evening hour is unknown but may probably be related with the increased level of hormones released after grazing and decrease in day light.

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Duration of oestrus in dry group did not vary significantly between timings of onset of oestrus within the breed but differed between the breeds. It averaged 27.80±0.99 and 30.1±1.40 hr in Muzaffarnagri and crossbreds, respectively, ranging from 6 to 60 hr. These findings are in agreement with those of Singh *et al.* (1983) in different sheep breeds. However, fairly high values were recorded by Aboul-Naga *et al.*(1985).

Analysis of variance revealed significant effect of breed ($P<0.05$) and time of onset of oestrus on duration of oestrus in lactating ewes. Oestrus duration averaged 21.25±1.11 and 23.56±2.49 hr in lactating Muzaffarnagri and crossbred sheep, respectively, with a range of 12-48 hr. The duration of oestrus was significantly higher in dry group in comparison to lactating group. Hunter (1962) reported similar results in dry and lactating ewes. However, Sahni and Roy (1967) did not observe any difference between the 2 groups of Bikaneri ewes. Time of onset of oestrus had a significant effect on duration of oestrus in lactating crossbreds indicating more oestrus duration (31.2±3.55 hr) in ewes exhibiting first time oestrus in morning in comparison to evening (19.06±1.87 hr). No reports are available in any of the sheep breeds for the effect of time of onset of oestrus on oestrus duration.

Age of the ewes had a significant effect on duration of oestrus and it was higher in adult group (above 2 years age) in comparison to young (< 2 years) age group (22.6±1.78 hr). The results obtained in this study are in close agreement with that of Steklenev (1967).

The duration of oestrus ranged between 24-36 hr in 60-70% ewes irrespective of genotype. The value for per cent frequency distribution in the same duration interval was higher in Sonadi ewes (Taparia 1972). Muzaffarnagri (20%) and crossbred (18%) ewes exhibited oestrus duration of less than 18 hr which is in close proximation with the findings of Gubanov (1971).

Observations on 202 oestrus cycles in 15 Muzaffarnagri ewes revealed the mean duration of oestrus cycle length of 16.75±0.26 days. On an average each ewe showed 13.47 cycles ranging from 9 to 26 in a year. Percentage of oestrus cycle according to distribution was 8.42, 61.58, 8.95 and 21.05

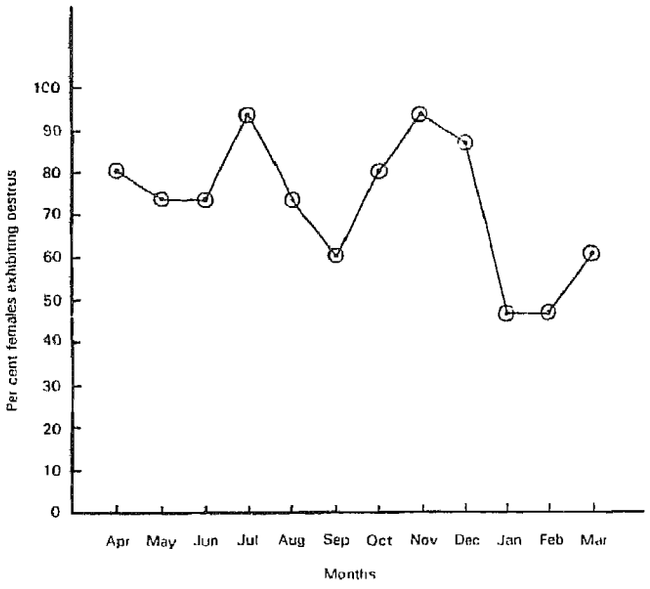


Fig. 1. Oestrus activity over the months in Muzaffarnagar and crossbred sheep.

for short, normal, long and missed groups respectively. The present findings coincide with the reports of Sahni and Roy (1967) in Bikaneri sheep and Aboul-Naga *et al.*(1985) in Rahmani ewes. However, a higher percentage of normal cycles were reported in different sheep breeds (Cetinkaya 1979, Mittal and Ghosh 1980). Oestrus cycle length differed significantly ($P<0.01$) between animals and between cycles which is similar as reported by Taparia (1972) in Sonadi sheep.

The maximum ewes (93.5%) exhibited oestrus in November (Fig.1) followed by July (92.5%) and least in January and February (46.0%) indicating that Muzaffarnagri sheep are nonseasonal in oestrus behaviour. However, 2 peaks in breeding seasons could be identified on the basis of periodicity of oestrus and availability of feed and fodder resources for better conception and better survival of neonatal lambs, viz. May-June and October-November.

On the basis of above findings it is concluded that Muzaffarnagri sheep are nonseasonal in oestrus behaviour, heat detection should be done both in the morning and evening and breeding should be practiced in specific months in lieu of throughout year breeding.

ACKNOWLEDGEMENT

Authors thank the Director of the Institute for providing necessary facilities.

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Indian Journal of Animal Sciences **70** (10): 1071-1073, October 2000

Studies on rumen function in uterine torsion in buffaloes

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Received: 8 November 1999; Accepted: 25 April 2000

Key words: Rumen, Uterine torsion, Buffalo

The present study was designed to evaluate rumen function in cases of uterine torsion compared to normal parturient buffaloes.

Animals and treatment protocol

Study was conducted on 19 Clinical cases of uterine torsion in buffaloes brought to the veterinary clinics, Haryana Agricultural University Hisar for treatment (group A). Five pregnant buffaloes maintained by the Buffalo Research Center at the University farm, under identical conditions of feeding and management were also included in the study to serve as the normal parturient controls (group B).

The diagnosis of torsion of uterus in the clinical cases was confirmed by rectal and vaginal examination and the degree, direction and site of uterine torsion determined. The delivery of the fetus was achieved by lapro-hysterotomy in all the cases and uterus dotorted through the laprotomy-wound after the withdrawl of fetus.

The cases of uterine torsion (group A) were randomly assigned to 3 subgroups namely, group 1 (n = 6), group 2(n = 6) and group 3 (n = 7). All the animals received supportive therapy (4 liters Ringer's lactate I/V during operation and next 2 days, 50 I.U. Oxytocin. I/V during operation, 50 ml chromostat I/V during operation, metranidazole 2 g I/V for 3 days, streptopenicillin 5 g I/M for 3 days and dressing of suture line with betadine). Group 2 received additional therapy of stomachic powder, while group 3 received 10 ml injection of B-complex and liver extract in addition to supportive therapy.

Collection of rumen liquor

Rumen liquor was collected with the help of stomach tube from all the animals before parturition / preoperatively (0 hr) and subsequently at 24, 48 and 72 hr after parturition / cesarean section. Rumen fluid was filtered through muslin cloth and was appropriately preserved for different parameters. The various preservatives used for ruminal fluid were 8% formaldehyde solution for total protozoal and total bacterial count and saturated mercuric chloride solution for total nitrogen and ammonia nitrogen.

Parameters and procedures

Rumen liquor hydrogen ion concentration (pH) was determined by digital pH meter. Protozoal count was determined with the help of McMester's counting chamber technique of Boyne *et al.* (1957). Counts of 30 fields / slide were made and means were taken for final calculation.

Counting of total number of bacteria per milliliter of ruminal fluid was done by Nigrosine slide technique of Gall *et al.* (1949) and total nitrogen concentration in the rumen liquor was determined by McKenzie and Wallace (1954) method. Ammonia nitrogen was estimated by adopting the technique of Bremner (1965).

Data was statistically analyzed using one-way analysis of variance and Duncan Multiple range test to compare effect of time within group, while t-test was used to compare means between group within time period.

The pH of rumen liquor (Table 1), though higher was not significantly different between uterine torsion affected and normal parturient buffaloes. After cesarean section in uterine torsion animals, pH became significantly higher than values in normal parturient animals and remained high till observation period of 72 hr. The pH of rumen liquor in normal parturient buffaloes showed a decline till observation period while pH in torsion cases remained high in all the treatment groups ranging between 7.44 ± 0.05 to 8.21 ± 0.15 in different groups.

The ruminal fluid pH is generally related to feed intake and decreases post feeding (Prasad and Pradhan 1990). In normal parturient animals, the pH tended to be lower after calving indicating normal feeding activity, while higher pH values in buffaloes with uterine torsion may be due to stress of dystocia associated with anorexia. The increase in ruminal fluid pH towards alkalinity may be because of the products of putrefaction and production of large quantities of ammonia

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Table 1. Rumen liquor pH values, protozoal concentrations, bacterial concentration, total nitrogen concentrations and ammonia nitrogen concentrations in buffaloes affected with uterine torsion (group A:, n=19) and normal parturient (group B: Control, n=5) buffaloes

Parameters	Group	0 hr	24 hr	48 hr	72 hr
pH	A	7.78±0.14	7.84* ±0.13	7.76** ±0.12	7.58**±0.08
	B	7.34^{a}±0.06	7.30^{ab}±0.12	7.08^{b}±0.06	7.4^{b}±0.05
Protozoal	A	3.65**±0.14	3.57**0.14	3.60**±0.15	3.33*±0.16
conc. (n × 10^5/ml)	B	6.90±0.09	6.70±0.33	7.00±0.13	6.90±0.21
Bacterial	A	4.24**±0.06	4.35**±0.08	4.48**±0.08	4.56**±0.13
conc. (n × 10^9/ml)	B	6.56±0.30	6.58±0.18	6.50±0.17	6.48±0.23
Total nitrogen	A	97.19**±4.00	92.94*±4.09	91.10**±3.34	88.92**±2.56
conc. (mg/100ml)	B	75.50±2.49	74.70±1.75	74.60±1.95	74.93±1.71
Ammonia	A	38.68*±2.54	36.62*±2.96	34.71±2.86	29.93±3.52
nitrogen conc. (mg/100ml)	B	24.57±1.06	24.53±0.97	25.45±1.09	23.87±0.99

One star (*, P<0.05) and two stars (**,P<0.01) indicate significant difference between groups within period, while alphabt superscripts (a,b,.) indicate significant difference between time periods within group (P<0.05).

(Prasad *et al.* 1972). Lower production of volatile fatty acids (VFA) in the rumen of dystocia affected buffaloes as reflected by lower plasma VFA in these animals (Dahiya 1998) could also be responsible for the increased rumen liquor pH.

Protozoal counts

Total protozoal count (Table 1) was significantly low in uterine torsion than normal parturient buffaloes. The protozoal count did not improve in any of uterine torsion cases till observation period. The low protozoal count in uterine torsion could be due to lack of proper feeding in buffaloes (Rajasekaran and Venkataswami 1981) or due to alkaline pH (Quinn *et al.* 1962).

Bacterial counts

The bacterial count (Table 1) was significantly (P<0.01) lower in the rumen liquor collected from the uterine torsion buffaloes than in the normal parturient ones and remained so till 72 hr of observation. The decline in mean total bacterial count in the uterine torsion animals could also be due to high rumen pH and decreased availability of substrate that is essential for the growth of rumen bacteria (Thomas 1983).

Total nitrogen

The buffaloes affected with uterine torsion had significantly higher concentration of total nitrogen in rumen liquor than in normal buffaloes on day of calving (Table 1). The values in torsion cases remained higher than normal animals even at 72 hr after cesarean section and treatment. The higher concentration of total nitrogen could be attributed to slow down of passage of ingesta along with nitrogenous fractions from rumen to omasum and abomasum due to decreased ruminal motility in uterine torsion in buffaloes (Singh 1991) and thus retention of nitrogen produced in rumen liquor.

Ammonia nitrogen

The ammonia nitrogen concentration (Table 1) in ruminal fluid was significantly higher in uterine torsion than normal parturient buffaloes at 0 and 24 hr of observation. The values at 48 and 72 hr were not significantly (P > 0.05) different between uterine torsion affected and normal parturient buffaloes. The high levels of ammonia nitrogen in uterine torsion is probably due to the disturbance in the balance between production and utilization of ammonia as whatever ammonia is produced in the rumen is not being utilized fully. The incorporation of ammonia into the synthesis of microbial protein is diminished because of decreased bacterial count in these animals. Alkaline pH, as observed in the present study, could also be responsible for higher ammonia nitrogen (Randhawa *et al.* 1991).

Supplementation of stomachic powder or injection of B-complex vitamins + liver extract did not affect pH, protozoal counts, bacterial counts, total nitrogen and ammonia nitrogen in uterine torsion animals (results not shown). This could be due to lack of rumen motility and feed intake, other wise supplementation of either stomachic powder or B-complex + liver extracts had been reported to improve some of these parameters particularly bacterial count in normal buffaloes (Singh *et al.* 1992).

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Indian Journal of Animal Sciences **70** (10): 1074-1076, October 2000

The effect of chemical defaunation on rumen bacterial, protozoal population and enzyme activities in the cattle and buffalo rumen

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Received: 24 March 1999; Accepted: 17 April 2000

ABSTRACT

The experiment was designed to study the duration of effectiveness of sodium lauryl sulphate on rumen microbial population and enzyme activities in a 4-factor RBD using 4 fistulated bullocks, rumen liquor samples were collected for counting number of bacteria and protozoa and for estimation of enzyme activities on first 10th and 20th day of administration of sodium lauryl sulphate. The enzyme activity was determined in the rumen liquor samples collected at 4 hr post feeding, whereas the protozoal and bacterial population was counted in sample collected at 0, 2, 4, 6 and 8 hr post feeding from defaunated and faunated animals. The bacterial population was significantly higher in defaunated animals and protozoa started reappearing on 20th day of defaunation. The bacterial and protozoal count did not differ significantly in cattle and buffalo bullocks.The carboxymethyl cellulase activity was higher in faunated animals, whereas, protease activity was significantly ($P<0.05$) higher in defaunated animal and amylase, xylanase and urease activity did not differ between faunated and defaunated animals.

Keywords: Bacterial population, Chemical defaunation, Enzyme, Protozoal population

The exclusion of protozoa from the rumen, is also beneficial for the performance of the animal under certain feeding regimes (Leng 1982, Mudgal *et al.* 1988). However, very few studies have been conducted on the duration of effectiveness of defaunating chemicals and the present experiment was designed to ascertain the duration of effectiveness of sodium lauryl sulphate in defaunating the rumen and its effect on the bacterial and protozoal population in cattle and buffaloes.

MATERIALS AND METHODS

Adult fistulated bullocks (2 cattle and 2 buffaloes) were divided into 2 species wise groups. One animal from each of the 2 species was defaunated using sodium lauryl sulphate @ 9.0 g/100 kg body weight/day for 3 consecutive days. To defaunate these animals 10% solution of the required sodium lauryl sulphate was administered by drenching for 3 consecutive days. The animals were not offered any food except drinking water for 24 hr before giving them the first dose of the chemical. After 8 hr of the first dose the 1/3rd of daily normal required quantity of concentrate mixture was offered and on second day of defaunation 2/3rd of daily requirement of concentrate was given. However, on third day of administration of the last dose of chemical, the whole quantity of concentrate and oat fodder was given. The defaunated animals were isolated from other animals. The bullocks were given 1-1.5 kg concentrate mixture and oat fodder to fulfil their energy and protein requirements (NRC 1988).

In the second phase of the experiment defaunated animals were switchover to faunated animals by inoculating the rumen liquor obtained from another healthy faunated animal, whereas sodium lauryl sulphate given to change them into defaunated category. The bacterial population (Warner 1962) and protozoal (Wintrob 1981) were counted. Immediately, after collection strained rumen liquor was processed (Agrawal *et al.* 1991). The rumen liquor samples collected at 4 hr of post feeding on 1st, 10th and 20th day after the last day of administering the chemical were analyzed for carboxymethyl cellulase (Miller 1959), protease, amylase, xylanase and urease activity (Lowry *et al.* 1951). The bacterial and protozoal population was counted in the rumen liquor sample collected at 0, 2, 4, 6, and 8 hr of post feeding on 1st, 10th, and 20th, day after the last day of administering the chemicals. The observation on bacterial and protozoal counts were statistically tested using 4 factor randomized block design (Snedecor 1967).

RESULTS AND DISCUSSION

The bacterial population differed significantly ($P<0.05$)

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Table 1. Effect of defaunation on total bacterial population in the rumen fluid of cattle and buffaloes

Particulars effect	Bacterial population ($\times 10^6$/ml RL)					Overall mean ±SEM
	0hr	2hr	4hr	6hr	8hr	
Pooled period effect						
1st day	14.67*	15.22	18.30	17.38	14.87	16.08a**0.32
10th day	14 30	14.95	18.56	16.88	15.55	19.08ab±0.32
20th day	16.95	18.96	21.28	21.68	19.53	16.68^{ba}±0.32
Pooled species effect						
Buffaloes	15.35	15.83	17.91	19.64	16.77	17.10**±0.26
Cattle	15.26	16.92	20.84	17.64	16.52	17.44±0.26
Pooled treatment effect						
Protozoa (+)	11.73	13.18	14.99	14.76	13.42	13.62*a±0.26
Protozoa (-)	18.87	19.58	23.77	22.52	19.87	20.93^{b}±0.26
Overall mean ± SEM	15.30^{a}	16.38^{ab}	19.38^{dc}	18.64^{c}	16.64^{ba}	17.27±0.42
	±0.42	±0.42	±0.42	±0.42	±0.42	

**Each is average of faunated and defaunated animals. Different superscripts a, b, c, d, in a column/row differ significantly (P<0.05).

between 1st, 10th, and 20th day period (Table 1), higher bacterial population on 10th day period might be because of the role of protozoa being taken up gradually by bacterial and again reduced on 20th day as the protozoa started reappearing on 20th day period. The bacterial population differed nonsignificantly in between cattle and buffalo bullocks probably because of identical conditions of feeding and management (Pant and Roy 1971). The bacterial count was significantly higher in defaunated animals compared to faunated (20.93×10^8 vs 13.62×10^8/ml RL) might be due to the engulfing activity of protozoa in faunated animals (Kurar and Madhu Mohini 1990, Nangia and Sharma 1994).

The protozoal population did not differ significantly in 1st, 10th and 20th day periods (Table 2). The protozoa were totally absent on 1st and 10th day period sampling but after 20th day few ciliates were observed in the rumen liquor which might be due to the direct contact of defaunated animals with faunated animals of this experiment and also non-experimental animals. The defaunated animals remained protozoa free on experimental period of 3-4 weeks when chemical manoxol was used for defaunation (Chaudhary *et al.* 1995).

The protozoal population was similar between 2 species since their rations was of moderate quality and not much different (Kurar and Madhu Mohini 1988). The smaller count of protozoa in the defaunated animals might be due to reappearance of some protozoa in the last (20th day) period of sampling. The protozoal population count was significantly ($P<0.05$) higher in faunated animals in comparison to defaunated animals (23.35×10^4 vs 0.37×10^4/ml SRL) which was very much expected.

The carboxymethyl cellulase activity was significantly ($P<0.05$) lower and protease activity was significantly ($P<0.05$) higher in defaunated animals (Table 3) probably because of higher bacterial count in defaunated animals since

Table 2. Effect of defaunation on total protozoal population in the rumen fluid of cattle and buffaloes

Particulars effect	Bacterial population ($\times 10^8$/ml RL)					Overall mean ±SEM
	0hr	2hr	4hr	6hr	8hr	
Pooled period effect						
1st day	14.74*	12.70	10.39	11.84	13.84	12.71**±0.26
10th day	14.16	12.47	10.34	12.31	15.89	12.64**±0.26
20th day	15.09	12.51	10.95	13.44	14.15	13.23**±0.26
Pooled species effect						
Buffaloes	14.16	12.45	10.16	12.18	13.92	12.57**±0.21
Cattle	15.17	12.66	10.96	12.88	14.04	13.14**±0.21
Pooled treatment effect						
Protozoa (+)	29.13	24.82	20.65	24.56	27.60	25.35^{b}±0.21
Protozoa (-)	0.20	0.30	0.47	0.50	0.35	0.37^{a}±0.21
Overall mean ± SEM	14.67^{c}	12.56^{b}	10.56^{a}	12.53^{b}	13.98^{c}	12.86±0.33
	±0.33	±0.33	±0.33	±0.33	±0.33	

**Each is average of faunated and defaunated animals. Different superscripts a, b, c, d, in a column/row differ significantly (P<0.05).

Table 3. Effect on defaunation on rumen enzyme activity (u/100 ml RL) in fistulated cattle and buffaloes

Enzymes	Cellular		Extra cellular		Total	
	Faunated	Defaunated	Faunated	Defaunated	Faunated	Defaunated
Carboxymethyl cellulase	93.10	64.35	39.77	23.16	132.87^{b}	87.511^{a*}
Amylase	127.06	84.64	169.25	210.09	296.31	294.73
Xylanase	165.86	141.67	40.90	63.31	206.76	204.98
Protease	39.47	56.62	0.00	0.00	39.47^{a}	56.62^{b*}
Urease	1.82	2.24	0.00	0.00	1.82	2.24

a,.b Means bearing different superscripts in a row differ significantly* ($P<0.05$).

the carbohydrate hydrolyzing enzymes are retained mostly in cell wall of bacteria (Coleman 1986). Amylase, xylanase and urease activity were not affected due to defaunation (Table 3). Williams and Wither (1991) also observed no difference in the fibrolytic activity of rumen liquor of defaunated and faunated animals. Since the protozoa did not reappear and their number did not increase to a significant extent and rumen metabolite production pattern did not differ significantly ($P<0.05$) between 1st and 20th day. It is concluded that the sodium lauryl sulphate was quite effective up to 3 weeks after administering its last dose.

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Indian Journal of Animal Sciences **70** (10): 1077-1080, October 2000

Influence of inbreeding level on economic traits of imported rabbit populations in Egypt

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Received: 20 November 1999; Accepted: 4 April 2000

ABSTRACT

Influence of inbreeding level on economic traits of 3 (5 075 records) imported rabbit breeds 2 740 Bauscat, 1 739 New Zealand White and 596 Californian after 7 years of importation, were analyzed by using 2 models. The 2 models included inbreeding coefficients (IC) of does as fixed effects or covariate. Inbreeding adversely affected litter size and mass at birth and weaning and pre-weaning mortality, while bunny weight and pre-weaning daily gain unexpectedly were significantly larger in >0.50% (IC) group than in the other groups studied. Linear regression coefficients of IC on pre-weaning traits were highly significant in the breeds studied. Inbreeding levels in imported populations can be avoided by combining inbreeding levels with selection to purge a population of the genes that cause inbreeding depression.

Key words: Economic traits, Inbreeding depression, Imported rabbits

The present work was carried out to estimate the effects of doe's inbreeding on preweaning performance of subsequent generations of close imported rabbit populations.

MATERIALS AND METHODS

The study was carried out in San El-Hagar Agricultural Company Farm, San El-Hagar area, Sharkeya Governorate, Egypt. The data set included records of 5 075 parturitions from 3 breeds: 596 Californian (Cal), 2 740 Bauscat (Bau), and 1 739 New Zealand White (NZW). The rabbits were imported in 1989. The herd was closed from 1989 to 1996. During this period selection of replacement does was based on weaning weight and litter size of their dams. The animals were reared under similar environmental and managerial conditions. They were fed *ad lib.* on a commercial pelleted rabbit diet containing 18% crude protein and 14% crude fibre, with 10.88 MJ/kg as digestible energy. Continuous access to freshwater was provided from automatic nipple drinkers.

The pedigree of each doe was traced back to the original imported stock and pedigree chart was prepared. The inbreeding coefficient (IC) of foundation stock was assumed to be zero. The coefficient of inbreeding (IC) for each doe was calculated as per Quass (1976) and grouped into 4 classes (non-inbred, <0.25, 0.25 to <0.50 and >0.50). Each calender year was divided into 4 seasons viz., winter (January to March), spring (April to June), summer (July to September) and autumn (October to December). The data were analysed for each breed separately using General Linear Fixed Models (SAS 1990) for the model including year, season, parity, IC classes and all interactions among fixed effect and random residual effects. The interaction effects were subsequently omitted from the model if they were nonsignificant. Traits included in the study were litter size (LS), litter mass (LM) and mean bunny weight at birth (BWB) and weaning (BWW), preweaning daily gain (DGW), stillbirths (Sb%), preweaning mortality rate (MR%), gestation length (GL) and sex ratio of young weaned (SR%).

Analysis of inbreeding coefficient (IC) as independent covariate was carried out using a model including the previous fixed effects, except IC classes (Model 2). Logarithmic transformations were applied to each fitness measure prior to regression analysis.

RESULTS AND DISCUSSION

Inbreeding of coefficient percentages

The percentage of inbred does under study ranged between 55 and 60% after 7 years of importation.

Inbreeding coefficient as fixed effect: The overall means observed of performance traits in the 3 breeds were similar to those of Farghaly (1996a) for LSB, LSW, LMB and LMW and Partridge *et al.* (1981) and Farghaly *et al.* (1994) and Farghaly (1996b) for Sb% and DGW and Farghaly (1996c)

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Table 1. Least-squrares means (KSM)±SE of productive and reproductive performance as affected by inbreeding levels in Bauscat breed

Traits[£]		Overall Means	No.	Inbreeding coefficient (IC) classes			
				Non-inbred	< 0.25	0.25 to< 0.50	> 0.50
Litter size at:							
Birth	(LSB)	7.05±0.06***	2725	8.25±0.05 A	8.30±0.10 A	8.05±0.08 B	3.95±0.12 C
Weaning	(LSW)	5.89±0.05***	1982	6.47±0.06 A	6.42±0.11A	6.33±0.10 A	3.52±0.04 B
Litter mass (cm) at							
Birth	(LMB)	398.15±2.65***	2543	450.31±2.96 A	455.42±5.72 A	428.16±4.66 B	241.19±4.24 C
Weaning	(LMW)	3444.40±26.71***	1979	3792.14±35.78 A	3567.28±66.72 B	507.90±7.32 C	708.29±9.26 D
Bunny weight (g) at:							
Birth	(BWB)	57.87±0.35***	2543	55.19±0.33 A	55.73±0.61 A	53.81±0.50 A	67.44±1.17 B
Weaning	(BWW)	602.94±3.59***	1979	601.69±4.46 A	570.3±8.49 B	507.90±7.32 C	708.29±9.26 D
Daily gain (cm) from.							
Birth to weaning	(DGW)	18.17±0.12**	1973	18.20±0.15 A	17.13±0.28 B	15.13±0.24 C	21.33±0.31 D
Mortality %							
Stillbirths	(Sb%)	6.53±0.29 NS	2740	5.83±0.65 AB	5.85±0.79 A	6.32±0.39 AB	8.07±0.65 B
At weaning	(MW%)	17.42±0.64***	2740	15.99±0.99 A	17.06±1.19 A	18.86±0.61 B	19.19±1.88 B
Gestation length	(GL)	32.09±0.02***	2740	31.95±0.03 A	31.98±0.06 A	32.44±0.06 B	
Sex ratio (males/ females)	(SR%)	49.40±0.41 NS	1827	49.31±0.56 A	48.44±1.10 A	50.77±1.03 A	49.05±1.01 A
Inbreeding coefficients	(%)	55.49		44.51	11.45	16.70	27.34

Least-squares means in the same row bearing different letters, differ significantly (*** P<0. 001, **P>0. 01 and NS=non significant); [£]key to abbreviations are given in materials and methods.

Table 2. Least-squares means (LSM) ±SE of productive and reproductive performance as affected by inbreeding levels in New Zealand White breed

Traits[£]		Overall Means	No.	Inbreeding coefficient (IC) classes			
				Non-inbred	< 0.25	0.25 to< 0.50	> 0.50
Litter size at:							
Birth	(LSM)	7.00±0.06***	1727	8.10±0.06 A	7.97±0.12 A	7.89±0.09 A	4.16±0.10 B
Weaning	(LSW)	5.93±0.06***	1261	6.49±0.08 A	6.65±0.16 A	6.05±0.12 B	4.05±0.09 C
Litter mass (g) at:							
Birth	(LMB)	390.48±3.04***	1601	433.75±3.59 A	438.01±8.32 A	418.55±4.81 B	256.66±5.18 C
Weaning	(LMW)	3416.20±34.56***	1257	3826.56±49.24 A	3446.97±83.27 B	30.38.88±64.44 C	2679.49±63.43 D
Bunny weight (g) at							
Birth	(BWB)	57 05±0.38***	1601	54.08±0.41 A	55.63±0.94 A	53.70±0.54 A	66.68±1.14 B
Weaning	(BWW)	604.04±6.35***	1257	609.01±6.19 A	532.89±9.65 B	537.58±26.75 B	684.08±11.66 C
Daily gain (g) from:							
Birth to weaning	(DGW)	18.21±0.21***	1253	18.46±0.21 A	15.92±0.31 B	16.11±0.89 B	20.54±0.39 C
Mortality %							
Stillbirths	(Sb%)	5.86±0.34 NS	1739	5.13±0.63 A	5.24±0.95 A	6.96±0.54 A	6.49±0.73 A
At weaning	(MW%)	17.78±0.70***	1739	14.48±1.52 A	17.82±0.79 A	16.78±1.15 A	24.55±1.90 B
Gestation length	(GL)	32.03±0.04***	1739	31.91±0.04 BC	32.13±0.09 AB	31.84±0.12 C	32.33±0.07 A
Sex ratio (males/ females)	(SR%)	50.74±0.54*	1138	49.14±0.72 AB	52.43±1.68 A	48.98±1.25 AB	48.53±1.34 B
Inbreeding coefficients	(%)	54.95		45.05	9.32	19.11	26.52

Least-squares means in the same row bearing different letters, differ significantly (***P<0.001, *<0.05 and NS=non significant) [£]key to abbreviations are given in materials and methods.

Table 3. Least-squares means (LSM)± SE of productive and reproductive performance as affected by inbreeding levels in Californian breed

Traits[£]		Overall Means	No.	Inbreeding coefficient (IC) classes			
				Non-inbred	< 0.25	0.25 to< 0.50	> 0.50
Litter size at:							
Birth	(LSM)	6.93±0.11***	592	8.16±0.11A	7.89±0.20 A	7.92±0.14 A	4.01±0.18 B
Weaning	(LSW)	5.97±0.10***	421	6.97±0.12 A	5.83±0.31 B	6.05±0.21 B	3.45±0.11 C
Litter mass (g) at:							
Birth	(LMB)	371.34±5.15***	549	417.97±6.45 A	413.27±11.94 A	406.84±7.21 A	231.84±8.41 B
Weaning	(LMW)	3306.89±54.87***	421	3815.67±68.92 A	3119.57±173.3 B	2980.85±102. 15 B	2519.38±108.72 C
Bunny weight (g) at:							
Birth	(BWB)	54.95±0.57***	549	51.71±0.71 A	53.28±1.47 A	51.81±0.71 A	64.81±1.56 B
Weaning	(BWW)	579.24±16.83*	421	564.25±9.53 A	541.58±18.33 A	515.62±15.87 A	713.32±81.13 B
Daily gain (g) from:							
Birth to weaning	DGW	17.48±0.56*	421	17.06±0.31 A	16.26±0.61 A	15.43±0.53 A	21.67±21.71 B
Mortality %							
Stillbirths (Sb%)		7.00±0.66 NS	596	5.99±1.42 A	6.66±1.30 A	6.91±0.96 A	8.62±2.55 A
At weaning	(MW%)	20.27±1.40***	596	14.31±1.22 B	17.17±2.07 AB	23.09±3.41 A	27.37±3.32 C
Gestation length	(GL)	32.14±0.06*	596	32.09±0.08 B	32.27±0.20 AB	31.72±0.11 C	32.53±0.14 A
Sex ratio (males/ females)	(SR%)	47.36±0.91 NS	374	48.06±1.30 A	44.04±2.72 A	48.07±1.78 A	46.54±2.45 A
Inbreeding coefficients	(%)	60.13		39.87	9.29	23.14	27.70

Least squares means in the same row bearing different letters, differ significantly (***P<0.001, **P<0.05 and nonsignificant). [£]key to abbreviations are given in materials and methods.

Table 4. Linear regression analysis (b±se) for inbreeding coefficients (%) on performance traits in three commercial (%) on performance traits in 3 commercial population

Traits[£]		Breed		
		Bauscat	New Zealand White	Californian
Litter size at:				
Birth	(LSB)	-0.064±0.002***	-0.060±0.001***	-0.065±0.002***
Weaning	(LSE)	-0.033±0.004***	-0.031±0.002***	-0.032± 0.004***
Litter mass at:				
Birth	(LMB)	-2.670±0.073***	-2.300±0.093***	-2.340±0.160***
Weaning	(LMW)	-13.280±0.084***	-11.970±1.090***	-10.860±1.860***
Bunny weight at.				
Birth	(BWB)	+ 0.176±0.012***	+0.195±0.013***	+ 0.201±0.020***
Weaning	(BWW)	+ 1.563± 0.116***	+ 1.460±0.219***	+ 2.510±0.677***
Daily gain from:				
Birth to weaning	(DGW)	+ 0.047±0.004***	+0.042±0.007***	+0.078±0.023***
Mortality %				
Stillbirths	(Sb%)	+0.029±0.010**	+0.010±0.012NS	+0.037±0.023 NS
At weaning	(MW%)	+ 0.258±0.021***	+ 0.260±0.023***	+ 0.257±0.035***
Gestation length	(GL)	+ 0.006±0.001***	+ 0.006±0.001***	+ 0.005±0.002**
Sex ratio (males/females)	(SR%)	+ 0.006±0.016 NS	-0.012±0.021 NS	-0.019±0.039 NS

***P<0.001, **P<0.01, *P<0.05 and NS= not significant; [£]key to abbrevations are given in materials and methods.

for GL.

The LSB, LSW, LMB and LMW decreased gradually by the increase of IC level (Tables 1-3) as all the traits have strong positive phenotypic association. Similar to that reported by Rastogi and Heyer (1992) in NZW, Cal and Checkered Giant rabbits. In general, poor LSB, LSW, LMB and LMW during inbreeding might occur because inbred does suffer higher mortality at the embryonic, fetal and preweaning period. In addition to, inbred does provide poorer maternal care for their litters. Such effect was noted by Ralls *et al.*

(1988) in mammals and by Lacy *et al.* (1996) in *Peromyscus polionotus*.

Unexpected results were that BWB, BWW and DGW were significantly larger in >0.50 IC group than other groups studied in all the breeds. It may be due to negative correlation between BWB and LSB (Farghaly 1996a).

The MW% increased when the IC was greater than 50%. Similar to that reported by Ralls *et al.* (1988) in mammals. It might be due to lowered resistance with increase inbreeding (Bielanski *et al.* 1992). The difference between inbreeding groups were not significant for stillbirths in Bau, New and Cal breeds. These findings were in agreement with results reported by Nunes and Polastre (1988) in Norfolk rabbits.

GL was the longest in group with IC >0.50 in Bau, NZW and Cal breeds. The effect of IC on GL may be due to indirect effect on LSB. SR% was not significantly affected by IC in breeds studied. The present results agreed with those reported by Brewer *et al.* (1990) in *Peromyscus* mice.

Inbreeding coefficient and interactions

The interactions between year and season were significant for LSW, LMB, LMW, BWW, DGW, MW% and SR% in Bau; LSB, LSW, LMB, LMW and BWB in NZW and LMW and MW% in Cal breeds of rabbits. The differences in preweaning performance and mortality traits among year and season may be attributed to the temporal variations especially in managements and climatic conditions from season subclasses year to another (Farghaly 1996a).

The Interactions between IC value and year were significant for LSW and LMW in Bau, and LSB, LSW, LMB, LMW, BWW and DGW in NZW breed. The Interactions between IC value and season were significant for LSB, LSW and BWB in Bau, and SR% in Cal breeds. The Interactions between IC value and parity were significant for LSB, LSW, LMW and Sb% in Bau, and LSW, LMB and LMW in NZW breeds only.

Inbreeding coefficient as a regression

The linear regression coefficients of IC as covariant on pre-weaning performance were highly significant, except Sb% in NZW and Cal and SR% in all breeds (Table 4). Chai (1969) and Poujardieu and Toure (1980) also reported an adverse effect of inbreeding on LSB, LSW, LMB and LMW. Antagonism trends were observed for BWB, BWW, DGW and GL in all the 3 breeds. The positive linear regression between IC and each of BWB, BWW or DGW in the present work confirms the above results. These results were in agreement with that reported by Lacy (1993) in *Callimico*. The small litters tended to increase the mean GL, while large litters tended to decrease the mean GL, such effect was noted by Farghaly (1996c).

In conclusions, the average effect of inbreeding was detrimental for pre-weaning performance. Inbreeding levels in close imported rabbit populations can be avoided by combining inbreeding levels with selection to purge a population of the genes that cause inbreeding depression, opening the population to outside sire and mating strategies are based not only on performance traits, but on pedigree data. Therefore, evaluating true value of doe's inbreeding depression for pre-weaning performance, adjustments might be made by gain of selection, under selection system of same population.

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Indian Journal of Animal Sciences **70** (10): 1081-1084, October 2000

Inheritance in nutritive value of rice straw quality

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Received: 24 January 2000; Accepted: 25 April 2000

Key words: Inheritance of digestibility, Rice straw

Rice straw is a most abundantly available crop residue in India. Farmers use straw from different cultivars of rice grown on the farm for feeding of animals. Straw of different rice varieties varies in their digestibility and intake and such variation may be attributed the genetic make up of the plant (Capper 1988, Singh 1993 and Rahal *et al.* 1997) (Sohane *et al.* 1999). A great deal of plant breeding research is underway in the different Agricultural Universities, ICAR institutes in India, IRRI in Philippines and other Asian countries to develop high yielding rice varieties but straw quality is not included as selection parameter. However, before breeding programmes are undertaken with a view to improve the nutritive value of straw, it needs to be clearly established whether significant differences exist among straw from different cultivars and/or digestibility is inheritable character and if so whether the differences are large enough to make significant improvement, so that farmers could harvest a straw of good quality rather than upgrading the crop residue of inferior quality through chemical treatments with additional cost involvement. Zerbini and Thomas (1999) reported that an small improvement of only 1% digestibility could result in increase in milk, meat and draught power outputs ranging from 3-8%. The present study therefore was designed to investigate inheritance of rice straw in relation to its nutritive value.

Four rice cultivars representing low nutritive value Ratna and IR-24 and high nutritive value PantDhan-6 and Narendra-359 were crossed in all possible combination (excluding reciprocals) in diallel fashion to generate 6 hybrid population during Kharif 1998 season, F_2 seeds were produce by selfing of F_1's and hand crossed seeds of F_1 were also made during off season 1998. Available F_1's, F_2's along with their parents were grown in randomized block design with 3 replications at the crop research centre of G.B. Pant University of Agriculture and Technology, Pantnagar, India during Kharif 1999. Standard cultural practices of raising a normal healthy crop were followed. Straw of parents, F_1's and F_2 were harvested at their maturity, collected, dried and ground using 2 mm sieve. A sample of 5 g of each genotype was incubated for 72 hr in the rumen of three rumen fistulated adult male cattle as suggested by Mehrez and Orskov (1977). Samples were incubated in duplicate for 3 periods in animals fed on *ad lib.* rice straw along with 1.5kg concentrate. The sample of each hybrid and their parents were analyzed for contents of their dry matter (DM), organic matter (OM), ash, (ISI 1962) crude protein (CP) (AOAC 1975), neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose (HC) (Goering and Van Soest 1970) before and after 72 hr incubation and by difference the *in sacco* digestibility of nutrients was calculated. The data obtained were statistically analysed (Snedecor and Cochran 1980).

The OM content varied with an average value of 85.14% in straw of parents which remained similar in F_1 and decreased in F_2 (Table 1). The parents having higher content of CP like PD-6 (6.12%) and N-359 (6.30%) when crossed with each other produced F_1 hybrid of lower CP content (5.87%) which further reduced to 5.06% in F_2. Similarly, crossing of parents having lower CP contents like IR-24 (4.81%) and Ratna (5.25%) produced hybrid with higher CP content (6.25%). Polygenes, the combination of which seem to be identical in superior parents with that of hybrid did not show improvement. These results are in agreement with those reported by Devasia *et al.* (1976); Singh *et al.* (1995) and Sohane *et al.* (1999). The mean digestibility of DM varies from 47.81 (IR-24) to 59.25% (PD-6) in parents with a difference of 11.44 units. However, this range narrowed down to 3.55 units in F_1 and 5.17 units in F_2 generation. The dry matter digestibility in crosses of superior varieties PD-6 (59.25) × N-359 (57.17) was 51.94% in F_1 and 53.02% in F_2 generation and reduced significantly ($P<0.01$) as compared with parents (negative heterosis) (Table 1). Similarly, hybrid (F_1) from inferior parents IR-24 (47.81%) and Ratna (47.90%) resulted to significantly ($P<0.05$) higher dry matter digestibility to 54.49 and 55.46% in F_1 and F_2 generation, respectively (positive heterosis). However, crossing of parents

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Table 1. Chemical composition (%DM basis) and *in sacco* digestibility of parents, F_1 and F_2

Parents	Chemical composition (%DM basis)						*In sacco* digestibility (%)				
	OM	Ash	CP	NDF	ADF	HC	DM	OM	NDF	ADF	HC
PD-6	83.55	16.45	6.12	74.50	48.87	25.63	59.25	61.90	54.54	52.02	59.36
N-359	87.20	12.80	6.30	76.51	52.15	24.36	57.17	59.23	53.31	53.88	52.10
IR-24	86.15	13.85	4.81	77.25	52.85	24.40	47.81	51.11	41.51	40.90	42.84
Ratna	83.69	16.31	5.25	74.25	50.58	23.67	47.90	50.12	43.42	42.08	46.28
Average	85.14	14.86	5.62	75.62	51.11	24.51	53.03	55.59	48.19	47.22	50.14
F_1 generation											
PD-6×N-359	84.50	15.50	5.87	75.85	50.45	25.40	51.94	54.36	46.62	43.93	51.98
PD-6×IR-24	85.10	14.90	5.25	76.80	49.85	26.95	53.11	55.05	47.58	45.94	50.62
PD-6×Ratna	87.25	12.75	5.45	77.15	50.15	27.00	51.76	54.52	48.20	49.64	45.51
N-359×IR-24	84.15	15.85	5.25	73.87	51.80	22.07	50.94	54.63	45.55	47.25	41.18
N-359×Ratna	85.12	14.88	6.12	75.26	53.28	21.92	53.34	55.84	48.39	48.56	46.98
IR-24× Ratna	85.15	14.85	6.25	76.34	50.38	25.97	54.49	56.01	48.85	50.90	44.36
Average	85.21	14.79	5.69	75.87	50.98	24.86	52.59	55.06	47.43	47.70	46.77
F_2 generation											
PD-6×N-359	84.73	15.27	5.06	75.69	50.25	25.43	53.02	54.02	51.28	49.70	52.80
PD-6×IR-24	83.97	16.03	5.31	77.37	51.35	26.02	53.20	53.59	50.71	51.93	48.30
PD-6×Ratna	86.55	13.45	5.63	74.43	48.34	26.09	50.27	53.71	48.83	48.04	50.29
N-359×IR-24	85.66	14.34	5.03	75.83	51.29	24.54	50.27	50.16	49.34	48.05	52.04
N-24×Ratna	83.21	16.79	5.62	74.93	52.27	22.66	51.12	51.93	50.53	49.37	53.20
IR-24×Ratna	81.67	18.33	5.84	76.00	50.97	25.03	55.44	55.77	54.21	54.32	53.98
Average	84.29	15.71	5.41	75.70	50.74	24.96	52.22	53.19	50.81	50.23	51.76
Overall maen	84.88	15.14	5.57	75.75	50.92	24.82	52.56	54.48	48.53	48.93	49.48
SEm±	0.685	0.648	0.190	C 731	0.854	0.588	0.922	1.128	1.258	1.247	1.401
CD at 5%	1.685	1.953	0.575	2.205	2.574	1.774	2.599	3.258	3.634	3.603	4.046
CD at 1%	2.857	2.70	0.795	3.048	3.558	2.453	3.449	4.388	4.894	4.85	5.449

Table 2. Correlation coefficient among nutrient composition, morphological characters and *in sacco* digesibility parameters traits of rice straw hybrid, F_1 and parents (n=16)

	Ash	CP	NDF	ADF	HC	DMD	OMD	NDFD	ADFD	HCD	Pl.ht	Pani. length	NO of tiller	Total wt.$^{-1}$	Grain yield^{-1}	Straw yield^{-1}
OM	-1.00**	-0.047	0.354	-0.013	0.263	-0.166	-0.010	-0.251	-0.152	-0.322	0 154	0 057	0.051	0.094	-0.028	0.025
Ash		0.047	-0.355	0.013	-0.263	0.166	0.009	0.251	0.152	0.322	-0.154	-0.057	-0.051	-0.094	0.029	-0.025
CP			-0.128	-0.073	-0.032	0.724**	0.743**	0.548*	0.567*	0.323	0.083	-0.018	-0.414	-0.161	-0.155	-0.261
NDF				0 201	0.540*	0.077	-0.037	0.004	0.105	-0.134	0.005	-0.022	-0.097	0.169	-0.011	0.109
ADF					-0.716**	-0.205	-0.250	-0.242	-0.104	-0.387	0.618*	0.624**	0.752**	0.709**	0.456	0 663**
HC						0.229	0.186	0.210	0.164	0.236	-0 527*	-0.553*	0.713**	-0.486	-0.398	-0.489
DMD							0.927**	0.843**	0.800**	0.594*	-0.252	-0.330	-0.606*	-0.451	-0.346	-0.515*
OMD								0.651**	0.629**	0.431	-0.323	-0.423	-0.551*	-0.466	-0 495	-0.559*
NDFD									0 923**	0.763**	-0.062	-0.109	-0.484	-0.427	-0 024	-0 383
ADFD										0.461	0.130	0.073	-0.349	-0.191	0.101	-0.189
HCD											-0.375	-0.385	-0.521*	-0.638**	-0.210	-0.541*
Plant height												0.981**	0.571*	0.631**	0.748**	0.663**
Panicle length													0.628**	0.653**	0.805**	0.698**
No. of tiller														0.670**	0.548*	0.702**
Total weight^{-1}															0.729**	0.973**
Grain yield^{-1}																0.820**

* And ** significant at 1 and 5 per cent lever of probability respectively.

of superior and inferior straw resulted to moderate digestibility in their F_1 which slightly further reduced in F_2. Similar trends were observed in organic matter digestibility. These results confirm the earlier observation obtained by Gupta and Singh (1991), Atharuddin (1993) and Sohane *et al.* (1999). The NDF, ADF and HC digestibility of low × low and low moderate × crosses were significantly ($P<0.05$) increased except for hemicellulose digestibility in N-359 × IR-24 and IR-24 × Ratna in F_1 generation. However, digestibility of above traits again increased in F_2 generation in all crosses except HCD in

Table 3. Morphological charcteristics for hybrid, their F_2 and parents of rice straw

Parents	Plant height (cm)	Panicle length (cm)	No. of tiller	Total plant weight^{-1}	Grain yield plant^{-1}	Straw yield plant^{-1}	Grain straw ratio
			Parents				
PD-6	72.0	19.4	10.8	45.33	18.66	26.66	1:1.448
N-359	93.2	23.6	14.4	85.00	36.33	49.06	1:1.348
IR-24	91.2	20.2	17.0	75.50	32.66	42.34	1:1.296
Ratna	82.2	18.0	14.4	71.25	28.33	42.95	1:5.158
			F_1 generation				
PD-6×N-359	84.2	20.6	13.0	52.33	21.00	31.33	1:1.561
PD-6×IR-24	79.8	17.4	13.0	51.66	23.33	28.33	1:1.221
PD-6× Ratna	86.2	20.0	14.0	81.00	31.33	49.66	1:1.620
N-359× IR-24	93.6	20.0	17.2	93.33	34.00	59.33	1:1.730
N-359×Ratna	101.6	21.2	15.4	96.66	40.00	56.66	1:1.476
IR-24× Ratna	98.2	22.0	12.0	91.82	42.00	49.82	1:1.186
			F_2 generation				
PD-6×N-359	90.8	20.8	12.65	59.70	39.90	42.86	1:1.160
PD-6×IR-24	89.8	21.0	13.60	92.12	30.88	61.24	1:1.983
PD-6×Ratna	92.6	21.2	13.60	72.16	39.33	52.38	1:1.343
N-359×IR-24	94.2	18.8	15.20	98.50	49.50	69.00	1:1.393
N-359×Ratna	101.8	20.6	16.60	97.92	45.92	72.00	1:1.561
IR-24×Ratna	88.8	17.8	14.20	91.95	47.72	64.22	1:1.345

PD-6 'IR-24. However, NDF, ADF and HC digestibility of high' high cross showed significant ($P<0.05$) depression in F_1 without any change in F_2. Crossing of two low straw quality varieties yielded straw of high ash content in F_2 (18.33%) which improved the DMD from 54.49 to 55.44% and cell wall (NDF) digestibility from 42.46 in parents to 48.85 in F_1 and 54.21% in F_1, due to higher HC digestibility.

Organic matter content of the straw, had perfect negative correlation (-1.00**) with ash content (Table 2). The CP content of straw was positively correlated with DMD, OMD, NDFD and ADFD. The NDF content of straw was positively correlated with HC, because latter being part of former component. The ADF content of straw was significantly negatively correlated with HC, might be due to the fact that ADF consists mainly of cellulose and lignin. However, the ADF was positively correlated with plant height, panicle length, no. of tiller plant -1, total weight of plant-1 and straw yield-1, because ADF consist of cellulose and lignin content to provide strength to the plant. The HC contents were negatively correlated with plant height, panicle length and no. of tiller plant-1. The dry matter digestibility was found significantly positively correlated with OMD, NDFD, ADFD and HCD, while negatively correlated with no. of tiller plant-1 and straw yield plant-1. Similar trend was found with organic matter digestibility. The digestibility of different nutrients was significantly ($P<0.01$) positively correlated, which means that the degradation of all nutrients contributed to the degradation of each other and thus towards DMD. The results of the present investigation are supported by Saleem (1973) and Srivastava (1990). The digestibility of DM, OM and HC significantly negatively correlated with plant height-1, no. of tiller plant-1, plant weight-1 and straw yield-1, which might be due to deposition of higher amount of ADF fraction. Morphological characteristics like, plant height, panicle length, no. of tiller, total plant weight-1, grain yield plant-1 and straw yield plant-1 were significantly positively correlated with each other.

It may be concluded from the present investigation that a considerable extent in inheritance exists in nutritive value of rice straw quality. The plant breeders may prefer hybrid formulation involving varieties having low nutritive value of straw, so that farmers can grow varieties of rice having both high grain yield potential as well as superior straw.

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Indian Journal of Animal Sciences **70** (10): 1085-1086, October 2000

Effect of feeding various combinations of soybean and sorghum straw on the growth performance of Murrah heifers*

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Received: 7 August1998; Accepted: 7 April 2000

Key words: Growth, Murrah heifers, Soybean straw

Simple physical mixing with conventional sorghum straw was tried to improve the utilization of soybean straw and feeding of such mixtures of varied proportions was tried with the objective to assess the growth performance and nutrient digestibility in growing Murrah buffalo heifers.

Murrah heifers (18) were intially selected and divided in 3 homogenous groups on the basis of live weights. A pilot trial on feeding of 100% soybean straw indicated lower feed intake and incomplete appetite of the buffalo calves and therefore, sole feeding of soybean straw was not considered in this study. Fresh soyabean straw and chaffed sorghum straw were considered for the formulation of 3 treatments, viz. soybean straw mixed with sorghum straw chaff in 60: 40 (T 1), 50: 50 (T 2) and 40: 60 (T 3) proportion by weight. The heifers under each group were fed either of these mixtures *ad lib.* alongwith 250 g concentrate mixture daily. The experiment was conducted for 9 weeks from 18 December, 1996 to 22 March, 1997. All the heifers were housed in well ventilated shed with routine sanitary measures. They were dewormed before the start of the experiment. Sufficient clean drinking water was provided twice daily.

The observations on the proximate analysis (AOAC 1990) of soybean straw, sorghum straw and concentrate mixture were recorded. Daily record on actual feed and water intake by each animal was maintained. Weekly observations on live weights and linear body measurements were noted. The data were analyzed by following the standard procedures of analysis of variance as described by Federer (1967).

Table 1. Chemical composition of feeds and fodders (% on DM basis)

Feed stuff	OM	CP	EE	CF	NFE
Soybean	88.50	10.60	1.50	42.12	34.28
Sorghum straw	89.90	3.60	2.51	37.60	46.19
Concentrate mixture	92.64	21.00	4.22	9.08	58.34

Table 2. Liveweigth gain, digestibility and body measurements of Murrah heifers on different diets

Attributes		T1	T2	T3
Initial BW (kg)		159.25	158.67	158.83
Final BW (kg)		176.60	175.35	174.40
Mean gain in BW (kg)		17.33	16.67	15.58
ADG (g)		275	264	247
Nutrient intake (kg) and digestibility (%)				
	DM	5.629	5.673	5.574
		(57.30)	(56.60)	(55.81)
	CP	0.412	0.368	0.317
		(58.25^{a})	(57.06^{ab})	(55.52^{b})
	EE	0.098	0.103	0.109
	CF	2.177	2.162	2.104
		(52.96)	(51.66)	52.61)
	NFE	2.234	2.390	2.400
		(56.53)	(56.61)	(56.33)
Gains in body measurements (cm)				
	Height	5.50	4.50	4.08
	Length	6.50^{a}	5.00^{b}	4.08^{b}
	Chest girth	5.50	5.00	4.08

Figures in parenthesis indicate per cent digestibility. Means bearing different superscripts differ significantly (P<0.05).

The CP content (Table 1) of soybean straw is comparatively higher than average values; this might be due to higher proportion of soybean grains in the whole mass of the straw used in this study. The results on the total gains in body weights (Table 2) showed nonsignificant differences amoung the treatments indicating equal response of soybean: sorghum straw combinations by growing buffalo heifers. Pachauri and Negi (1976) reported similar results for crossbred calves when fed different soybean: wheat *bhoosa*

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combinations. The daily growth rate of 275, 264 and 247 g is in agreement in with values reported by Pacola *et al.* (1985) in fattening cattle fed soybean with burnt sugarcane tops and cotton seed oil.The lower values of 190 g / day were reported by Kumar and Garg (1995) in Murrah heifers fed with sole soybean straw ration without any concentrates. The various combinations of soybean and sorghum straw did not affect the mean gains in body height and chest girth of the growing Murrah heifers suggesting equal response to different roughage combinations for normal growth. However, significant difference ($P < 0.05$) in body length of heifers under 3 treatments was recorded. Nevertheless, body length has no direct correlation with body weight and it is the function of skeleton growth (Prajapati *et al.* 1991). Further, the heifers responded equally either to body weight and chest girth, the factors which are highly correlated with each other.

The values of digestibility of DM, CF, EE and NFE from various treatment rations did not show any significant difference (Table 2). However, CP digestibility in T 3 was significantly lower than the corresponding values in T 1 and T 2. The intake of lower CP and higher energy due to higher proportion of sorghum straw in T 3 might have resulted lower nitrogen retention and, therefore, lower CP digestibility. Pachauri and Negi (1976) observed similar findings in their study with significantly higher DCP intake and nitrogen retention in soybean straw than that in wheat straw diet. Thus soyabean straw may be utilized as a coarse roughage provided the amount of crude proteins is proportionate.

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Indian Journal of Animal Sciences **70** (10): 1087-1089, October 2000

Effect of different flooring materials on body weight and measurement of crossbred (LWY × *desi*) piglets*

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Received: 29 September 1999; Accepted: 12 April 2000

Key words: Body measurements, Body weights, Crossbred piglets, Flooring materials

The concrete floor is not favourable to the growth of the piglets (Kloppel *et al.* 1983, Heyde and Hendricks 1984). The present investigation was undertaken to assess the effect of different flooring materials like paddy husk, chaffed paddy straw and *kachcha* floor along with concrete flooring on body weights, body measurements and different types of ailments.

Weaned piglets (36), 2 to 3 month old, were randomly selected, irrespective of sexes for all the groups equally from the AICRP on pigs at JNKVV. The growth performance and body measurements were studied under the 4 types of flooring systems i.e. available cemented concrete floor (T_1); paddy husk flooring (T_2); *kachcha* floor by means of filling with black cotton soil on concrete floor (T_3) and chaffed paddy straw filling floor (T_4). The experiment was done for 18 weeks. Amongs the 36 piglets 10 each were allotted for concrete and paddy straw flooring while 8 each were allotted for *kachcha* and paddyhusk flooring system. The feeding was done as per schedule of AICRP on pigs.

The fortnightly body weights and body measurements (length, height, heart and paunch girth) were taken on each piglet during the whole experimental period.

To measure the feed efficiency of piglets of each flooring groups, the record of total feed given and the left over was weighed.To measure the incidence of ailments the skin scrapings for the incidence of mange were taken at monthly intervals. The incidence of diarrhoea and hoof abnormalities were also recorded.

The body weights and measurements data were put under analysis of variance using 1-way classification, to identify the effect of flooring on these parameters (Snedecor and Cochran 1976). Feed conversion efficiency for each treatment was calculated (Sapra and Shingari 1991). The incidence of diarrhoea, hoof abnormalities and occurrence and severity of mange was recorded for each treatment.

The different flooring significantly influenced the growth of piglets (Table 2). The body weight showed significant effect of flooring from sixth stage onwards while for body length it was from seventh stage onwards (Table 2). However, the significant effect of flooring on height was recorded during, first 2 and last 2 stages only. Body length showed significant effect from seventh stage onwards which was in concurrence with the later stage results of height (Table 1).

The results of heart girth showed significant growth during early stage (Third onward) indicating early muscular growth as compared to skeletal growth. However, paunch girth showed significant growth from seventh stage onwards which was similar to body length results.

The results of mean and their standard error of different flooring for different experimental stages for body weights and mesurements are presented in Table 1. Almost all the parameters showed highest growth on *kachcha* flooring indicating its superiority for faster growth over other types of flooring. The next in order, in respect to growth, was that on paddy husk flooring followed by paddy straw and with minimum growth on cement concrete flooring (Table 1).

The results of 'modified multiple range test' (Kramer 1957) showed body weight being only significantly higher on *kachcha* flooring compared to cement concrete flooring. There was no significant difference on body weight growth when compared with paddy straw and husk flooring. Similar poor growth rate in piglets on concrete slat flooring when compared with different types of flooring like asphalt, straw and wire mash floor, were reported by Heyde and Hendricks (1984).

The body length showed significantly higher length only during last stage on *kachcha* flooring compared to cement concrete flooring. The results on paddy husk and straw flooring although close to *kachcha* flooring, did not differ significantly from concrete flooring. The concrete floor showed the shortest length compared to *kachcha,* paddy husk and straw floorings. Similar shorter body length in exotic piglets were reported by Bartussek *et al.* (1992), when raised

*A part of M.V. Sc. and A.H. Thesis submitted to Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur in 1996.

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Table 1. Fortnightly mean ± S.E. values of body weight and measurement of piglets raised on different flooring

Stages (fortnightly interval)	Treatments	Body weight	Body length	Body height	Heart girth	Paunch girth
I	Concrete floor	11.48±0.99	50.038±1.727	33.782±1.524	46.101±1.473	44.323±1.981
	Paddy husk floor	12.175±1.11	51.277±1.930	38.100±1.701	46.355±1.651	44.45±2.209
	Kachcha floor	13.25±1.11	51.112±1.930	43.497±1.701	46.672±1.651	43.180±2.209
	Paddy straw floor	11.30±0.99	47.244±1.727	43.688±1.524	44.958±1.473	46.228±1.981
II	Concrete floor	13.10±1.27	53.975±1.803	38.862±1.651	50.038±1.371	47.625±1.727
	Paddy husk floor	15.00±1.42	56.992±2.006	43.022±1.828	51.117±1.676	47.785±1.930
	Kachcha floor	15.438±1.42	57.150±2.006	47.942±1.828	52.865±1.676	49.847±1.930
	Paddy straw floor	13.650±1.27	53.848±1.803	45.466±1.651	49.174±1.498	47.371±1.727
III	Concrete floor	14.45±1.50	55.626±1.752	42.926±1.625	51.562±1.727	51.562±2.006
	Paddy husk floor	17.363±1.68	60.325±1.955	46.197±1.803	59.850±1.930	52.547±2.235
	Kachcha floor	17.625±1.68	61.120±1.955	49.847±1.803	57.627±1.930	55.087±2.233
	Paddy straw	16.30±1.50	56.642±1.752	46.990±1.625	53.848±1.727	51.054±2.006
IV	Concrete floor	16.30±1.73	57.785±1.828	45.466±1.625	53.340±1.879	53.340±2.260
	Paddy husk floor	20.25±1.94	63.025±2.032	50.325±1.828	63.025±2.108	56.197±2.514
	Kachcha floor	21.50±1.94	64.135±2.032	50.642±1.828	58.737±2.108	58.262±2.514
	Paddy straw floor	19.25±1.73	61.087±1.828	47.625±1.625	56.794±1.879	55.626±2.260
V	Concrete floor	17.29±1.79	60.833±1.+955	50.038±1.625	56.261±1.701	55.880±2.286
	Paddy husk floor	22.813±2.00	66.200±2.184	53.657±1.803	63.182±1.905	56 832±2.565
	Kachcha floor	23.375±2.00	67.787±2.184	53.340±1.803	64.135±1.905	60.960±2.565
	Paddy straw floor	20.70±1.79	65.405±1.955	49.911±1.625	59.436±1.701	58.420±2.286
VI	Concrete floor	18.06±2.14	63.881±1.545	53.340±1.625	59.436±1.498	59.436±2.006
	Paddy husk floor	26.25±2.39	69.532±2.209	56.675±1.803	61.595±1.676	59.372±2.235
	Kachcha floor	26.68±2.39	70.327±2.209	57.310±1.803	66.040±1.676	64.135±2.235
	Paddy straw floor	24.30±2.14	68.707±1.545	54.102±1.625	62.357±1.498	62.484±2.006
VII	Concrete floor	19.55±2.21	65.659±2.032	53.721±1.676	60.858±1.524	59.563±2.184
	Paddy husk floor	28.00±2.47	72.072±2.286	64.135±1.701	64.135±1.701	62.707±2.438
	Kachcha floor	28.625±2.47	73.025±2.286	60.485±1.854	68.740±1.701	69.057±2.438
	Paddy straw floor	26.70±2.21	73.660±2.032	58.166±1.676	64.135±1.524	69.088±2.184
VIII	Concrete floor	22.60±2.36	67.945±2.108	55.880±1.701	61.722±1.955	61.087±2.362
	Paddy husk floor	30.563±2.62	73.972±2.336	63.025±1.905	69.215±2.184	66.675±2.641
	Kachcha floor	33.00±2.62	77.152±2.336	63.817±1.905	70.010±2.184	70.010±2.641
	Paddy straw floor	30.70±2.35	77.622±2.108	61.849±1.701	67.437±1.955	72.517±2.362
IX	Concrete floor	25.15±2.45	70.866±2.336	59.690±1.651	63.373±2.184	62.230±2.540
	Paddy husk floor	35.25±2.74	78.582±2.616	64.930±1.854	71.915±2.463	69.215±2.844
	Kachcha floor	35.625±2.74	82.867±2.616	65.722±1.854	75.247±2.463	72.961±2.844
	Paddy straw floor	33.15±2.45	81.153±2.336	64.262±1.651	69.469±2.184	75.184±2.540

on slatted floor in comparison to other floorings.

Similar significant superiority for height on *kachcha* floor was observed from seventh stage onwards in comparison to piglet raised on other 3 floorings. Here also the shortest height was consistantly recorded in piglets raised on concrete flooring.

However, piglets raised on *kachcha* flooring recorded significantly larger diameter for heart girth from the third stage onwards when compared with piglets raised on concrete flooring. The other 2 flooring results although superior to concrete flooring, did not show significant differences.

The paunch girth of piglets raised on paddy straw started differentiating significantly with larger diameter from seventh stage onwards, compared to concrete flooring. *Kachcha* and paddy husk did not differ significantly but were close to paddy straw flooring results. The results for paunch girth were different from other body measurement results exhibiting superiority on *kachcha* flooring. This seems to be due to consumption of some paddy straw by piglets resulting in more fat deposition.

The growth pattern can easily be visualized from the fortnightly mean results of body weights and measurements (Table1). The fastest growth was consistantly observed in piglets raised on *kachcha* floor followed by paddy husk and straw. The poorest growth recorded was on cement concrete flooring which is generally being used by almost all organised pig raising sectors. It is therefore, a high time to decide whether one should continue to raise piglets on cement concrete floor or to switch to *kachcha* flooring.

The feed efficiency measured on feedlot basis showed

Table 2. Fortnightly analysis of variance on body weight and measurement of piglets Raised on different floor

Stage (fortnightly interval)	Source of Variation	Body weight Df	Body length (MS)	Body height (MS)	Heart girth (MS)	Paunch girth (MS)	(MS)
I	Bet. floors	3	6.777	32.399	215.012**	5.180	14.516
	Error	32	9.871	29.703	23.199	22.077	38.974
II	Bet. floor	3	10.832	29.632	137.599**	22.406	10.929
	Error	32	16.192	32.445	26.935	22.774	29.638
III	Bet. floors	3	20.296	64.928	73.115	123.122*	27.670
	Error	32	22.529	30.993	26.361	29.812	40.309
IV	Bet. floors	3	45.062	70.683	53.832	144.722*	36.735
	Error	32	33.007	33.135	26.451	35.257	50.806
V	Bet. floors	3	69.579	82.657	36.961	118.038*	42.503
	Error	32	31.889	37.812	26.309	28.993	56.864
VI	Bet. floors	3	148.028*	78.812	33.193	65.845*	47.593
	Error	32	45.619	39.032	26.367	22.303	40.154
VII	Bet. floor	3	165.459*	132.638*	84.638*	92.728*	212.141**
	Error	32	48.772	41.509	27.799	22.999	47.935
VIII	Bet. floors	3	196.286*	191.838**	127.728*	178.399*	239.586**
	Error	32	55.086	44.070	28.806	37.812	55.883
IX	Bet. floors	3	197.025*	266.734**	68.922	228.638**	315.192**
	Error	32	60.216	55.083	27.458	43.103	64.322

highest efficiency by the group of piglets raised on *kachcha* flooring while it was lowest in cement concrete. Similar feed efficiency was recorded on paddy husk and paddy straw flooring in between above 2 flooring results. This was expected as the fastest growth recorded on *kachcha* flooring with no incidence of mange.

Further, the incidence of disease prevalence observed as diarrhoea, the mange infestation and hoof abnormalities on different flooring were also recorded to ascertain the suitability of flooring. The results indicate that the incidence of diarrhoea was lowest (30.0%) on cement concrete flooring and was highest (62.5%) on *kachacha* and paddy straw flooring. However, it is interesting to note that in spite of double the occurrence of diarrhoea in *kachcha* flooring in comparison to cement concrete, the growth was fastest in *kachcha* flooring thus completely nullifying the adverse effect of diarrhoea.

The infestation of mange was recorded in terms of per cent incidence and severity depending upon the body area affected. The incidence was observed 100.0% on cement concrete flooring followed by 40.0% on paddy straw and paddy husk floor system but no incidence in *kachcha* flooring system. These results further strengthen the view point expressed above that *kachcha* flooring may be recommended. Similarly the severity is highest in cement concrete flooring which may be the reason for poor growth due to loss of energy in overcoming the stress of mange infestation.

The hoof abnormalities recorded as cracks and deformities on hooves showed 60.0% cracks and 20.0% deformities on cement concrete flooring. Similar higher incidence of hoof abnormalities on concrete and other types of hard floors have been reported by Kornegay and Lindermann (1984). These findings further discourage the use of cement concrete flooring.

From the results, it may be concluded that *kachcha* or paddy husk flooring may be preferred over cement concrete or paddy straw flooring for raising piglets as it resulted in significantly faster body growth inspite of high incidence of diarrhoea, lowest incidence of mange and minimum abnormalities of hoof with higher feed efficiency.

ACKNOWLEDGEMENTS

The authors thank the AICRP on Pigs at Livestock Farm, JNKVV, Jabalpur, for permitting to conduct research. They also thank Dr H K B Parekh, former Dean, Veterinary College, Jabalpur, for the statistical analysis and guidance.

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Indian Journal of Animal Sciences **70** (10): 1090-1093, October 2000

Influence of diet composition, starvation and feeding frequency on gastric evacuation rates in Catla, *Catla catla* (Hamilton) fingerlings

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Received: 27 September1999; Accepted: 25 April 2000

ABSTRACT

Experiments were conducted to evaluate the influence of protein source (fish-meal, soybean and casein), protein (30%, 35% and 40%) and lipid (5%, 10% and 15%) levels in the diet, feeding frequency (1, 2 and 3 meals/day) and starvation period (5, 10 and 15 days) on gut evacuation time in catla, *Catla catla* fingerlings. Casein-based diet emptied faster compared to soybean and fish-meal based diets. The food passage time varied between 9.14 and 10.55 hr for different protein levels in the diet. The gut evacuation time decreased as content of lipid increased in the diet and vice versa in feeding frequency. However, the effect of starvation period on the evacuation time was insignificant.

Key words: *Catla catla*, Diet composition, Feeding frequency, Gut evacuation rate, Starvation period

A clear understanding of nutritional requirements of fish and feeding schedules is must to develop suitable cost effective balanced diet in fish culture. The quality and quantity of feed given greatly influences the fish growth and thus the fish production. Gut evacuation rate particularly in temperate waters and the influence of various parameters like temperature, size of meal, frequency of feeding, composition of diet and energy content of the diet were studied (Windell 1967, Persson 1982, Brodeur and Pearcy 1987, Elliott 1991).

In this study the Indian major carp, *Catla catla* was selected as it is a fast growing species, and contributes more than 50-60% of the total fish production. Even though food habits and nutritional aspects of this species are well known, little information is available regarding the influences of diet composition and other related aspects on digestion rates. The study was conducted to evaluate the influence of protein source, protein and lipid level in the diet, feeding frequency and starvation period on food evacuation rates in catla fingerlings.

MATERIALS AND METHODS

Fingerlings of catla, (6.0- 8.0 cm and 2.01- 4.28 g) were obtained from Bhadra Reservoir Fish Farm, Shimoga, Karnataka. Approximately 1 000 fishes were placed in each of two 2000 L FRP (fibre glass reinforced plastic) rectangular tanks with water circulation facilities through a biological filter. The fishes were maintained on a conventional feed (dough of rice bran and groundnut-cake, 1: 1) for 1 month before being used in the experiments.

Protein source

Three groups of 50 fishes each were stocked in FRP (fibre glass reinforced plastic) circular tanks (0.6 m diameter × 0.76 m height) with constant acration. Three diets containing 30% protein using fish-meal, soybean and casein sources were prepared separately using Pearson square methods. (De-silva and Anderson 1995). Each group was fed with pelleted feed (Table 1) for 1 week to acclimatize to their respective diet. Tanks were cleaned every day to remove the feaces and approximately one-third of the total volume of water in the tank was replaced. The fish were starved for 12 hr, then they

Table 1. Composition of basal diet (30%protein)

Ingredients	Percentage
Fish-meal	27.00
Oil-cake	27.00
Rice-bran	34.00
Tapioca flour	11.00
Vit. and min.mixture	1.00

*Vitamin mix (mg/kg diet): Thiamin 20, riboflavin 20, pyridoxine 20, folic acid 5, calcium pentothenate 200, chloride 2000, niacin 150, ascorbic acid 100, cholecalciferol 2, menadione sodium disulphite 10, inositol 400, biotin 2.

*Mineral mix (mg/100g diet): Aluminum chloride 18, zinc sulphate 375, cuprous chloride11, manganese sulphate 80, potassium iodide 17, cobaltous chloride 105, calcium phosphate 18, sodium chloride 6, ferrous carbonate 1.75, sodium selenite 0.02.

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were fed with their respective diet to satiation for 20 min and excess feed was removed by siphoning. Immediately a random sample of 5 fishes was removed, and sacrificed to examine the amount of feed in the digestive tract.The gut contents in the anterior bulged portion of the alimentary tract were removed and weighed to the nearest mg after removing excess water by absorbent paper. This procedure was continued every 2 hr until the guts of sampled fish were empty.

Protein level

Three diets were formulated to contain 30%, 35% and 40% protein using fish-meal. The gastric evacuation experiments were carried out as described above.

Lipid level

Fish-meal based diets with 30% protein were prepared with 5, 10 and 15% sardine oil. The fishes were divided into 3 groups of 50 each and acclimatized to their respective diet for 1 week. Evacuation experiments were carried out as described above.

Starvation period

Three groups of fishes of 50 individuals in each group were starved for 5, 10 and 15 days separately. After starvation period fish were fed with fish-meal based diet for 20 min and sampling was carried out as described above.

.Feeding frequency

Out of 3 groups of fish, first group was fed once in a day, whereas second group twice and third group thrice in a day with fish-meal based diet containing 30% protein. After acclimatization, gastric evacuation experiments were carried out as described earlier.

Statistical analysis

In all the cases linear model was used to describe the depletion of the gut contents with time. Regression analysis was used to determine the relationships between percentage of food remaining in the gut and the time after initial feeding. Analysis of variance (2-way ANOVA) was used to test the significant difference between the regression coefficient.

RESULTS AND DISCUSSION

The results were expressed on a wet weight basis as the amount of food material ingested and the remaining in the gut was small and no attempt was made to express the data in terms of dry weight. There was a gradual decrease in percentage of food recovered from guts with time. Higher the value of instantaneous rate (b), shorter the time taken for complete evacuation (100%) of food from the gut (Table 2). Protein sources and protein levels had influence on the gut evacuation time.Amoung protein sources casein based diet evacuated at faster rate (7.19 hr) compared to soybean (10.89 hr) and fish-meal based diets (9.14 hr). The diet incorporated with plant protein (soybean) required longer duration for complete evacuation from the gut because it contain more fibre, whereas casein evacuated at faster rate due to its pure simple protein. Fish-meal, took lesser time for evacuation compared to soybean. There was no significant difference ($P< 0.05$) in evacuation rate due to fish-meal and soybean, however, there were significant differences ($P< 0.05$) in evacuation rates due to other 2 combinations.

The food passage time varied between 9.14 and 10.55 hr

Table 2. The instantaneous rate of evacuation (b) and time (hr) to the various levels of evacuation using linear model

Treatment		b	Time (hr) to percentage evacuation		
			25%	75%	100%
Protein source	Casein	0.1994^{a}	0.92	5.10	7.19
	Fishmeal	0.1820^{ab}	2.27	6.85	9.14
	Soyabean	0.1709^{b}	3.46	8.41	10.89
Protein level (%)	30	0.1820^{a}	2.27	6.85	9.14
	35	0.1556^{ab}	1.55	6.91	9.58
	40	0.1662^{b}	3.03	8.05	10.55
Lipid level (%)	5	0.1639^{a}	3.19	8.27	10.81
	10	0.1577^{b}	3.14	9.67	12.93
	15	0.1128^{bc}	4.79	12.18	15.87
Starvation period (days)	5	0.1487^{a}	2.33	7.94	10.74
	10	0.1282^{a}	3.29	9.80	13.05
	15	0.1132^{a}	2.83	10.20	13.88
Feeding frequency (meals/day)	1	0.1820^{a}	2.27	6.85	9.14
	2	0.1851^{b}	1.61	6.11	8.36
	3	0.2131^{c}	1.68	5.59	7.54

Note: Figure with same superscripts are not significantly different.

for different protein levels in the feed. There was significant difference ($P< 0.05$) in gastric evacuation rate due to 30 and 35% protein levels and 30 and 40% protein levels in the feed. Similar results were observed in rohu, *Labeo rohita* (Annappaswamy 1997). Anderson (1999) reported that gastric evacuation is regulated by the dietary energy and type ingredient used.

In different lipids levels in the feed, 5% lipid level evacuated at faster rate (10.8 hr) compared to 10 (12.93hr) and 15% (15.87hr) lipid level in the feed. There were significant differences ($P< 0.05$) in evacuation rate due to 5 and 15%, 10 and 15% lipid levels. Addition of oil along with feed led to a reduction in the proportion of meal emptied during the 24hr following feeding, which is in agreement with results of experiment conducted with rohu, *Labeo rohita* (Annappaswamy 1997) cod, *Gadus morhua* (Santos and Jobling 1988), rainbow trout *Salmo gairdineri* (Windell *et al.* 1969) and plaice, *Pleuronectes platessa* (Jobling 1980). Thus an increase in the lipid of the diet increased the time required for evacuation. Hunt and Stubbs (1975) reported that the fatty feeds are known to delay digestion. On contrary to present findings, De-silva and Owoyemi (1983) reported that density of diet or lipid had no effect on evacuation time in *Sarotherodon mossambicus*. Windell *et al.*(1972) also made similar observation for rainbow trout, *Salmo gairdineri*.

The fish starved for 5,10 and 15 days prior to feeding took 10.74, 13.05 and 13.88 hr for complete evacuation of food from the gut respectively. Prolonged starvation prior to feeding delayed the initiation of digestion (Fange and Grove 1979, Annappaswamy 1997). It is probably due to absence of digestion juice when the food enters in the gut (Elliott 1972). Long periods of fasting decreased the evacuation rates of fish (Windell 1968). Starvation had effect on functioning of enzyme secreting organs in the fish body (Darnell and Meierotto 1962). However, evacuation rate did not differ significantly ($P< 0.05$) due to starvation period. Russel and Wootton (1993) also observed that in *Phoxinus phoxinus* (cyprinidae), starvation for 4 to 16 days had no significant effect on the rate of evacuation of the first satiation meal consumed after the starvation period.

The rate of evacuation increased with feeding frequency. The food passage time was 9.14, 8.36 and 7.54 hr for 1 meal, 2 meals and 3 meals per day respectively. The evacuation rate due to 1 and 3 meals per day differed significantly ($P< 0.05$). Similar observations were made by Annappaswamy (1997).The identical initial stomach contents showed significantly higher stomach evacuation rates under 3 meals/day condition than under 1 meal/day condition in *Coregonus lavaretus*. Multiple meals of whole shrimp produced higher evacuation rates in pacific cod than the single meal (Paul *et al.*1990). Multiple meals have been shown to be digested (evacuated) faster than a single meal of the same weight (El-shamy 1976). It is assumed that in nature fish do not consume a single meal and then wait for the gut to get completely empty before they resumed feeding.

ACKNOWLEDGEMENTS

Authors thank the ICAR (Indian Council of Agricultural Research), New Delhi,for the financial support in carrying out this investigation. We also indebted to Dr S L Shanbhogue, Director of Instruction (Fisheries) for the providing facilities.

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Indian Journal of Animal Sciences **70** (10): 1094-1098 October 2000

Limno-chemical parameters vis-a-vis productivity in Pong reservoir, Himachal Pradesh

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Accepted: 10 April 2000

ABSTRACT

Pong reservoir (15000 ha) in Himachal Pradesh, was assessed during August 1991 to July 1992 for its limno-chemical features and productivity status. Total alkalinity (48 to 96 ppm), temperature (13 to 31°C), pH (7.1 to 8.3) and specific conductivity (96.4 to 195.8 μmhos/cm^{-1}) indicated medium productivity. The values of chemical parameters were generally high during summer and showed decline during monsoon period. The reservoir exhibited thermal and biogenic chemical stratification in respect of oxygen, bicarbonate and specific conductivity. The annual carbon production was to the tune of 93725 tonnes or 6248 kg/ ha/ annum. The conversion ratio of gross carbon to fish (496 t) was 0.23% in this reservoir. The reservoir could be developed for increasing yield if better management norms are adopted.

Key words: Limnology, Productivity and reservoir management

Though general limnological information are available for some impoundments in penninsular India (David *et al.* 1969; Subba Rao and Govind 1964; Srinivasan 1971; Pathak 1979), it is only recently that reservoirs in north India have been subjected to similar observations. Mathew (1975) made a limnological study of Govindgarh lake and related the fish production with total carbon synthesised in the lake. Sarkar *et al.* (1977) and Kaushal *et al.* (1984) presented limnological features of Gobindsagar. A detailed knowledge of limnological parameters, trophic status and of the kinetics of reactions taking place in trophogenic and tropholytic zones, is thus a pre-requisite for the evaluation of the productivity and in turn the scientific management of reserviors. The present communication reports the seasonal changes in physico-chemical features and carbon synthesis of Pong reservior.

DESCRIPTION OF THE RESERVOIR

Pong reservoir came into being in 1974 as a result of construction of an earth core gravel shell dam across the river Beas in Kangra district of Himachal Paradesh, at 32°-25'N latitude. The water sprea area at F.R.L. (433.12 m above mean sea level) is 240 km^2. The reservoir has a maximum depth of 97.84 m and a mean depth of 35.7 m. The reservoir has a total catchment area of 12562 km^2. It impounds 8570 million m^3 of water of which 7290 million m^3 constitute the live storage capacity. The reservoir is fed with monsoon run-off and cold snow melt.

The reservoir was divided into 3 zones viz., lotic, intermediate and lentic zone for the present studies.

MATERIALS AND METHODS

Surface water samples for the study of limno-chemical parameters were collected at monthly intervals from each zone during August 1991 to July 1992. Samples from depth profile from surface to 30 m were collected in lentic zone with the help of a VanDorn water sampler and analyses were done following methods as described by Jhingran *et al.* (1969). Primary production was studied in littoral and limnetic regions of lentic by 'dark and light bollte' technique *in situ*.

OBSERVATIONS

Inflow outflow and water level

The reservoir level fluctuated by 13.41 m from a maximum of 422.17 m in October 1991 to a minimum of 408.76 m in June 1992. The rising trend of water was noticed in July with timely rains. Yearly rainfall varied from 84.3 cm in 1991-92 to 132.2 cm in 1990-91 with 40.2 to 74.2% of it occurring during July-September indicating that the South-West monsoon has a greater influence in the reservoir catchment.

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Table 1. Monthly mean and annual zonal variation in physico-chemical features of surface water of Pong reservoir

Months/ zones	Transparency (cm)	Temp. (°C)	pH	Dissolved oxygen (ppm)	Free Co_2 (ppm)	Total alkalinity (ppm)	Hardness (ppm)	Dissolved organic matter (ppm)	Phosphate (ppm)	Specific conductivity (μmhos/cm)
Aug. 1991	96.7	29.7	7.9	8.5	2.0	70	49	4.7	0.11	161.7
Sept. 1991	106.3	29.0	8.2	9.5	0.6	73	61	2.5	0.23	140.8
Oct. 1991	122.3	26.3	7.9	9.3	2.0	68	69	4.0	0.14	168.6
Nov. 1991	246.0	22.7	8.1	8.8	2.0	67	68	3.8	0.12	164.1
Dec. 1991	232.3	17.3	7.9	8.7	2.0	71	71	3.8	0.14	157.5
Jan. 1992	167.0	14.7	7.8	9.2	2.0	80	73	3.7	0.13	159.3
Feb. 1992	181.7	15.0	7.9	9.1	2.0	75	71	3.9	0.12	149.3
Mar. 1992	243.0	17.0	8.0	9.5	1.3	78	69	4.1	0.14	159.3
Apr. 1992	162.5	22.1	7.6	9.5	1.3	75	75	3.9	0.12	159.3
May 1992	235.5	24.5	7.4	9.2	Nil	79	72	4.1	0.16	148.4
June 1992	165.5	24.7	7.4	9.3	Nil	71	70	4.1	0.16	151.7
July 1992	127.5	28.2	7.5	8.5	4.0	72	69	3.9	0.15	147.8
Lotic	142.0	21.5	8.3	9.2	1.8	74	69	3.7	0.14	148.3
	(45-278)	(13-29)	(7.1-8.0)	(8.6-9.6)	(Nil-2)	(62-96)	(50.76)	(2.4-4.6)	(0.12-0.22)	(96.4-182.7)
Intermediate	127	23.5	7.8	9.0	1.7	71	67	4.0	0.12	160.3
	(45-211)	(13-31)	(7.3-8.3)	(8.4-9.6)	(Nil-4)	(48-78)	(48-80)	(2.2-4.8)	(0.10-0.24)	(138.9-187.5)
Lentic	247	23.6	7.9	8.9	1.3	74	68	3.9	0.17	158.3
	(121-423)	(15-31)	(7.8-8.2)	(8.4-9.6)	(Nil-4)	(64-94)	(50-78)	(2.8-4.6)	(0.10-0.20)	(131-195.8)

Figures in parenthesis represents range.

The total inflow and outflow were 8270 and 7800 million m^3 respectively. The inflow varied from 130 million m^3 in December to 2190 million m^3 in August where as outflow fluctuated between 430 million m^3 in August and 880 million m^3 in October.

Characteristics of bottom soil

Soil was alkaline in reaction with pH ranging from 7.3 to 7.6. The reservoir soil was in general, sandy in texture and poor in organic carbon (0.24 to 0.57%) and available nitrogen (14.2-17.0 mg/100g), but available phosphorus (4.0-12.0 mg/100g) was fairly high.

Physico-chemical features of water

Clarity of water was high in the reservoir, the mean secchidise reading ranged between 96.7 and 246 cm (Table 1). Highest water transparency of 423 cm was recorded in lentic zone. Transparency declined during the monsoon period in all the zones from higher values in summer. After monsoon, water clarity showed gradual increase due to settling of suspended particles attaining a peak in November. Thereafter transparency declined during December-February due to North-East monsoon.

The mean surface water temperature ranged from 14.7 (January) to 29.7°C (August) with an annual fluctuation of 15°C. The wide seasonal variation in water temperature had a great bearing on the heat cycle of the reservoir. The water temperature in lentic zone covering a belt of 30 m depth showed near homothermal condition, except during the period of May to July, as the variation in water temperature never exceeded 3°C. Thermal stratification was recorded in May and July, 1992 (Table 2). In May, the temperature difference from surface to bottom was 9.5°C consisting of thermocline between 8 and 9 m; the drop of temperature being @ 1.0°C m^{-1}. During July, 3 thermoclines were oberved; water temperature dropped from 29°C at 10 m to 27°C at 11 m @ 2.0°C m^{-1}, from 24°C at 26 m to 23°C at 27 m and from 23°C at 28 m to 22°C at 29 m showing a decline @ 1.0°C m^{-1}.

Water always remained alkaline in reaction with surface pH varying from 7.1 to 8.3 (Table 1). The narrow range of fluctuation in pH from7.8 to 8.2 in lentic zone indicated a strong buffering capacity of the reservoir. Free carbon dioxide varied from nil to 4 ppm. It was absent during May-June in lotic and intermediate zones and during March-June in lentic zone. Total alkalinity was high, ranging between 48 and 96 ppm, with higher values in summer and lower values in post monsoon months (Table 1).

Dissolved oxygen ranged from 8.4 to 9.6 ppm (Av. 9.04 ppm) indicating high rate of photosynthetic activity in the reservoir. It showed peaks in September and March-April. The distribution of dissolved oxygen at different depths showed an almost uniform distribution, difference in concentration never exceeding 1 ppm except May to July. The decline in oxygen in lentic zone from 9.4 ppm at surface to 7.6 ppm at 30 m indicated the presence of a weak oxycline in this zone during summer (Table 2).

The nutrient status of the reservoir with respect to phosphate was optimum. Its value varied from 0.10 to 0.24 ppm. The increase in values of phosphate with the onset of monsoon may be due to enriched water received from the

Table 2. Major physico-chemical features at various depths of Pong reservoir

May 1992						July 1992					
Depth (m)	Temp. (°C)	pH	Dissolved oxygen (ppm)	Total alkalinity (ppm)	Specific conductivity (μmhos/cm)	Depth (m)	Temp. (°C)	pH	Dissolved oxygen (ppm)	Total alkaline (ppm)	Specific conductivity (μmhos/cm)
S	27.5	7.9	9.4	94	193.2	S	29.5	7.9	8.4	66	156.3
2	27	7.8	9.4	94	192.3	2	29.5	7.8	8.4	66	156.1
4	27	7.8	9.2	88	191.6	4	29.5	7.8	8.4	66	155.9
6	26	7.8	9.2	88	190.3	6	29	7.8	8.4	66	155.2
8	25	7.7	9.0	88	189.2	8	29	7.8	8.4	66	154.7
9	24	7.7	8.8	84	187.1	10	29	7.8	8.4	66	154.1
10	24	7.6	8.8	84	187.1	11	27	7.5	8.2	68	150.8
11	23.5	7.6	8.8	84	185.3	12	27	7.4	8.0	68	148.2
12	23.5	7.6	8.8	84	185.3	14	26	7.4	8.0	68	147.8
14	23	7.6	8.6	82	183.6	16	25	7.4	8.0	68	147.3
16	22	7.5	8.6	78	179.8	18	25	7.4	8.0	70	146.9
18	22	7.5	8.4	78	177.9	20	24	7.3	7.8	70	146.8
20	21	7.4	8.2	78	173.7	22	24	7.3	7.8	70	146.1
22	20.5	7.4	8.2	72	170.5	24	24	7.3	7.8	70	145.7
24	20	7.4	8.0	72	168.3	26	24	7.3	7.8	72	145.3
26	19	7.4	7.8	66	164.4	27	23	7.2	7.6	72	144.9
28	19	7.4	7.8	66	162.2	28	23	7.2	7.6	72	144.2
30	18	7.4	7.6	66	158.4	29	22	7.0	7.4	72	144.0
						30	22	6.9	7.2	72	143.8

catchment area. Organic matter ranged from 2.2 to 4.8 ppm with an average of 3.9 ppm. Values of dissolved organic matter more than 1 ppm are generally considered to be indicative of high production potential. The specific conductivity varied between 96.4 to 195 μmhos/cm^{-1}. It was, in general, high in lentic and low in lotic zones (Table1). No specific seasonal trend was noted.

Chemical stratification

Depth studies showed a clear indication of biogenic chemical stratification, an index of the productivity of the reservoir, in respect of oxygen, pH, total alkalinity and specific conductivity in May and July (Table 2). The value of dissolved oxygen was high, at 9.4 ppm in surface and dropped to 7.6 ppm at 30 m depth, in May. pH with 7.9 at surface and 6.91 at 30 m depth showed narrow range of its variation. The values of specific conductivity increased with depth, the maximum variation was observed in October, ranging from 144.67 at surface to 173.61 umhos/cm^{-1} at 30 m depth. The stratification of specific conductivity, however, indicated reverse trend during April-July, with values ranging from 193.2 in surface to 158.4 μmhos/cm^{-1} at 30 m depth in May.

Table 3. Monthly variations in primary carbon production (mg C/m^2/day)

Months	Gross production	Net production
August, 91	281.16	187.44
September	375.0	249.96
October	468.72	281.16
November	468.72	249.96
December	499.92	296.88
January, 92	515.52	296.88
February	499.92	312.48
March	531.24	281.16
April	468.72	218.64
May	468.72	218.64
June	437.40	249.96
July	437.40	234.24

Primary productivity

The data of net and gross production based on surface and sub-surface samplings of lentic zone have been given in Table 3. Gross primary production ranged from 281.16 to 531.24 mg C/m^2/ day and net production varied from 187.44 to 312.48 mg C/m^2/ day. The over all values of net production were high during winter and low during monsoon month. The average gross production during the year was 0.454 g C/m^2/ day. The total annual production is thus calculated to be 165.84 g C/m^2/ year or 93725 tonnes/year for the entire reservoir.

Depth-wise analysis indicated decrease in productivity from surface to sub-surface. Gross and net production were more in littoral regions.

DISCUSSION

It is apparent from the observations that the inflow into the reservoir was the most important factor which had influenced the ecology. It primarily altered the depth of

reservoir which is an important influencing factor on the productivity of lakes (Ryder *et al.* 1974). It has been observed that the productivity of lakes is governed by the effect of replenishment of water also. The reservoir level in Pong reservoir fluctuated by 13.41 m mainly due to draw down of 7800 million m^3. The frequent floods, common in the area, also affected the growth of phytoplankton in the photosynthetic zone repeatively within the year in turn the productivity of the reservoir. Similar observations have also been presented by Singh and Desai (1980).

The basin soil is alkaline and contains low values of organic carbon (0.24 to 0.57%) and available nitrogen. The basin soil thus appeared to have very little effect on the quality of water as the later mainly derived its nutrients from the catchment.

Total alkalinity (48 to 96 ppm), temperature (13 to 31°C), pH (7.1 to 8.3) and specific conductivity (96.4 to 195.8 $\mu mhos/cm^{-1}$) shows that Pong reservoir is medium productive. The total alkalinity above 90 ppm and specific conductivity above 200 $\mu mhos/cm^{-1}$ considered to indicate high fish productivity. Mathew (1975), however, reported eutrophic status of Govindgarh on the basis of limnological features other than alkalinity which was 40 ppm. The nutrient enrichment of the reservoir was ensured by the allochthonous inputs from the catchment area. A significant increase in the values of organic matter and phosphate in October is attributed to the allochthonous input into the reservoir.

The impoundment presents thermal stratification in summer months. A narrow thermocline was formed in colum region, between 8-9 m, in May and three thermoclines in July. However, the differences between the surface and bottom temperatures are not of that magnitude as in the temperate regions. Thermal stratifocation in reservoirs has been reported by Srinivasan (1971); Pathak (1979); Gupta (1984) and Kaushal *et al.* (1984). The reservoir exhibited a clinograde oxygen curve during summer while in other seasons there was a near uniforum distribution of oxygen. The slow decline in oxygen limiting to 1.8 ppm in May could be due to low oxidation of organic matter in the sediments. Konar and Tilaiya reservoirs (Anon 1970), despite the presence of thermal stratification, also did not show much variation in oxygen from surface to bottom. The high bottom temperature prevailing at the bottom of tropical lakes accelerates the rate of chemical reactions and hence consumption of oxygen is more than the temperate reservoirs (Ruttner 1953). Biogenic-chemicla stratification in respect of total alkalinity and specific conductivity also indicated productive character of the reservoir. The stratification of specific conductivity having low values on the surface and high values at the bottom continued up to March but indicated reverse trend during the months of April-July. This occurrence of reverse stratification of specific conductivity could be due to nonmixing of reservoir water with the influx of cold snow melt starting from the month of April, forming different layers of water in the reservoir.

The average fish production of Pong reservoir (August, 1991 to July, 1992) was 33.07 kg/ha/year and average carbon production 93725 tonnes or 6248 kg/ha/year. Odum (1960) stated that a harvest of 1.2% of primary production as fish would be excellent. Carbon values divided by 0.44 (Waldickuk 1958) indicated total plankton biomass in this reservoir as 213011 tonnes. Considering that 100 kg of dry plankton yield 1 kg of fish the harvest from pong reservoir is expected to be 2130 tonnes. The annual yield in terms of carbon was thus calculated as 142 kg/ha/year. In Pong reservoir, the conversion ratio was 0.23%. In Nagarjunasagar, the conversion ratio was 0.24%. Srinivasan (1966) found a ratio of 0.1% for Stanley reservoir and Mathew (1975) reported a meagre ratio of 0.07% in Govindgarh lake.

The delineation of limno-chemical parameters as given in foregoing paragraphs tend to suggest that Pong reservoir is medium productive. The present yield 33.07 kg/ha/year, from the reservoir could be due to inadequate stocking, nonutilization of certain food niches and limited spawning habitat. The fish production in the reservoir is also affected by the dominance of cat fishes like *Mystus seenghala* and *Wallago attu* which feed at higher trophic levels and consequently result in a greater loss of energy stored at primary producer level. It is therefore essential to stock the reservoir with fishes which feed at lower chains for greater utilization of energy and increasing the yield.

ACKNOWLEDGEMENTS

The authors are indebted to Dr A G Jhingran, former Director and Dr V R Desai. Director, Central Inland Capture fisheries Research Institute, Barrackpore, for encouragement.

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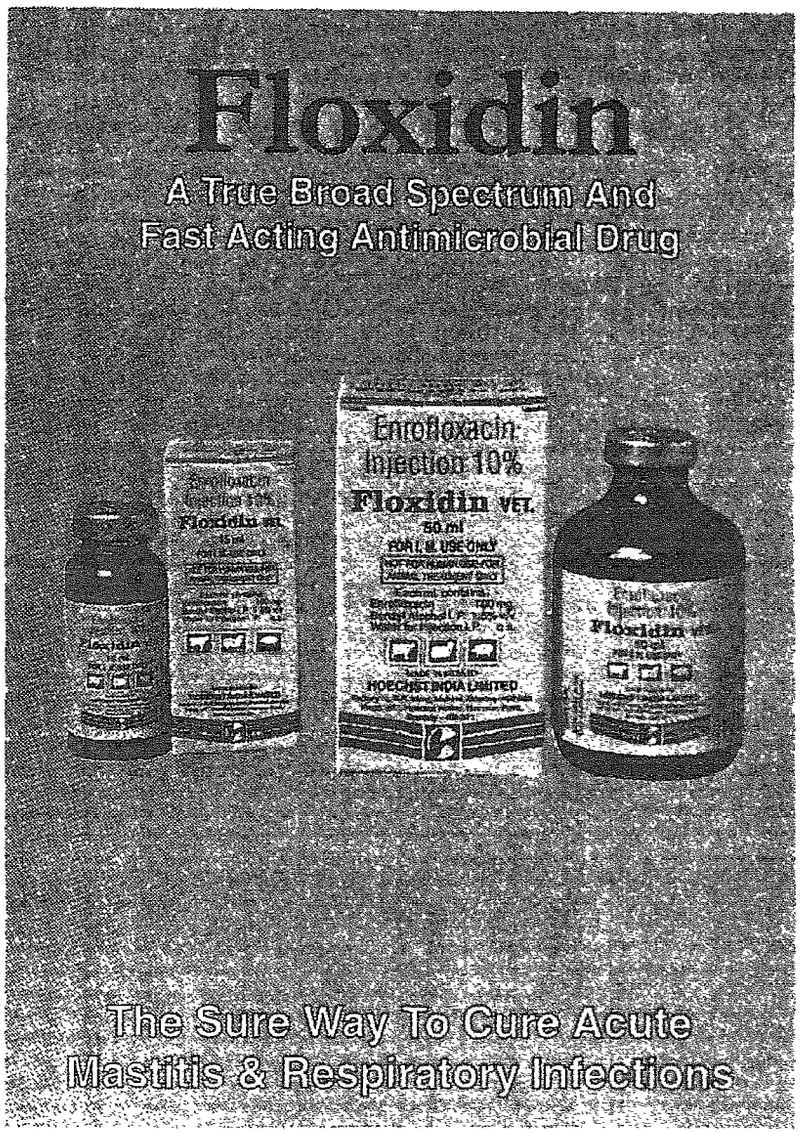
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Restriction profile studies on local isolates of IBD virus • Pathogenic attributes of *Escherichia coli* isolates from free-ranging sambars (*Cervus unicolor*) • Pathology of Ochratoxicosis in quails • Characterization of *Escherichia coli* derived from cattle milk samples using random amplification of polymorphic DNA analysis • Fowlpox in the trachea of laying hens • Incidence of retention of placenta with special reference to subsequent repdoructive performance in Frieswal cows • Evaluation of direct and correlated responses to family index selection for part period egg production in egg type chicken • Some unusual findings in goats fed *Coprinus fimetarius* treated paddy straw • Thyroid hormone levels during reproduction, development of embryo and juvenile in Indian major carps, *Catla catla* (Ham.) • Changes in thyroid hormones and 5 monodeiodinase activity during embryonic and larval developments in freshwater catfish *Heteropneustes fossilis*

THE INDIAN JOURNAL OF ANIMAL SCIENCES

is indexed in the AGRINDEX, published by the FAO; the *Science Citation Index* and the *Current Contents* (Agriculture, Biology and Environmental Sciences), published by the Institute of Scientific Information, Philadelphia. It is also indexed in the abstracting journals of the CAB International as well as all the major abstracting services of the world.

Published by Shri A Chakravarty, Director (Directorate of Information and Publications of Agriculture), Indian Council of Agricultural Research, Krishi Anusandhan Bhavan, Pusa, New Delhi 110 012; Laser typeset at Xpedite Computer Systems, A-1, Ground Floor, Pandav Nagar, New Delhi 110 008; and printed in India at the Bhagwati Printers Pvt. Ltd., B-233, Naraina, Phase-I, New Delhi 110 028.