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Haemagglutinating activity of canine parvovirus

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ABSTRACT

Haemagglutination is an important characteristic of non-defective parvovirus. The HA test is simple, convenient and comparatively more accurate. The present study was aimed to standardize the HA test using erythrocytes of different species, and to analyze the effect of different incubation temperatures, buffers and pH on the HA reaction. The porcine erythrocytes when used at an incubation temperature of 4°C gave best results. Highest HA titer was obtained using PBS , PBSS and PBS with 0.1% BSA as diluent in the pH range of 4-6. Also its specificity was ascertained by haemagglutination inhibition test and a HI titer of 3120 was obtained. Using the standardized reagents, HA test was used to detect CPV in the fecal samples from clinical cases and experimentally infected pup.

Key words: Canine, CPV, Haemagglutinating activity, CRFK, Parvovirus

Canine parvovirus (CPV) enteritis, a highly contagious and often fatal disease of dogs, is caused by CPV-2, which is believed to be a genetic mutant of feline panleukopenia virus (Kramer *et al.* 1980). The disease is characterized by 2 prominent clinical forms (i) enteritis with vomition and diarrhoea in dogs of all ages, (ii) myocarditis and subsequent heart failure in pups of less than 3 months of age (Carmichael *et al.* 1980).

The haemagglutination (HA) is an important characteristic of non-defective parvovirus. The HA is simple and rapid test for detecting CPV in faeces (Carmichael *et al.* 1980). The HA by CPV was reported by several workers (Appel *et al.* 1979, Johnson *et al.* 1979, Gagnon and Povey 1979). This test was performed using erythrocytes of porcine (Carmichael *et al.* 1980), rhesus monkey (Burtonbuoy *et al.* 1979) and African green monkey (Gagnon and Povey 1979). It was reported by Senda *et al.* (1986) that erythrocytes from other species such as horse, shrew, mouse, hamster, cat, sheep and dog can be used for this test after certain modifications. Formalin fixed erythrocytes extended the useful life of erythrocytes without any adverse influence on specificity and sensitivity of HA (Mathys *et al.* 1983).

The present study was aimed to standardize the HA test for diagnosis of the CPV infection in dogs.

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MATERIALS AND METHODS

The HA activity was studied using the fecal samples from clinical cases (n=15) and the daily faecal sample from experimentally infected pup (0 day to 8 day). The HA activity was also analyzed in the 7 passages of cell culture adapted CPV. The presence of CPV in the different passages was confirmed by polymerase chain reaction assay and hence served as reference virus source. The specificity of HA test was confirmed by performing the haemagglutination inhibition (HI) test.

Faecal samples

Faecal samples (15) from dogs showing symptoms of diarrhoea, anorexia, pyrexia and vomition were collected from the Veterinary College, Pantnagar, and a private veterinary clinic at Haldwani. Besides this, the fecal sample of a mongrel pup inoculated with cell culture adapted CPV was also collected from 0 day till 8 day post inoculation (DPI). The faecal samples were suspended in equal amount of Eagles minimum essential medium (MEM) and filtered through a nylon sieve to remove coarse particles. The samples were then centrifuged at 5 000 rpm for 20 min at 4°C and the supernatant was carefully pipetted out. The supernatant was filtered through millipore filter (pore size 0.45μm) and was used as inocula for isolation and detection of CPV.

Preparation of porcine erythrocytes

The blood was collected from non-descript local breed of pig in equal amount of Alsever's solution and was kept at 4°C overnight. It was then centrifuged at 2 500 rpm for 10

min. The plasma and buffy coat was removed and the erythrocytes were washed 3 times with 0.015 M PBSS, pH 7.0. Thereafter a 1.0% (V/V) suspension of RBC was prepared in PBSS with 0.1% BSA.

Virus as antigen

The isolation and cultivation of canine parvovirus was done in the crandell feline kidney (CRFK) cells procured from National Centre for Cell Science, Pune. The cells were grown in MEM with non-essential amino acids L-glutamine, in Earle's salt and Dulbecco's modified Eagles Medium (DMEM) with L-glutamine and 1000 mg glucose per litre in 50 : 50 ratio supplemented with 10% heat inactivated fetal calf serum. The cell culture adapted CPV was used as antigen for performing the HA test.

The HA test was attempted, taking into account the following parameters:

Erythrocytes: The erythrocytes were obtained by collecting blood from swine, sheep, goat, poultry and human and a 1% V/V suspension was prepared for use.

Temperature: The test was performed by incubating the plate at 4-6°C, 23-26°C and 37°C.

Buffers: Normal saline solution (0.9% NaCl), phosphate buffer solution (0.015M), phosphate buffer solution with BSA (0.015 M PBS+0.1% BSA) and phosphate buffer saline solution (0.015% PBS+PBS+0.9%NaCl) were used as different buffers.

HA test

The test was performed in 'U' bottomed microtiter plates, as per Carmichael *et al.* (1980). A 2-fold serial dilution of the test sample starting from 1:2 to 1:4096 was made in ice-cold buffer solution (50µl/well). Then 50µl of 1% pig erythrocytes were added to all the wells. The plate was then incubated at 4°C for 4-6 hr. The HA titer was expressed as the reciprocal of highest dilution of virus showing agglutination.

HI test

The test was performed as per Carmichael *et al.* (1980) with slight modifications. The hyper-immune serum (raised in pups) was inactivated at 56°C for 30 min a water bath. A 1:10 dilution of serum in PBSS was treated with 0.1 parts of 50% pig erythrocytes to remove non-specific haemagglutinins. It was allowed to stand for 2 hr at room temperature and then centrifuged at 1500 rpm. The pellet was discarded and the supernatant serum was subjected to a 2-fold serial dilution in micro titer plate using PBSS as diluent. To 25µl of each serum dilution, an equal volume of CPV antigen containing 4HA units was added and incubated for 2-16 hr at 4°C. The HI titer was expressed as the reciprocal of highest serum dilution inhibiting the haemagglutination reaction.

RESULTS AND DISCUSSION

Out of the 15 faecal samples from clinical cases, only 10 agglutinated porcine erythrocytes. The excretion of the virus in fecal sample of experimentally infected pup started by 3 DPI and continued till 8 DPI the HA title below 1:8 was considered negative. The HA titer in the CRFK cells adapted CPV increased from 256 in the first passage to 2048 in the third passage and then remained constant till seventh passage.

Amongst the erythrocytes of different species, erythrocytes of pig exhibited the characteristic haemagglutination. Erythrocytes from other species did not give agglutination reaction. It is possible that the erythrocytes of species other than pig do not have the receptors that can bind to the virus particles. Similarly Gagnon and Povey (1979), Timbol *et al.* (1983), Celer (1984) and Senda *et al.* (1986) used the porcine erythrocytes for performing the haemagglutination test. On incubating the plate at different temperatures, it was found that the best result (HA titer-2048) was obtained at 4°C followed by incubation temperature of 23°C-26°C (HA titer-1024) and the least HA titer (256) was obtained at 37°C (Table1). Gagnon and Povey (1979) also suggested that incubation temperature of 4°C was optimum for performing the HA test. It can, therefore, be concluded that receptor sites are better available at this temperature, may be due to their configuration.

Table 1. Effect of incubation temperature on HA reaction using different buffers

Incubation temperature	HA Titre		
	PBSS	PBS	PBS with 0.1% BSA
37°C	256	256	256
23°C to 26°C	1024	1024	1024
0°C	2048	2048	2048

The effect of different buffers at different pH was analyzed and it was found that best results were obtained with PBS, PBS with 0.1% BSA and PBSS in a pH range of 4-6 (Table 2). It suggested that slightly acidic pH is favourable for haemagglutination reaction. These buffers were comparable and can be effectively used for haemagglutination test.

Table 2. Effect of pH on HA reaction using different buffers

pH	HA titre		
	PBSS	PBS	PBS with 0.1% BSA
4	1024	1024	1024
5	1024	1024	1024
6	1024	1024	1024
7	1024	1024	512
8	1024	512	256
9	128	128	128

The specificity of haemagglutination reaction can be confirmed by haemagglutination inhibition test (Mathys *et al.* 1983, Veijalainen *et al.* 1986). In the present study, haemagglutination inhibition test was performed to confirm that the haemagglutination reaction was due to the parvovirus. Immune serum raised in dogs against parvovirus inhibited the haemagglutination reaction. The haemagglutination inhibition titer was 3120. Thus the observations of the present study suggest that HA test can be used easily and successfully for the detection of parvovirus in faecal samples of dogs excreting the virus.

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Seasonal histomorphological studies on the thyrotrophs of Gaddi sheep

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ABSTRACT

The cytoplasm i.e. granules of large, round or polyhedral TSH cells stained mildly or intensely with aniline blue, periodic acid Schiff and Gomori's aldehyde fuchsin stains. The number of thyrotrophs increased significantly during winter (210±12.74 cells/mm^2) when compared with summer (126±12.24 cells/mm^2). The average diameter of TSH-cells varied from 9.14±0.11 µm during winter to 8.35±0.20 µm during summer. Its nucleus measured 4.59±0.09 µm during summer but enlarged to 4.86±0.08 µm during winter. This physiological increase in size of the TSH cell and their population during winter might be associated with the increased BMR.

Key words: Animal reproduction, Histomorphology, Sheep, Thyrotrophs, TSH

The thyroid gland secretes thyroxine and triiodothyronine, composed of iodinated amino-acid from the basophilic beta cells in the anterior pituitary gland. Information on the thyroid hormone action on seasonal changes (Thrun *et al.* 1997, Fujita *et al.* 1996) are available. The present study was undertaken to explore the histomorphology and histophysiology of the thyrotrophs in relation to the seasonality in the reproduction of Gaddi sheep of Himalayas.

MATERIALS AND METHODS

Pituitary glands from 44 apparently healthy, adult (around 2.5-4.0 years old), cyclic, non pregnant pleuriparous Gaddi sheep were collected. Immediately after the slaughter of the animal the tissues were preserved in 10% neutral buffered formalin, chilled alcohol and Zenker's solution and processed for routine paraffin embedding (Luna 1968). The sections were cut at 5-7 µ thickness and stained with haematoxylin and eosin (Luna 1968), Crossman's modification of Mallory's triple stain (Crossman 1937), Periodic Acid Schiff reaction (Bancroft and Stevens 1996), and aldehyde fuchsin method (Cameron and Steele 1959). The sagittal sections of adenohypophysis were divided into 6 regions for descriptive purpose and quantitative cytoanalysis, viz. rostro-dorsal, rostro-ventral, mid-dorsal, mid-ventral, caudo-dorsal and caudo-ventral parts. The transverse sections were also divided into 6 regions, viz. dorsoperipheral, mid-peripheral, ventro-peripheral, dorso-medial, mid-medial and ventromedial parts. The total number of TSH-cells was counted (under oil immersion) and converted in per mm^2 from Crossman's modification of Mallory's triple stained sections in 5 randomly selected fields. Values were averaged season-wise. The average diameter of cells and nuclei for atleast 12 cells from each animal was recorded. The data were subjected to Tukey-Krammer multiple comparison test.

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RESULTS AND DISCUSSION

The thyrotrophs were round, polygonal or angular cells with distinct cell boundaries. They tend to appear angular due to the pressure imposed by neighbouring cells. Roy (1970) described these cells as irregularly polyhedral shaped cells while Khan (1995) described these as round elongated or irregular shaped cells in the buffalo pituitary gland. They were commonly located in the rostral, mid-dorsal regions of the pars distalis adenohypophysis. This conforms with the results of Gomez *et al.* (1989) who observed these cells in the anterior area in sagittal sections as an antero-caudodorsal band, and in transverse sections in the ventral and medial regions. They were located deep in the parenchymal cell cords and were not always in close contact with the sinusoids as also reported by Prasad and Singh (1980) in the pars distalis adenohypophysis of non-pregnant buffalo.

The cytoplasmic granules of these cells appeared coarse and clumped in the supranuclear zone. However, in few cells they appeared fine and homogeneously stained throughout the cells. This heterogeneity of the cytoplasmic granulation might be indicative of the functional activity of the different cells. The cytoplasm of these cells stained intensely with the periodic acid Schiff reagent as also reported by Khan (1995) in buffalo. They also stained selectively with Gomori's

aldehyde fuchsin technique, which conforms with the observations of Roy (1970) in buffaloes. In contrast, the thyrotrophs of cattle did not stain specifically with the aldehyde fuchsin (Jubb and McEntee 1955).

The number of the thyrotrophs in the pituitary gland of sheep significantly increased during winter (210±17 cells/mm^2). This result conforms with the observations of Hifny *et al.* (1983) who concluded that the percentage of TSH-cell population were high with highest cellular activity during winter (Table 1). Perez and Alvarez (1997) also recorded a reduced thyroid secretion in the Holstein breeding bull during high environment temperature. The diameter of these cells increased significantly ($P \leq 0.5$) during winter (9.14±0.1185 µm). Khan (1995) measured 8.8±0.28 µm average diameter of these cells in buffaloes, which was larger (10.3µm) in females during the luteal phase and more so in pregnancy (10.6-10.7 µm), when the metabolic rate increases to nourish the embryo.

Table 1. Micromorphometrical parameters of TSH cells of pars distalis adenohypophysis of sheep

Seasons	Total cell count (per mm^2)	Cell diameter (µm)	Nuclear diameter (µm)
Spring	186±18.09^{ab}	8.73±0.11^{ab}	4.64±0.10
Summer	126±12.24^{a}	8.35±0.20^{a}	4.59±0.09
Monsoon	180±13.39^{ab}	8.63±0.17^{ab}	4.68±0.08
Autumn	184±27.03^{ab}	8.86±0.16^{ab}	4.73±0.12
Winter	210±17.74^{b}	9.14±0.11^{b}	4.86±0.08

Values (mean ± SE) bearing different superscript vary significantly ($P \leq 0.05$).

Generally the thyroid glands are more active during winter as indicated by their increased size, histological changes (Breit *et al.* 1998) and elevation of circulating thyroid hormone (T_4 and T_3 levels. In this study the increased activity of the thyrotrophs during winter as evidenced by cell count and diameter, might be due to the increased basal metabolic rate of the animal that is regulated through the positive feed back effect on thyroid gland. This increased basal metabolic rate might help in the regulation of body temperature during the cold winter.

The Gaddi sheep showed seasonality in their reproductive behaviour i.e. autumn is the breeding seasons while summer is anestrous season (Paramasivan 2000). In other seasons data variation was not significant. Karsch *et al.* (1995) postulated that the thyroid hormones are necessary only during a limited period in the late breeding season to promote seasonal reproductive suppression in ewes. Thrun *et al.* (1997) further observed a reduced activity of thyrotrophs as the thyroid hormones were not needed to maintain suppression of the reproductive neuroendocrine axis once the anestrous was established, and also it did not influence the onset of the subsequent breeding season of the ewes.

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Anatomical and correlative biometrical study of head and pituitary gland of Gaddi sheep (*Ovis aries*)

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ABSTRACT

Studies were conducted on 36 heads of adult female pleuriparous Gaddi sheep of 2.5-3.5 years of age. The sheep has long head (cephalic index 66.1) with a prominent nose bridge; the dorsal and ventral length of the head being equal and the cranial cavity almost rectangular and flat. The pituitary was elongated pyriform in shape. Its length, width and thickness were affected by interorbital distance. The skull base length affected length and width while the cranial width affected thickness and width of the pituitary gland. The given regression equation can be utilized to predict the size of the pituitary gland on the basis of known external parameters of head within the safe limits ($P \leq 0.05$).

Key words: Anatomy, Biometry, Head parameters, Pituitary gland, Sheep

Gaddi sheep are the backbone of livestock industry of North-Western Himalayan residents, as it provides apparel and food, besides other animal utilities. Present paper records the gross biometrical parameters of the head and pituitary gland of female Gaddi sheep to find out the mathematical correlation between the external parameters of head and the size (length, width and thickness) of pituitary gland.

MATERIALS AND METHODS

Studies were conducted on 36 heads of normal, cyclic, pleuriparous, nonpregnant Gaddi sheep of 2.5-3.5 years of age. The heads were collected from the local abattoir immediately after sacrifice for another study (Paramasivan 2000). The gross parameters on the head, viz. head length, head width, facial length , cranial length, cranial width, cranial height, head height, skull base length and interorbital distance were recorded with the help of a meter scale and unstretchable (nylon-Ethicon) thread (Archana 1997). The skulls were then carefully opened and the pituitary was dissected out. The length (anteroposterior), width (laterolateral) and thickness (dorsoventral) of the pituitary gland were measured by Vernier callipers. The data recorded on each animal was statistically analyzed to find out the correlation and regression coefficient (Snedecor and Cochran 1997) between the different external (head) parameters and the pituitary parameters.

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RESULTS AND DISCUSSION

The head of the female Gaddi sheep examined from the anterodorsal aspect, appeared long and narrow rostrally and broad caudally. It was widest at the level of the caudal margin of the orbit. It narrowed down towards anterior margin abruptly and then gradually up to the level of anguli oris. Hence, it took an ovoid/spheroidal shape at rostrum. The integument here was non wooly covered with only hair.

Table 1. Biometry of head and pituitary gland of Gaddi sheep (n=36)

Parameters	Mean±SE (cm)	Range (cm)	CV%
Head length (X_1)	24.8±1.68	20.5-27.5	06.92
Head width (X_2)	16.4±0.85	15.0-18.0	05.37
Facial length (x)	13.4±1.62	08.5-15.5	12.35
Cranial length (x)	11.5±1.10	10.0-14.5	09.87
Cranial width (X_5)	11.3±0.95	09.5-13.0	08.65
Cranial height (x)	09.7±1.05	08.5-12.5	11.13
Head height (x)	11.5±1.38	09.0-16.5	12.29
Skull base length (X_3)	24.5±1.53	21.5-28.0	06.38
Interorbital distance (X_4)	11.1±0.64	09.5-12.0	05.93
Pituitary			
Length (Y_1)	1.44±0.18	1.12-1.91	12.90
Width (Y_2)	1.01±0.12	0.75-1.18	12.43
Thickness (Y_3)	0.96±0.23	0.69-1.31	24.06

x Correlation coefficient with any of the parameters of the pituitary gland were not significant. X_1-X_5 were significantly correlated and therefore were used to predict various (Y_1-Y_3) pituitary parameters.

Caudal to the supraorbital process it became pyramidal with flat top along the occiput. The integument here was mostly wooly. The orbital margin was quite prominent with the orbit facing rostrolaterad. The head appeared triangular shaped with a rounded apex at the rostrum, when seen from the lateral aspect. The dorsal nasal area was convex. An elongated triangular view was apparent from the ventral aspect also. The integument on the lateral and ventral aspects of the face was covered with ordinary hair; directed ventrocaudally on lateral, and caudally along the ventral surface. The frontonasal junction was present as a depression while the cranial cavity was well marked off behind the level of the orbits. The biometrical measurements on the head are shown in Table 1. It was evident that the head length measured nearly 50% more than width indicating a dolicocephalic (long type) head in sheep with a cephalic index of 66.1% as in cattle, buffalo, camel and yak (Rao 1967, Gupta and Sharma 1990, Gupta *et al* 1991, Singh 1984, Archana 1997). The cranial length was slightly shorter than the facial length. However, the cranial length and cranial width were not much different. The cranial height was shorter than the length and width both. Thus indicating nearly an equidimensional flat shape of the cranium.

The cranial index in sheep was 98.2%. The height of head and the cranial length were similar but little more than the cranial width and the interorbital distance. The skull base length was equal to the head length indicating a typical triangular shape of the head, as seen in profile.

The pituitary gland was pyriform located in the interpeduncular fossa of the brain connected with the tubercinerium by means of its stalk. It was broad anteriorly but blunt and narrow caudally as reported by Paramasivan *et al.* (2001). May (1970), however, described it as irregularly conical in shape. The morphometrical data (Table 1) revealed that the pituitary width length per cent ratio was 73.8% clearly indicating an elongated ovoid shape of the pituitary gland of sheep which was similar to that of goat (Adams *et al.* 1964, Singh 1973 and Khatra and Nanda 1983). Varadin and Ljubica (1984) and Paramasivan (2000) have further indicated variability in the various measurements of the pituitary gland of sheep during different seasons of the year. They further correlated it with the breeding season and anoestrous in these animals.

There was no correlation between head length and pituitary width, cranial length and height and facial length with any of the pituitary parameters (Table 2). Similarly the skull base length bore no significant correlation with the pituitary thickness as also the cranial width with the pituitary length. The pituitary length and width were highly correlated with interorbital distance and skull base length, however, the thickness was correlated with interorbital distance but not with the skull base length. The cranial width also correlated significantly with the pituitary width and thickness. Taking

Table 2. Correlation between the head parameters and the pituitary parameters (n=36)

Parameters	r	R^2	SD	P
Head length vs pituitary length	0.488	0.238	0.165	0.016*
Head length vs pituitary width	0.281	0.079	0.192	0.184 NS
Head length vs pituitary thickness	0.442	0.195	0.217	0.035*
Head width vs pituitary length	0.483	0.233	0.166	0.017*
Head width vs pituitary width	0.349	0.122	0.123	0.095 NS
Head width vs pituitary thickness	0.567	0.323	0.199	0.004**
Cranial height vs pituitary length	0.115	0.013	0.188	0.593 NS
Cranial height vs pituitary width	0.172	0.029	0.129	0.423 NS
Cranial height vs pituitary thickness	0.001	1.872	0.242	0.995 NS
I.O distance vs pituitary length	0.693	0.481	0.137	0.002**
I.O distance vs pituitary width	0.516	0.266	0.133	0.009**
I.O distanc vs pituitary thickness	0.570	0.325	0.199	0.005**
Facial length vs pituitary length	0.289	0.083	0.181	0.172 NS
Facial length vs pituitary width	0.298	0.089	0.126	0.157 NS
Facial length vs pituitary thickness	0.362	0.157	0.222	0.061 NS
Skull base length vs pituitary length	0.571	0.326	0.156	0.003**
Skull base length vs pituitary width	0.539	0.290	0.111	0.007**
Skull base length vs pituitary thickness	0.098	0.009	0.241	0.657 NS
Cranial width vs pituitary length	0.404	0.163	0.173	0.050 NS
Cranial width vs pituitary width	0.451	0.203	0.118	0.027*
Cranial width vs pituitary thickness	0.433	0.188	0.218	0.039*
Cranial width vs pituitary length	0.284	0.081	0.182	0.179 NS
Cranial width vs pituitary width	0.251	0.063	0.128	0.237 NS
Cranial width vs pituitary thickness	0.090	0.008	0.241	0.684 NS

I.O Distance-Interorbital distane, *P≤0.05, **P≤0.01, NS, nonsignificnat.

Table 3. Regression equations for the prediction of pituitary (Y_1, Y_2 and Y_3) parameters from the known parameters of skull (X_1, X_2 X_3 X_4 and X_5)

Head parameters	Correlation coefficient r	Regression	Calculated F value	R^2
Pituitary length (Y_1)				
Head length (X_1)	0.4877**	(0.1285+0.0526X)	0.6860	0.23787
Head width (X_2)	0.4828*	(-0.2322+0.1018X)	0.6.680	0.2331
Skull base length (X_3)	0.5713**	(-0.2236+0.0679X)	10.662	0.3264
Interorbital distance (X_4)	0.6932**	(-0.7305+0.1949X)	20.350	0.4806
Pituitary width (Y_2)				
Interorbital distance (X_4)	0.5157**	(-0.0980+0.1008X)	07.969	0.2659
Skull base length (X_3)	0.5387**	(-0.0650+0.0444X)	08.993	0.2902
Cranial width (X_5)	0.4512*	(0.3495+0.0549X)	05.624	0.2036
Pituitary thickness (Y_3)				
Head length (X_1)	0.4418*	(-0.5248+0.0597X)	05.094	0.1952
Head width (X_2)	0.5686**	(-1.4880+0.1497X)	10.036	0.3234
Interorbital distance (X_4)	0.5700**	(-1.3090+0.2037X)	10.106	0.3249
Cranial width (X_5)	0.4332*	(-0.2343+0.1063X)	0.4852	0.1877

*$P \leq 0.05$, **≤ 0.01, N=36, df=2.

into account the significantly correlated parameters of the head, a functional regression between the external parameters of the head and that of the pituitary was calculated. The regression equations so computed have been summarized in Table 3. The F values as also the R^2 were significant. Therefore it is concluded that the given regression equations can be utilized to determine the length, width and thickness of the pituitary gland within the prescribed (5% probability) safe limits using the external parameters of head in Gaddi sheep.

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Histopathological study after selenium toxicity in buffalo calves

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ABSTRACT

Subacute selenosis was experimentally induced in 6 buffalo calves by oral administration of selenourea @ 0.75 mg/kg body weight. The calves died within 25 days. Clinical symptoms observed were loss of appetite and body weight; alopecia on tail and feet of animals. Congestion, inflammatory, haemorrhagic and necrotic changes in kidney, liver, lungs, skin, heart, duodenum, rumen, spleen, prescapular lymphnode and cerebrum were observed.

Key words: Buffalo, Histopathology, Selenosis

Selenium (Se), a component of the enzyme glutathione peroxidase, is an antioxidant as that of vitamin E (Allway 1973). The selenium and vitamin E are synergic in action decrease lipid peroxidation and thus protects damage to cellular component.

Selenium prevents muscular dystrophy in calves and lambs, reproductive disorder in cows and hepatosis dietetica in growing pigs (Shamberger 1983), but the clinical evidence of Se deficiency has not yet been recorded in India. On the contrary Se toxicity has been reported in dairy animals in Haryana (Arora *et al.* 1975) and in Punjab (Gupta *et al.* 1982 and Dhillon and Dhillon 1991).

However, the systemic histomorphological study after subacute selenosis is meagre in buffalo calves. Therefore, the present study was undertaken to elucidate the histomorphological changes on various organs of buffalo calves after experimental subacute selenosis.

MATERIALS AND METHODS

Non descript male buffalo calves (9), 5-8 months of age, weighing between 48-102 kg, were kept under hygienic conditions for 15 days prior to the commencement of study. The animals were fed with berseem and provided water *ad lib.* The animals were dewormed by using albendazole bolus 10 days before experimentation. Selenium as selenourea was used to produce experimental toxicosis. The buffalo calves were divided into 2 groups, viz. control calves (3) and subacute selenosis calves (6). Selenium toxicity was produced experimentally in 6 male buffalo calves by administration of selenourea @ of 0.75 mg/kg body weight (providing 0.48 Se mg/kg body weight). The Se salt was dissolved in 100 ml of tap water and drenched to the animals in the morning for 30 days or till death. Tissue samples were collected from kidney, liver, lungs, skin, heart, intestine, spleen, lymphnode, brain (cerebrum) and fixed in neutral buffered formalin for histomorphological studies.

After complete fixation the tissues were processed for paraffin blocks by cedar wood oil schedule (Luna 1968) to get 5µm thick sections on glass slides. The sections were stained with haematoxylin and eosin.

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RESULTS AND DISCUSSION

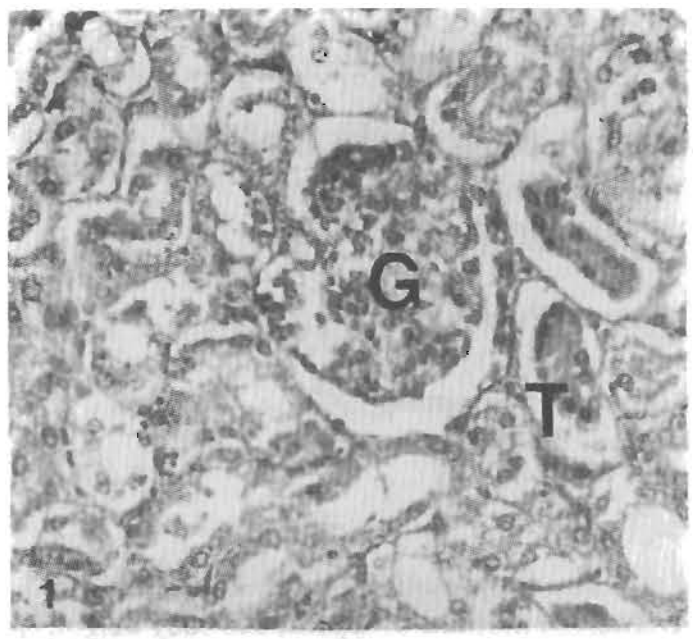
Clinical symptoms

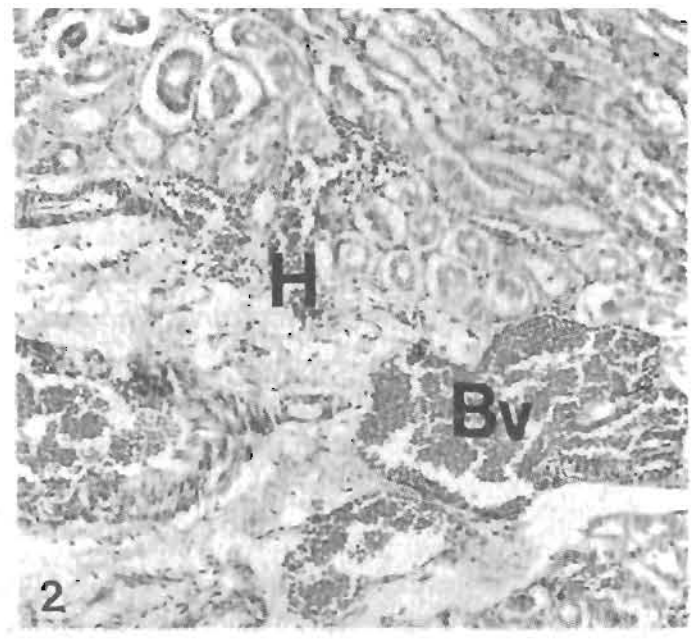
All the 6 buffalo calves died within 25 days. The clinical symptoms observed were loss of appetite and body weight, alopecia particularly from tail and feet. Sub normal body temperature and mild convulsions were observed. Few animals showed cynotic conjunctivitis with watery discharge without any blindness (Clark 1993). Additional symptoms were polydipsia, polyurea, difficult respiration, watery diarrhoea and frothing from nostrils. The symptoms observed in subacute selenosis were almost identical to the report of Kumar *et al.* (1987).

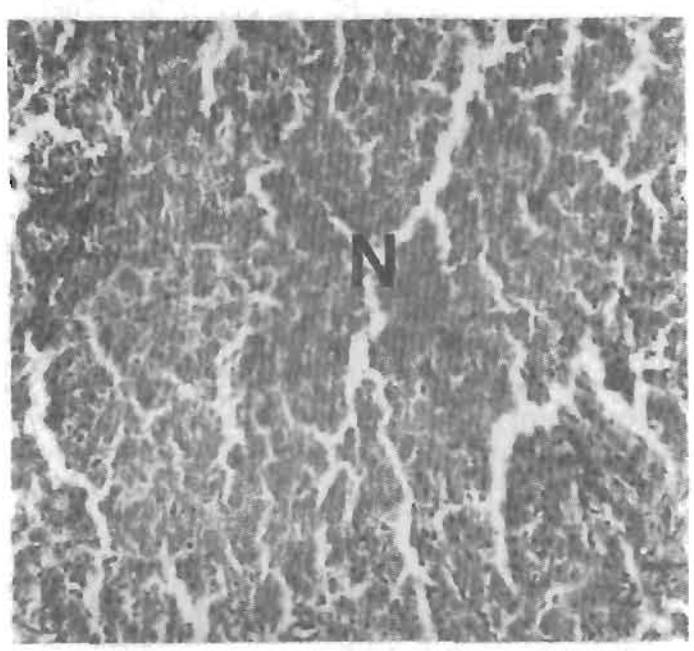
On postmortem examination kidneys, liver and spleen showed haemorrhagic and necrotic foci. Lungs were pale and oedematous. Gastrointestinal tract showed catarrhal and haemorrhagic enteritis Haemonlogic spot were observed on epicardium interventricular septum and endocardium. No lesions were observed in brain of buffalo calves.

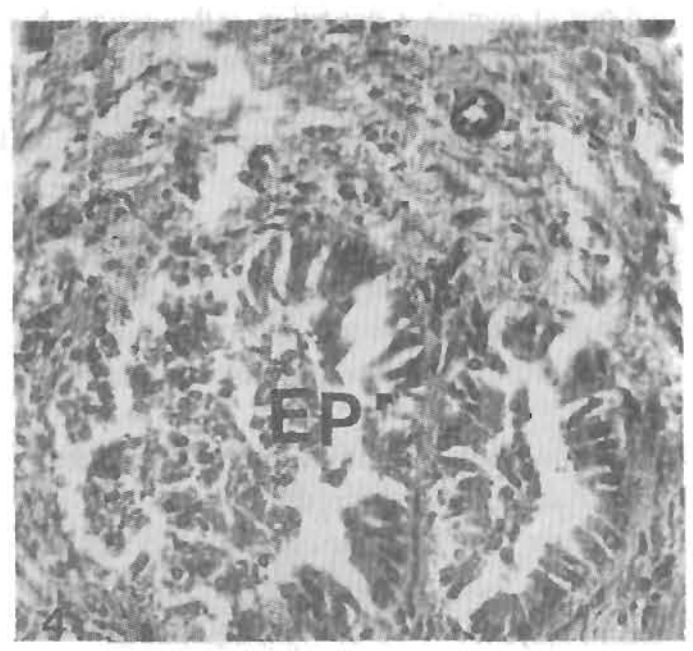
The histopathological changes observed during subacute selenosis are discussed here.

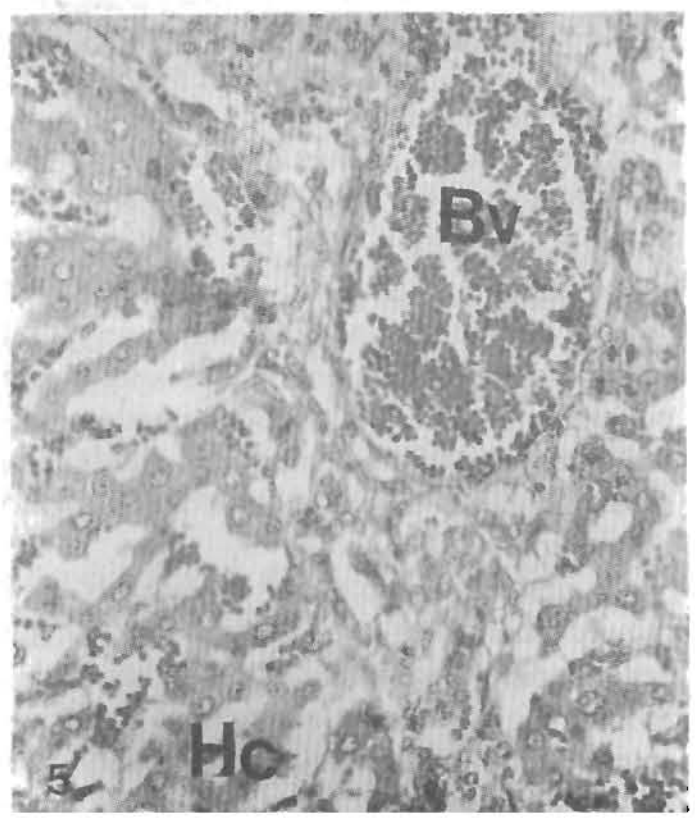
Kidney: The cortex and medulla of kidneys showed severe venous congestion and haemorrhage (Fig. 2). There was

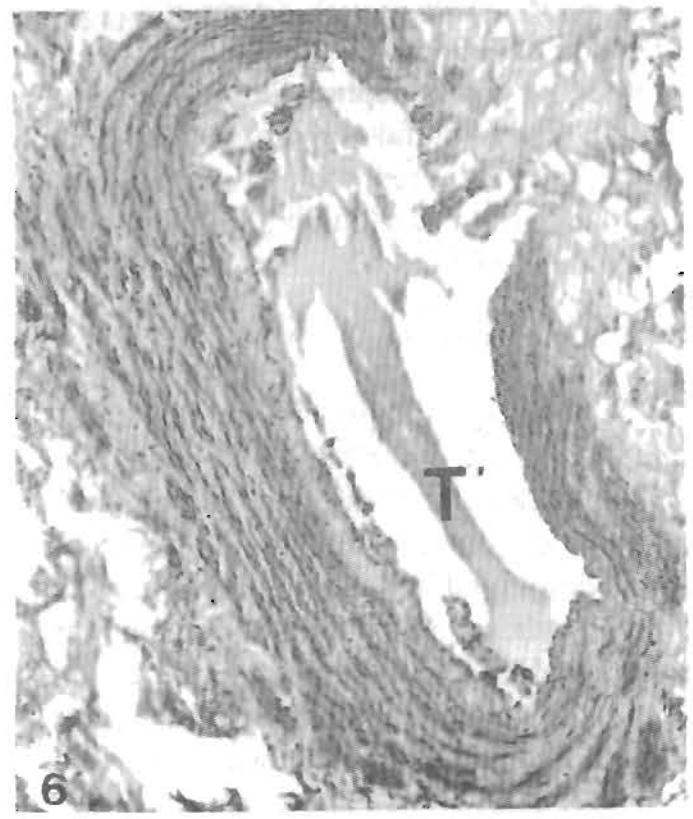




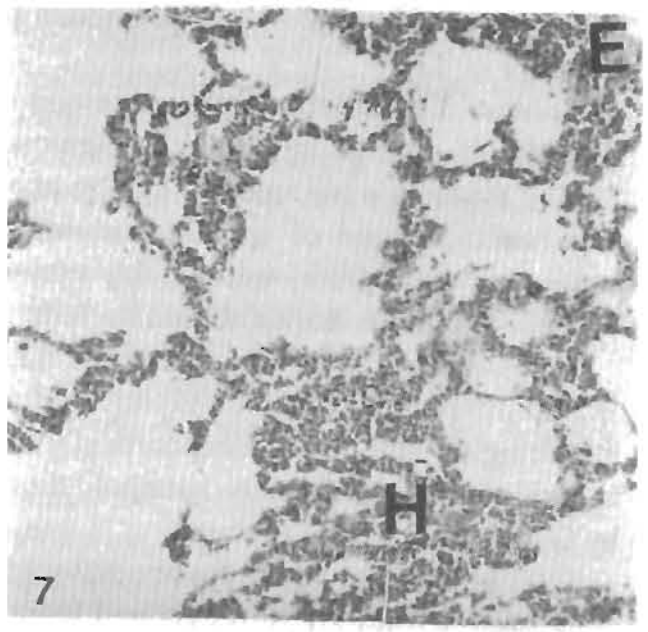


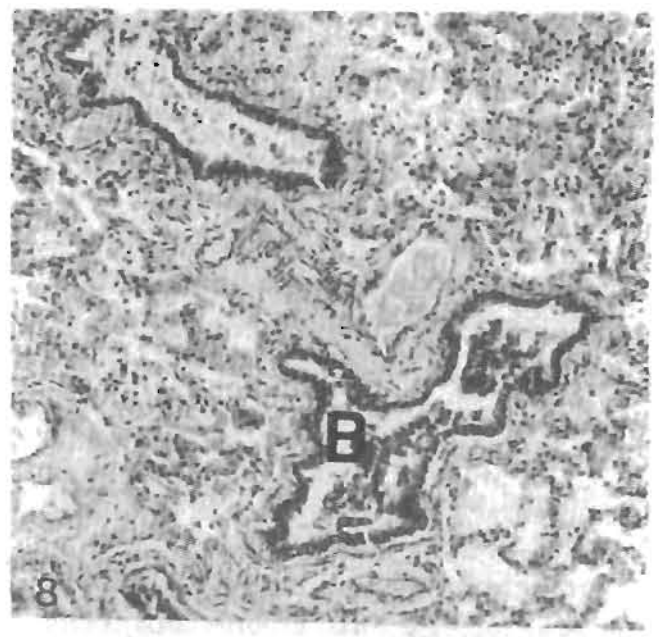


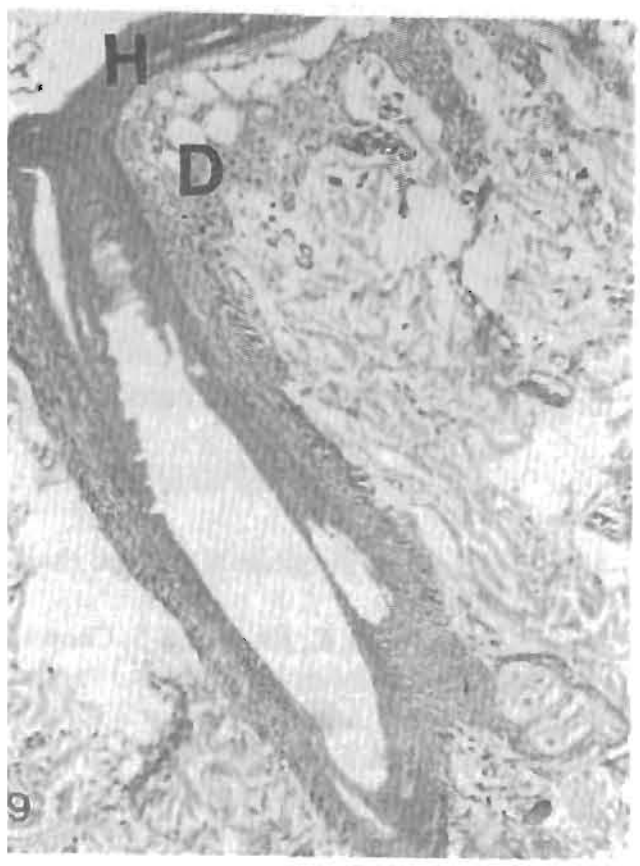


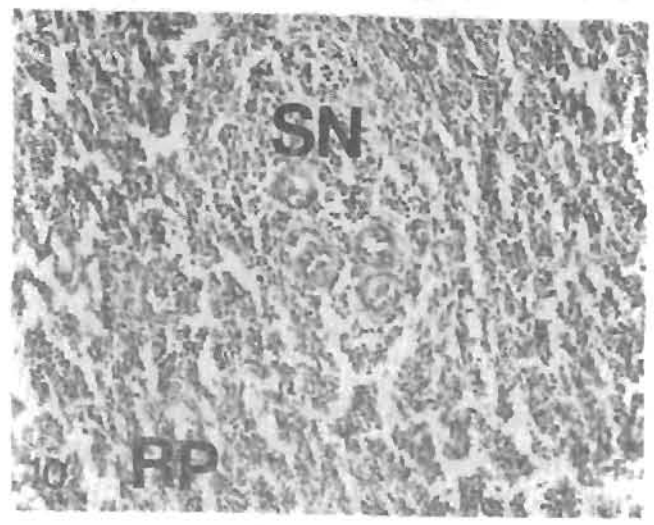


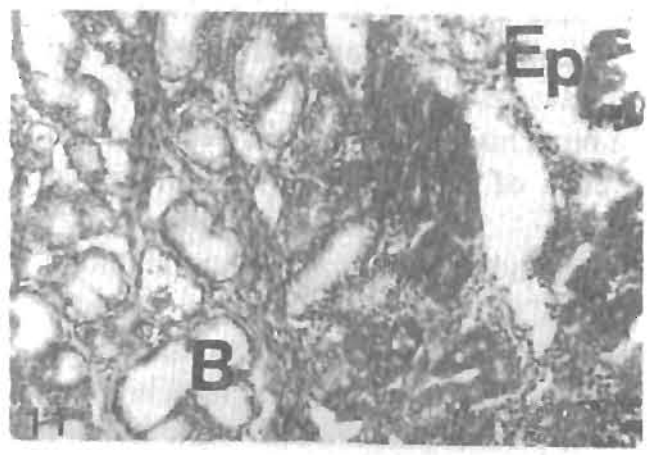
Figs 1-6. 1. Kidney showing congestion and haemorrhages in glomeruli (G) and desquamated and degenerated tubular epithelium (T). Haematoxylin and eosin × 280. **2.** Kidney showing congestion of blood vessels (Bv), haemorrhage (H) in glomeruli and degeneration of tubular epithelium. H&E × 140. **3.** Liver showing necrosed area (N) surrounded by inflammatory reaction. H&E × 140. **4.** Section of liver showing proliferation and exfoliation of epithelium (EP) of bile ducule. H&E ×280. **5.** Liver showing congestion of blood vessel (Bv), haemorrhage and mild degeneration of hepatocyte (Hc). H&E × 280. **6.** Artery with inital stage of thombus formation (T). H&E × 140.

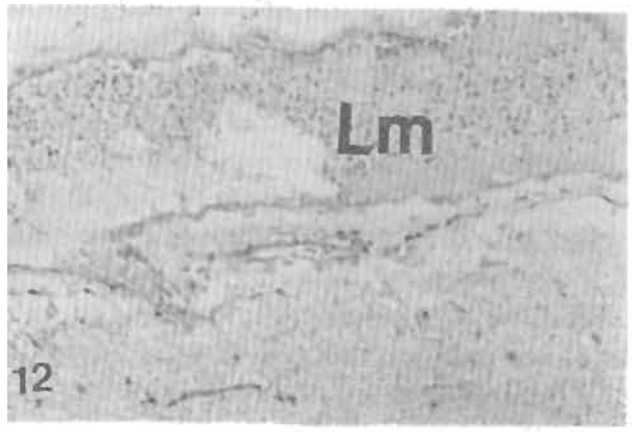


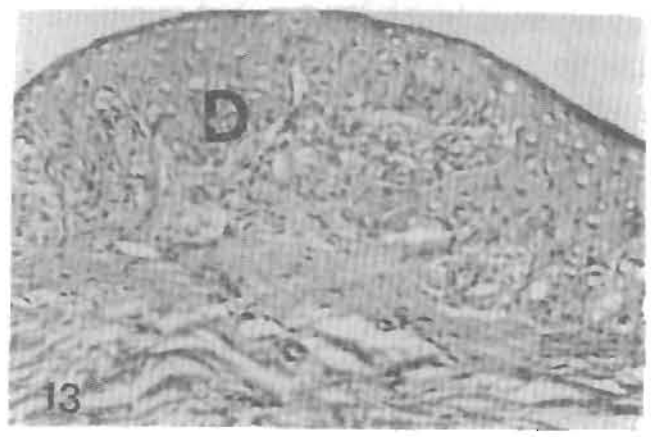












Figs 7-13 **7.** Lungs showing severe congestion and interstitial haemorrhage (H) and eden) H&E × 140. **8.** Lungs showing epithelial desquamation of bronchiole (B), congestion and interstitial edema. H&E × 140. **9.** Skin showing vacuolar degeneration of epithelium (D), hyperkeratosis of epithelium (H), subepidermis tissue showing odema and disruption H&E × 280. **10.**Spleen showing degeneration and atrophy of splenic nodule (SN) and red pulp (RP). H&E × 140. **11.** Duodenum showing desqumation of epithelium (Ep) and degeneration of Brunner's glands (B). H&E ×140. **12.** Cerebrum showing congestion and haemorrhage in leptomeninges (Lm). H&E ×140. **13.** Rumen showing vacuolar degeneration of epithelium (D). H&E ×70.

marked atrophy and necrosis of glomeruli. At location there was fibrosis seen close to degenerated glomeruli. Both proximal and distal convulated tubules showed various stages of degeneration. There was hyalinisation, necrosis and desquamation of tubular epithelium (Fig. 1). Shortridge *et al.* (1971) also reported similar changes in kidneys of lamb and cattle respectively. These changes may be due to excessive accumulation of selenium in kidney. Infilteration of neutrophils, plasma cells and macrophages were seen in the interlobular stroma. Similar findings were observed by Rattandeep (1999) in buffalo calves.

Liver: The liver showed congestion, haemorrhage and disorganisation of hepatic lobules (Fig.5). The hepatocyte showed vacuolar degeneration with a karyorrhexis and karyolysis. There was focal aggregation of inflammatory cells and dilatation of hepatic sinusoids with proliferation of Kupffer cells (Fig.3). Similar were the reports of Rosenfeld and Beath (1946) and Caravaggi *et al.* (1970) in lambs. There was proliferation of arterial wall with thrombus formation (Fig.6) as reported earlier by Kalra *et al.* (1977) in dairy animals. This may be due to degenerative changes involved in the wall of blood vessels (Dhillon *et al.* 1990). Proliferation and exfoliation of epithelium of bile ductuli were also seen (Fig. 4).

Lungs: The lungs showed severe venous congestion and haemorrhage (Fig. 7). There was fibrinous exudate in septal tissue and transudate in alveoli and in interlobular and subplural tissue. The lung appeared as solid mass without any evidence of lung alveoli (Fig.8). These findings concurred with the reports of Caravaggi *et al.* (1970) and James *et al.* (1982) in sheep. The bronchial epithelium was necrosed and exfoliated. The pleura of lungs were very thickened (Rattandeep 1999).

Skin: The skin of legs and dorsum showed hyperkeratosis (Fig.9). The stratified squamous epithelium showed karyorrhexis and karyolysis (Verma 1995). The number of sebaceous gland decreased. The majority of hair follicles were devoid of hair shaft. The parenchyma of sweat gland showed mild hypertrophy as reported by Mensink *et al.* (1990) in piglets after selenium toxicity.

Heart: The cardiac muscle fibre and purkinje fibre showed mild degeneration. Some congestion and haemorrhage in between the cardiac muscle fibres were also noticed. Gardinear (1996) and Verma (1995) reported same histopathological lesions, but Gardinear (1996) also reported the atrophy of heart muscles in sheep.

Duodenum: The duodenal villi showed mild congestion, haemorrhage and cellular infiltration. There was marked necrosis and exfoliation of intestinal mucosa and duodenal glands showed marked degenerative changes (Fig.11). Rosenfeld and Beath (1946) and Shortridge *et al.* (1971) reported similar findings in sheep. The tunica muscularis had varying degree of degeneration with increased stromal tissue.

Rumen: The ruminal epithelium showed the vacuolar degeneration as reported by Rattandeep (1999) in buffalo calves (Fig. 13).

Spleen: The splenic trabeculae were thickened with decrease in number and size of splenic nodule (Fig. 10). Similar findings were reported by Qin *et al.* (1994) in pigs. The reaction zone of splenic nodule was unclear. The connective tissue fibres were thickly arranged. The arteriole wall became much thickened and occluded.

Prescapular lymphnode: Lymphatic nodules degenerated and disintegrated without visible germinal center and intermingled with surrounding cortical tissues. It may be due to toxic effect of selenium on lymphoid tissue as also reported by Glenn *et al.* (1964) in sheep.

Cerebrum: Cerebral cortex showed mild neuronal degeneration with karyorrhexis and karyolysis. There was mild congestion in leptomeninges (Fig. 13). Maag (1960) and Qin *et al.* (1994) observed similar findings with encephalomalacia in cattle and pigs.

It is concluded that oral administration of selenourea @ 0.75mg/kg body weight causes severe damage of body tissues in buffalo calves.

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Effect of FSH-E and FSH-P administration through single or multiple injections on superovulatory response and embryo recovery rate in cattle

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ABSTRACT

Crossbred cattle (Holstein Friesian × Sahiwal, n=18) were superovulated with single or multiple injections of FSH-E and FSH-P. Superovulatory response did not vary significantly between FSH-E and FSH-P either in single or in multiple injection schedules. The large anovulatory follicle on the day of flushing also did not vary significantly between the groups but increased rate of superovulatory response and reduction in large anovulatory follicles was found while using multiple injection schedule of FSH-P. The mean recovery of embryo in group 1-A, group 1-B, group 2-A and group 2-A and group 2-B was 5.8±1.51, 4.75±1.19, 3.0±1.79 and 5.0±2.03, respectively, difference in recovery rate were nonsignificant. Animals injected with FSH-E through single injection (group 1-A) or FSH-P through multiple injections (group 2-B) had better embryo recovery rates.

Key words: Animal reproduction, Embryo recovery, FSH, Superovulation

Superovulation in cattle has been induced with multiple injection (Moore 1975, Danner and Oxender 1980), as well as with single injection of HAP (horse anterior pituitary extract). Total doses of 18 mg of HAP and 36 mg of FSH-P were injected (I/M) over 4 days, 2 injection/day and PG was administered on day 3. Injections were given on days 6 to 13 of the oestrous cycle. The overall ovulation rates (mean ±SE) for HAP and FSH were 8.8±0.7 and 15.1±1.0 respectively (n=231: P<0.01). Donaldson and Lloyd (1991) reported the efficacy of 3 injections at 24 hr intervals using FSH for superovulation in cattle. There are also few reports (Mills *et al.* 1971, Smith Jr. *et al.* 1973, Vincent *et al.* 1973) on efficacy of a single I/M injection of FSH for superovulation. Hell *et al.* (1985) reported that single injection of FSH-P, induced a superovulatory response similar to that of a 5-day, twice daily treatment protocol. In the present study the single and multiple injections of FSH-E and FSH-P were evaluated in relation to superovulatory response and embryo recovery rate in crossbred cattle.

MATERIALS AND METHODS

The animals were selected from the livestock research centre (LRC) of the university. The animal constituted 18 crossbred cattle (Holstein Friesian × Sahiwal) weighing about 300 to 350 kg. All the animals had normal genitalia and had 2 consecutive oestrous cycles of normal health (21±2 days) before the start of the treatments. The treatment groups were divided into following small groups.

Group 1 (FSH-E)

1. Group. 1-A (FSH-E@ 50mg, single dose S/C, n=5)
2. Group. 1-B (FSH-E@ 50mg, 8 divided doses I/M, n=4)

Group 2 (FSH-P)

1. Group. 2-A (FSH-P@ 560mg, single dose S/C, n=5)
2. Group. 2-B (FSH-P@ 560mg, 8 divided dose I/M, n=4)

Luteolysis was induced by using dinoprost tromethamine @ 50mg I/M in 2 split doses of 25mg each at 48hr and 60hr, respectively, after gonadotrophin treatment.

RESULTS AND DISCUSSION

Superovulatory response in the present study following single (group 1-A) and multiple (group 1-B) FSH-E injections was 9.2±1.43 and 7.5±0.75, respectively, (Table 1) while the ovulation rate following single (group 2-A) and multiple (group 2-B) FSH-P injection was 6.4±2.33 and 9.75±1.29, respectively (Table 1). There was no significant difference in the ovulation rate when FSH-E and FSH-P were administered through single S/C or multiple I/M injections. Most workers have reported satisfactory superovulatory response in cattle with, multiple injections of FSH given in decreasing dose schedule (Seidel *et al.* 1978, Yadav *et al.* 1986, Totey *et al.* 1988, Staigmiller *et al.* 1992), increasing dose schedule

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Table 1. Ovarian response in crossbred cows superovulated with FSH-E or FSH-P (single and multiple injection regimen)

Treatment	Animal#	No. of CL			No. of UOF		
		Rt	Lt	Total	Rt	Lt	Total
Group 1-A	1	4	3	7	1	1	2
FSH-E (50mg)	2	2	4	6	1	1	2
Single, s/c injection	3	4	3	7	1	1	2
	4	5	7	12	0	0	0
	5	6	8	14	0	0	0
Mean±SE				9.2±1.43			1.2±0.44
Group 1-B	1	3	3	6	0	1	1
FSH-E (50mg)	2	4	5	9	1	0	1
Multiple, i/m injections	3	3	3	6	1	0	1
	4	6	3	9	0	1	1
Mean±SE				7.5±0.75			1.0±0.0
Group 2-A	1	2	3	5	1	0	1
FSH-P (560mg NIH)	2	7	6	13	1	1	2
Single, s/c injection	3	0	1	1	1	0	1
	4	1	0	1	1	1	2
	5	5	9	12	1	0	1
Mean±SE				6.4±2.33			1.4±0.22
Group 2-B	1	8	6	14	1	0	1
FSH-P (560mg NIH)	2	4	3	7	1	0	1
Multiple, i/m injections	3	4	5	9	1	0	1
	4	5	4	9	0	1	1
Mean±SE				9.75±1.29			1.0±0.0

Rt, Right ovary; Lt, left ovary; CL corpus luteum; UOF, anovulatory follicle (no significant difference between the groups).

(Critser *et al.* 1980) or in a constant dose schedule (Schams *et al.* 1979; Tahira and Hackett 1993). In the present study single S/C injection of FSH is as effective for superovulation in cattle as multiple I/M injection is in agreement with observations of Schalenberger *et al.* (1974) who reported ovulation rate and number of transferable embryos following single S/C and multiple I/M injection of FSH in cows to be 19.8 vs 15.5 and 8.8 vs 8.1 respectively. These findings, therefore, suggest that superovulation could be effectively induced in crossbred cattle with single S/C injection resulting into significant reduction in cost of superovulation in terms of material and time.

The embryo recovery rate in the present study was not significantly influenced by the source of FSH (FSH-E or FSH-P) or the route and frequency of FSH administration. The mean embryo recovery rates in group 1-A, group 1-B, group 2-A and group 2-B were 5.8±1.51, 4.75±1.19, 3.0±1.79 and 5.0±2.03 respectively (Table 2). These rates were similar to that reported by Staigmiller, *et al.* (1992) and were higher than values reported by Agarwal *et al.* (1992) following multiple I/M administration of FSH. The mean embryo recovery rate following superovulation with single S/C injection of FSH-E and FSH-P was 5.8±1.51 and 3.0±1.79, respectively, (Table 2) which were similar to the rate of 3.1±1.0 reported by Lovie *et al.* (1994) but far below the recovery rate of 19.8±5.1 reported by Schalenberger *et al.* (1994) for superovulation with single S/C injection of folltropin.

The mean number of transferable embryos in group 1-A, group 1-B, group 2-A and group 2-B were 4.0±1.62, 2.0±0.79, 2.4±1.43 and 2.25±1.95 respectively. There was no significant difference between the groups indicating that single S/C injection of FSH is as efficacious for superovulation in crossbred cattle as multiple i/m injections. These findings are in agreement to that of Schalenberger *et al.* (1994).

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Table 2. Recovery of embryos/ova in crossbred cows superovulated with FSH-E or FSH-P (single and multiple injection regimen)

Treatment	Animal#	No. of ova/embryos recovered						
		M/CM	EB	B	EXB	UFO	DEG	Total
Group 1-A	1	1	0	0	0	0	1	2
FSH-E (50mg)	2	0	1	0	0	0	1	2
Single, s/c injection	3	1	1	2	0	2	0	6
	4	6	3	1	0	0	0	10
	5	3	0	2	2	1	1	9
Mean±SE								5.8±1.51
Group 1-B	1	0	1	0	0	1	0	2
FSH-E (50mg)	2	2	1	0	0	0	0	3
Multiple, i/m injections	3	1	0	2	0	1	2	6
	4	2	2	2	1	0	1	8
Mean±SE								4.75±1.19
Group 2-A	1	0	0	0	0	0	0	0
FSH-P (560mg NIH)	2	2	1	0	1	0	1	5
Single, s/c injection	3	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0
	5	1	7	0	0	2	0	10
Mean±SE								3.0±1.79
Group 2-B	1	2	6	2	0	2	0	12
FSH-P (560mg NIH)	2	0	0	1	1	1	0	3
Multiple, i/m injections	3	0	0	0	0	2	1	3
	4	0	0	0	0	1	1	2
Mean±SE								5.0±2.03

M/CM. Morula/compact morula; EB, early blastocyst; B, blastocyst; EXB, expanded blastocyst; UFO, unfertilized ova; DEG, degenerated ova/embryo (no significant difference between the groups).

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Effect of buserelin on pregnancy rate in embryo recipient crossbred cattle

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ABSTRACT

Effect of buserelin on pregnancy rate following frozen and fresh embryo transfer was studied at the livestock research station (LRC) and at farmers' door. Embryos (124)—frozen (n=80), fresh (n=44) were transferred to the synchronized recipients non-surgically on day 7 of the heat. The recipient animals were subjected to gonadotrophin releasing hormone (GnRH) treatment on days 3, 5 and 7 post embryo transfer in groups 2, 3 and 4, and group 1 was kept untreated that served as control. Pregnancy rate was significantly higher ($P<0.05$) in group 3 (days 5 post ET) compared to group 1, 2 and 4 both in frozen as well as in fresh embryo transfer but pregnancy rate declined when buserelin was injected on day 3 or day 7 post embryo transfer. Pooled results (frozen + fresh embryo) also yielded significant ($P<0.05$) higher pregnancy rates in the group 3 compared to other group.

Key words: Buserelin, Cattle, Pregnancy, Embryo transfer

Gonadotrophin releasing hormone (GnRH) stimulates anterior pituitary to release gonadotrophin (LH and FSH), which in turn stimulates ovulation, luteinization of ovulated follicle and formation of corpus luteum to secrete progesterone to maintain the conceptus (Hafez 1993).

The effect of buserelin (synthetic GnRH) on conception rate in cattle when injected after insemination is widely proven by several studies. These findings suggested that buserelin improves fertility when administered 11-13 days post-mating. The effect may be explained on the basis of prolonged life span of the corpus luteum, which increases the probabilities of maternal recognition of pregnancy. Thus, the conceptus is protected from early embryonic death and pregnancy can be expected in repeat breeders (Moller Holtkamp 1995).

The hormone status of the cow contributes to the strength of her luteolytic drive during the maternal recognition of pregnancy, so manipulation of this critical period represents an appropriate technique for treating embryo mortality and studies have shown that cows suffering from early embryo loss generally have lower plasma and milk progesterone levels during the period day 10 to day 16 following mating (Lamming *et al.* 1989). These animals may suffer an enhanced luteolytic activity (Lamming and Mann 1993) which could contribute to high rate of embryo loss.

In the present study, GnRH was evaluated in relations to the conception rate following embryo transfer in cattle.

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MATERIALS AND METHODS

Study was conducted at the livestock research centre (LRC) of the university and at farmers' door. Embryos (124: frozen n=80; fresh n=44) were transferred to synchronized recipient cattle non-surgically on day 7 post heat. To evaluate the effect of buserelin on pregnancy rate following embryo transfer, the recipient cows for fresh as well as for frozen embryos were allocated to one of the following 4 experimental groups treated with buserelin (10 Fg, I/M).

Group 1: Control, no buserelin treatment (fresh embryo, n=14; frozen embryo, n =20)

Group 2: Buserelin, day 3 post ET (fresh embryo n=12; frozen embryo, n = 20).

Group 3: Buserelin, day 5 post ET (fresh embryo n=10; frozen embryo, n=20).

Group 4: Buserelin, day 7 post ET (fresh embryo, n=8; frozen embryo, n=20).

Data generated during the study were analyzed by the chi-square test to asses the effect of buserelin on pregnancy rate following embryo transfer.

RESULTS AND DISCUSSION

Our findings indicated that administration of buserelin (10Fg I/M) in recipient cows on day 5 post embryo transfer results in significant increase in pregnancy rate. However, when given on day 3 or 7 post ET it resulted in decreased pregnancy rates (Table 1).

Studies have indicated that administration of buserelin in

Table 1. Effect of buserelin on pregnancy rate in embryo recipient crossbred cattle

Treatment	Recipient (n)	Pregnancy	
		(n)	(%)
Frozen embryos			
Group 1 control	20	5	(25.0)
Group 2 3rd day post ET	20	4	(20.0)
Group 3 5th day post ET	20	7	(35.0)[c]
Group 4 7th day post ET	20	3	(15.0)
	80	19	(23.8)
Fresh embryos			
Group 1 control	14	5	(35.7)
Group 2 3rd day post ET	12	3	(25.0)
Group 3 5th day post ET	10	6	(60.0)[b]
Group 4 7th day post ET	8	2	(25.0)
	44	16	(36.4)
Frozen+fresh embryos			
Group 1 control	34	10	(29.4)
Group 2 3rd day post ET	32	7	(21.9)
Group 3 5th day post ET	30	13	(43.3)[a]
Group 4 7th day post ET	28	5	(17.9)
	124	35	(28.2)

a=P<0.25; b=P<0.30; c=P<0.50.

cows between day 11 and 13 post breeding results in significant increase in pregnancy rate (Elemer 1983, Drew and Peters 1994, Bostedt *et al.* 1995). The use of buserelin is designed to limit the oestradiol production from developing ovarian follicles during the period when it would increase the strength of luteolytic drive in the mated or embryo recipient animal. Moller-Holtkamp (1995) reported that buserelin if injected too early or too late in relation to maternal recognition of pregnancy cannot be expected to be effective in prevention of luteolysis. In the present study the beneficial effect of buserelin was observed when it was injected on day 12 post estrus i.e. day 5 post embryo transfer in recipient cows. This effect was not observed when buserelin was injected 2 days earlier or 2 days later.

Findings of the present study are important in the light of observation of Ellington *et al.* (1991) who has reported a slight increase in pregnancy rate when buserelin was injected on the day of embryo transfer but failed to observe any significant effect of buserelin when administered on day 4 to 7 post ET. Thus, use of buserelin (synthetic GnRH) improves pregnancy rates significantly when injected on day 5 post ET but results on pregnancy rates prior or later to this period were nonsignificant.

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Efficacy of different antibiotics on conception rate in repeat breeding cows

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ABSTRACT

Post-insemination effect of 3 antibiotics on conception rate was studied in 150 Holstein × Hariana crossbred repeat breeding cows. Animals were divided in group 1, group 2 and group 3 having 30, 50 and 70 cows, respectively. Group 1 and group 2 cows were given 15 ml terramycin and gentamycin solution i/u, respectively, at 16 to 20 hr post insemination, whereas, group 3 received 1.25 g dicrystecin dissolved in 15 ml distilled water. The conception rate in group 1 was 16.7%, 26.7 and 16.7% after first, second and third insemination respectively. In group 2 the conception rate was 42.0, 26.0 and 8.00% whereas, in group 3, it was 50.0, 24.3 and 8.57 during first, second and third inseminations, respectively. The cumulative conception rate achieved in group 1, group 2 and group 3 was 60.0, 70.0 and 82.8% respectively. The services/ conception also improved in group 3 (1.50) and group 2 (1.55) as compared to group 1 (2.0). This study indicated that even in the absence of clinical infection, some mild infection may be present in female genitalia which may cause embryonic death. Therefore, post-insemination treatment with antibiotics may be successful in improving the conception rate.

Key words: Animal reproduction, Antibiotics, Conception, Cow, Infection, Repeat breeding

Average calving interval of 1 year is optimum for economical dairy husbandry (Pelissier 1976). This is possible when cows are maintained under good and hygenic managemental condition so that genital organs are healthy but free from infectious pathogens. These pathogens are cause of early embryonic mortality which results into low reproductive efficiency, increase in calving interval and repeat breeding consequently. Calving problems and unhygenic conditions during parturition is mostly associated with above incidence (Maurer and Echtern Kamp 1985). The infections caused by these pathogens are clinical but some times may be sub-clinical which is very difficult to diagnose without histological studies. An incidence of 20-50% embryonic and fetal death has been noticed in apparently normal healthy animals of all domestic species including bovines (Arthur *et al.* 1989). Therefore an experiment was designed to treat such repeat breeding cows with different antibiotics post-insemination.

MATERIALS AND METHODS

Holstein Friesian cross repeat breeding cows (150) which were apparently healthy and fed with balanced ration but did not conceive after 3 inseminations, were used. All cows were between second to fourth parity. Oestrus was detected by single teaser bull in all the sheds twice a day between 0630 to 0830 and 1700 to 1900 hr. Although in some of the cows doughy feeling of uterus and inflammation of cervix was noticed but the oestrus mucus discharge was clear and without flakes. Cows were divided randomly into 3 groups comprising 30, 50 and 70 cows in group 1, group 2 and group 3, respectively. All the animal were artificially inseminated (AI) with good quality frozen semen straws (0.25ml) after 10-15 hr of standing oestrus. Group 1 cows were infused 15 ml terramycin group 2 received 15 ml gentamycin, and group 3 received 15 ml (1.25 g) dicrystecin intrauterine after 16-20 hr of insemination. Pregnancy was confirmed by rectal palpation after 45-60 days post AI.

Statistical analysis was done by using Chi square test according to Snedecor and Cochran (1989) to find out difference in conception rate in different groups.

RESULTS AND DISCUSSION

Data pertaining to treatment of repeat breeding cows with different antibiotics and results on conception rate has been shown in Table 1. There was significant improvement ($P<0.05$) after first AI (50%, 35/70) and in overall conception rate (82.8%, 58/70) in dicrystecin treated group than terramycin and gentamycin. The response was poor with terramycin treatment during first (16.7%) second (26.7%) as well as overall (60.6%) AI. It is clear from above results that

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Table 1. Effect of different antibiotics on conception rate

Treatment	No. of animal	Conception rate (%) after			Over all CR (%)	Services (s) per conception
		1st AI	2nd AI	3rd AI		
Terramycin	30	16.7[a]	26.7	16.7	60.0[a]	2.00
Gentamycin	50	42.0[a]	26.0	8.0	76.0[a]	1.55
Dicrysticin	70	50.0[b]	24.3	8.57	82.8[b]	1.50

Figure with superscript differ significantly ($P<0.05$).

terramycin was less effective in overcoming the uterine infection. This indicated that repeat breeding cows had some mild infections because even low grade bacterial infection causes embryonic loss even in significant number.

Namboodiripad *et al.* (1976) also suggested good results with broad spectrum antibiotic for intrauterine use to treat repeat breeding cows similar to our study. Similarly Awasthi and Tiwari (1999) obtained 20% improvement in first AI conception rate when cephalexine was infused intrauterine after 12-24 hr post AI. The overall conception rate observed in present study was 76.0% (38/50) and 82.8% (58/70) in gentamycin and dicrystecin treated groups which differ from the result of Awasthi and Kharche (1987) who reported 60.0 and 40.0% conception rate in repeat breeding cows treated with gentamycin and streptopenicillin, respectively. Verma *et al.* (1994) and Mohanty *et al.* (1992) also observed increased conception rate in repeat breeding cows when treated with gentamycin. Singh and Singh (1994) also observed beneficial effect of gentamycin in improving the conception rate in repeat breeding cows but when both gentamycin and streptopenicillin was used, latter was comparatively more effective in improving conception rate in repeat breeding cows (Jain and Thakur 1993) which was similar to the present finding. We also observed lower service per conception in dicrysticin treated cows (1.50) than gentamycin (1.55) and terramycin (2.0). Thus it may be concluded that post-insemination antibiotic therapy after 16-20 hr is beneficial in improving conception rate of repeat breeding cows and dicrysticin may be used as a drug of choice.

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Use of spermatozoa from epididymus of slaughtered rams for *in vitro* fertilization of ovine oocytes

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ABSTRACT

The oocytes harvested from the ovaries obtained from a local slaughterhouse were matured *in vitro*. The oocytes reaching to metaphase II stage were 63.21%. The spermatozoa used in this study were obtained from the cauda epididymus of 2-3 testes collected from local abattoir. The washed samples were having 70-80% motility, rising up to about 90% after swimup treatment. Sperm motility was maintained throughout the capacitation period in all the 3 media. At the time of insemination above 80% of the spermatozoa exhibited progressive motility in all the 3 media. The oocyte penetration rates were 46.5, 53.4 and 54.5, respectively, for oocytes in TCM-199, Hams F10 and Hams F12 respectively. Out of the oocytes incubated further only 7,4 and 5.7% cleaved. It can be concluded that the spermatozoa collected from the epididymus of dead/slaughtered rams can be used successfully for the *in vitro* fertilization of oocytes in sheep.

Key words: Animal reproduction, *In vitro* maturation, *In vitro* fertilization, Spermatozoa, Sheep

In vitro fertilization can be used as a conservation tool for rare and endangered animal species. By collecting the gametes from animals after their death, their *in vitro* handling and *in vitro* embryo production, embryo transfer or their preservation may help in their conservation. *In vitro* maturation and *in vitro* fertilization has a very great potential for the generation of large number of embryos for research and for the application of other technologies. In bovines the capacity to produce embryos by IVM/IVF has progressed very rapidly during the past few years. However, in sheep only a limited number of offsprings have been produced using this technique. IVF can prove to be an invaluable technique not only for the supply of more number of embryos from valuable animals than what was/is possible with conventional MOET programmes, but also IVM-IVF embryos are calculated to be 5 times cheaper than the embryos from superovulated donors (Wolliams and Wilmut 1989).

Garde *et al.* (1994) have used epididymal sperms of postmortem rams up to 24 hr to determine the percentage of penetration by *in vitro* sperm penetration assays using zona free hamster eggs, and found that penetration rates did not differ much at 0-24 hr postmortem. Lot of work in the last few years has been reported on *in vitro* fertilization. Very little information is available on the utilization of gametes from dead animals. The use of dead animal ovary and epididymus for the production of viable young ones shall help to restore population of rare and endangered species. As such the present study was undertaken with the objective to study the fertilization rates of *in vitro* matured oocytes with the epididymal sperms obtained from slaughtered rams.

MATERIALS AND METHODS

Ovaries were collected from a local slaughterhouse in Srinagar city and transported to the laboratory within 3-4 hr of slaughter in Dulbecco's phosphate buffer saline (DPBS) in a thermos flask. In laboratory each ovary was freed from the surrounding tissues and overlying bursa. Each ovary was given 3 washings in DPBS and 2 washings in oocyte harvesting medium (DPBS+ 4 mg/ml BSA +50 IU/ml penicillin). The oocytes were harvested by one of the techniques, puncture, slicing or aspiration.

While harvesting the oocytes by puncture and slicing methods, the ovary was kept completely dipped in the medium in a 35 mm petri-dish. In aspiration visible follicles were aspirated using a 20-gauge hypodermic needle attached with a sterile disposable syringe having 2ml of medium. The media along with the collected oocytes was then transferred to a 35-mm petri-dish. In all the 3 techniques, the petri-dishes were kept undisturbed for 5 min, allowing the oocytes to settle down. Excess media was taken out by a syringe without

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disturbing the oocytes to settle down. Excess media was taken out by a syringe without disturbing the oocytes at the bottom of petri-dish. The petri-dish was then examined under an inverted microscope and the total number of oocytes harvested were counted. The oocytes were graded as good, fair and poor on the basis of cumulus cells and cytoplasm.

Good: Oocytes with many complete layers of cumulus cells and uniform cytoplasm.

Fair: Oocytes with thin or incomplete layers of cumulus cells and uniform cytoplasm.

Poor: Oocytes with few or no cumulus cells.

The number of good, fair and poor oocytes obtained were recorded for each ovary.

In vitro *maturation*

The good and fair oocytes were pooled and given 2 to 3 washings in maturation medium (Hams F10, or TCM-199) supplemented with 10% fetal calf serum, 0.1 IU/ml human menopausal gonadotrophin and 50 IU/ml penicillin. Ten oocytes were placed in 2 ml of maturation medium and incubated at 38.5°C under 5% with saturated humidity for 24-26 hr.

After the end of incubation, a portion of oocytes was freed from the cumulus cells by continuously pippeting in and out of a capillary, so that completely denuded oocytes were obtained. The denuded oocyte were fixed in ethanol and acetic acid solution (3:1) for 24 hr, stained with 1% aceto orecine stain and examined under a high power microscope. The maturation state of oocytes was evaluated on the basis of nuclear maturation. The oocytes were classified as germinal vesicle (GV), germinal vesicle breakdown (GVB), metaphase-I (M-I), metaphase-II (M-II) and degenerated (Dg).

Collection of spermatozoa

Sheep testes obtained from the abattoir in DPBS and taken to laboratory in a thermos flask at room temperature. They were washed with normal saline and cauda epididymus was isolated. The area below the deferent duct was identified, and was supposed to have mature sperms. This area was trimmed free of the tissues covering and washed with sterile DPBS. A prick was made on the convoluted tubules with a sterile hypodermic needle. The gushing fluid rich in sperms was collected with a micropippete and mixed with 4 ml of washing medium. Spermatozoa were collected from 2-3 epididymi of different rams mixed and used for a single insemination. Three types of media were used for sperm preparation and *in vitro* fertilization i.e. TCM-199, Hams F10 and Hams F12. The sperms were given 2 washings by centrifugation at 1500 rpm for 10 min in media supplemented with 4 mg/ml BSA and 50 IU/ml penicillin. About 100µ of loose pellet was overlaid with 2ml of fertilization medium, supplemented with 4 mg/ml BSA, 50 IU/ml penicillin and 50 IU/ml heparin and kept in CO_2 incubator (38.5°C) at an angle of 45°. The sperms were allowed to swim up for 2 hr.

In vitro *fertilization*

The matured oocytes were washed once in fertilization medium and then transferred to 2 ml of fertilization medium. The highly motile spermatozoa from the upper layers were added to the oocytes at the concentration of 1-2 x 10^6/ml approximately. The mixture of gametes was incubated for 18-22 hr. The oocytes were fixed in acid-alcohol for 24 hr, stained with 1% aceto-orcine stain and examined under high power microscope for the sperm penetration and fertilization. The sperms in perivitalline space or in vitellus, swollen sperm head in ooplasm or a male and female pronuclei were observed and recorded.

Culturing of zygotes

After coincubation of sperms and oocytes for 18-22 hr some of the oocytes were washed and transferred to 100 l droplets of culture medium pre-equilibrated under 5% CO_2. The droplets under light paraffin liquid, containing 5 oocytes/droplet, were incubated for further 44-48 hr. The cleavage rates were observed and recorded.

The data were analyzed as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Oocyte recovery

The oocytes were collected by puncturing the ovarian surface by an 18 gauge hypodermic needle or by slicing the ovary using a scalpel blade or by aspiration of the visible follicles. All the data were pooled and the average number of good, fair and poor oocytes recovered per ovary were 4.88±0.24, 1.91±0.15 and 1.41±0.75 respectively.

In vitro *maturation*

Oocytes recovered were matured *in vitro* and their nuclear maturation was evaluated. About 63% oocytes were in metaphase-II stage followed by metaphase-I stage (Fig. 1)

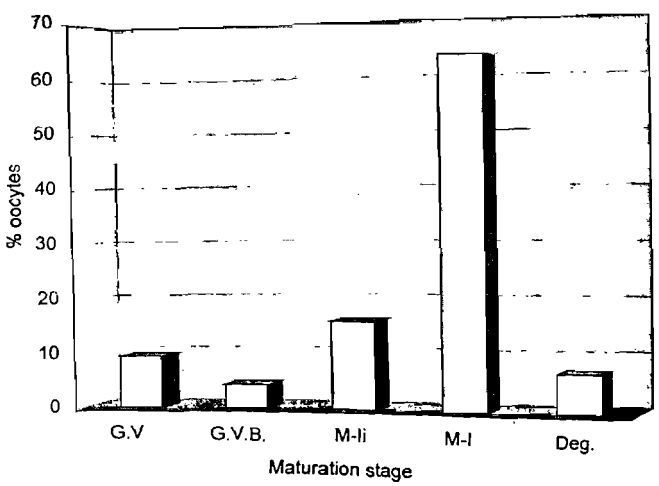

Fig. 1. *In vitro* maturation rates of ovine oocytes.

Table 1. *In vitro* fertillization of *in vitro* maturd oocytes by epididymal spermatoza in three different media

Media used	Total No.of oocytes	Oocytes (%)			
		penetrated	with polyspermy	incubated	cleaved
TCM-199	260	121.00 (46.54)	4.00 (1.54)	43.00	3.00 (7.00)
Hams-F10	148	79.00 (53.38)	1.00 (0.67)	50.00	2.00 (4.00)
Hams-F12	198	108.00 (54.54)	3.00 (1.51)	35.00	2.00 (5.7)

In vitro *fertilization*

The spermatozoa used in this study were obtained from the cauda epididymus of 2-3 testes collected from local abattoir. The washed samples were having 70-80% motility, rising up to about 90% after swimup treatment. Sperm motility was maintained throughout the capacitation period in all the 3 media. At the time of insemination above 80% of the spermatozoa exhibited progressive motility in all the 3 media. The oocyte penetration rates were 46.5, 53.4 and 54.5, respectively, for oocytes in TCM-199, Hams F10 and Hams F12 respectively. Out of the oocytes incubated further only 7,4 and 5.7% cleaved (Table 1).

In vitro fertilization can be used as a conservation tool for rare and endangered species. In such species a male is as precious as a female. The spermatozoa used in this study were collected from the cauda epididymus of slaughtered rams many hours after their death. The washed samples were having 70-80% motility and at the time of semination of oocytes above 80% spermatozoa showed progressive motility in all the 3 media, These findings are comparable to those reported by Crozet *et al.* (1987). However, they used fresh semen from rams of proven fertility. Our results are in full agreement with Garde *et al.* (1994) who used epididymal spermatozoa of postmortem rams (0-24 hr postmortem) to determine the percentage of penetration by *in vitro* sperm penetration assay using zona free hamster eggs. He found that live gametes capable of fertilization can be obtained many hours after the death of rams. Del Campo *et al.* (1994) have also used epididymal sperms for IVF in Llama successfully. Our findings demonstrate that all the 3 media can be used successfully for *in vitro* capacitation of spermatozoa and *in vitro* fertilization. Bondioli and Wright (1980) used synthetic oviductal fluid and minimum essential medium for sperm capacitation but no more than 14% of the oocytes were penetrated by the spermatozoa. Murzamadiev *et al.* (1986) used different media for sperm capacitation in sheep and found TCM-199 with sheep serum better for survival and capacitation of spermatozoa.

The fertilization rates obtained in our study were comparable to those reported by Murzamadiev *et al.* (1986) and Szollosi *et al.* (1988) who also obtained 50% and 45.7% fertilization rates, respectively, in sheep oocytes. However our results were lower than Cognie *et al.* (1991), who obtained 82.6% penetration rates. The differences might be attributed to the fact that these authors used *in vivo* matured oocytes whereas in our study *in vitro* matured oocytes were used. Fukui *et al.* (1988) obtained 43.89% fertilization rates affected by ram from which spermatozoa were taken. In our study also spermatozoa were collected from epididymus of different rams and the lower fertilization rates may be attributed to the sources of spermatozoa and ram effect. Our findings demonstrated that all the 3 media can be used for the spermatozoa preparation and *in vitro* fertilization of matured oocytes. However Hams F10 and Hams F12 proved slightly better than TCM- 199.

The polyspermy rates in our study were 1.54, 0.67 and 1.51% for TCM-199, Hams F10 and Hams F12, respectively, which are lower than reported by Cognie *et al.* (1991), who obtained 21% polyspermic oocytes and Cheng *et al.* (1986), who obtained 3% polyspermic oocytes. It indicated that the edididymal spermatozoa do not lead to more polyspermy as was thought earlier.

We compared the 3 media for culturing of presumed zygotes. The commercial media were used without any addition or alteration, except the addition of fetal calf serum. The percentage of oocytes cleaving were lower than Crozet *et al.* (1987), Shorgan *et al.* (1990), Galli and Moor (1991) and Watson *et al.* (1994), All these authors obtained a cleavage rate of about 34.81%. The lower rate of cleavage may be because of the absence of some energy substrates like sodium pyruvate, calcium lactate in the culture medium. It has been reported that first cell division requires pyruvate. This may be supplied by cumulus cells and oviductal cells *in vivo*. Oviductal pyruvate and lactate levels increases shortly after ovulation. This increase occurs only in pregnant oviducts implying acknowledgement by the maternal system that an embryo is present (Neider and Corder 1983). The other causes of lower cleavage rates can be attributed to the source of serum used. As reported by Pinlyopummintr and Bavister (1994) that different types and sources of protein supplementation in culture media can greatly influence development of mammalian embryo *in vitro*, ranging from marked stimulatory to completely inhibitory effects. In present study fetal calf serum was used, while sheep serum (Walker *et al.* 1986) or

human serum (Walker *et al.* 1988) have been reported to be superior than fetal calf serum. The presumptive zygotes were cultured in the droplets of medium, under light weight paraffin oil, which is reported to cause a low level toxicity to the embryo (Kane 1988). No attempt was made to co-culture the embryos with any cell type in this study. The co-culture of ovine embryos on monolayers of oviductal cells (Gandolfi and Moor 1987), trophoblastic vesicles (Rexroad and Powell 1988) and other reproductive tract cells (Rexroad and Powell 1993) are reported to increase the cleavage rates and number of embryos blastocyst stage.

It can be concluded that spermatozoa can be obtained from the epididymus of slaughtered/dead animals many hours after their death. These spermatozoa from valuable animals can be used for fertilization or can be preserved for future use.

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Comparative efficacy of ivermectin, albendazole, levamisole and rafoxanide against gastrointestinal nematode infections in goats

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ABSTRACT

A study on the efficacy of 4 anthelmintics was carried out using a faecal egg count reduction test (FECRT) in 107 goats on a breeding farm at Ol'Magogo, Naivasha, Kenya. The goats were randomly assigned to 7 groups; the first acted as untreated controls and the other groups received ivermectin (IVM) @ 0.2 mg/kgbodyweight once orally, albendazole (ABZ) @ 5.0 mg/kg, levamisole (LEV) @ 15 mg/kg and rafoxanide (RFX) @ 15 mg/kg orally as 2 doses 24 hr apart. Other groups received albendazole and levamisole (ABZ+LEV) and, levamisole and rafoxanide (LEV+RFX) at the same doses and schedules as and when used individually. Faecal egg counts were measured at treatment (day 0) and 10 and 21 days afterwards. Decreasing efficacies, as measured by 3 different formulae were recorded at day 21 for IVM, ABZ and ABZ+LEV (>96%), LEV (<54%), RFX (<60%) and LEV+RFX (<65%). Calculations of 95% confidence limits indicated that resistance was present in the LEV, RFX and LEV+RFX groups. The predominant use of LEV on the farm for a long period at dose rates recommended for sheep may have contributed to the development of resistance.

Key words: Anthelmintic resistance, Benzimidazoles, Goats, Haemonchosis, Levamisole, Nematode, Parasites

Gastrointestinal (GI) nematode parasitism is one of the major factors limiting goat productivity in Kenya. Goats are raised extensively on natural pasture, and goat farmers rely on anthelmintics for the control of gastrointestinal parasites. However, reports of the poor efficacy of these drugs against common nematodes of goats are numerous (Mwamachi *et al.* 1995, Wanyangu *et al.* 1996, Waruiru *et al.* 1998). This lack of effectiveness may be due to the resistance of some parasite population, especially *Haemonchus contortus* and/or due to underdosing when the anthelmintics are used at dose rates established for sheep (McKenna 1984). When such anthelmintic failures occur in goat farms, it is necessary to ascertain the adequate dosages for goats of the drug used. In June 1999, a faecal nematode egg count reduction test (FECRT) performed using levamisole and rafoxanide on goats at Ol'Magogo and 3 other farms within the locality suggested a high level of resistance (43% at 14 days after treatment) at Ol'Magogo farm. The intensity of GI nematode infections on this farm was moderate in most animals, the overall mean faecal strongyle egg per gram (epg) of faeces was 1240 and faecal cultures revealed 5 genera (*Haemonchus, Trichostrongylus, Cooperia, Nematodirus* and *Oesophagostomum*) with *Haemonchus contortus* being the predominant species. Keeping in view the above facts, three broad spectrum anthelmintics and a salicylanilide were tested on goats at Ol'Magogo farm, to check if their efficacy had declined.

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MATERIALS AND METHODS

Study site and background information

The study was conducted on a breeding herd of about 450 goats at Ol'Magogo Government farm, located at Naivasha and formerly managed by the Small Ruminant Collaborative Research Support Program (SR-CRSP). The animals were the Kenya Dual Purpose Goat breed (KDPG) consisting of 1/4 East African, 1/4 Galla, 1/4 Toggenburg and 1/4 Anglo-Nubian breeds. They were maintained on natural pasture during the day and housed at night, and water was provided *ad lib.* Over 5 years, about 6 treatments were undertaken annually using either levamisole (LEV), bezimindazoles (BZs) (thiabendazole, albendazole) or thiabendazole plus rafoxanide combination.

Anthelmintic treatment

In August 1999, goats (107) of mixed sexes and aged between 1-2 years were taken from pasture and a 3- week delay was observed before the start of the experiment to ensure the development of the possible immature stages of gastrointestinal nematodes. Goats were then randomly

assigned to 7 groups. Group 1 was untreated control; group 2 was given IVM (ivermectin) @ 0.2 mg kg^{-1} bodyweight once orally; group 3 was given ABZ (albendazole) @ 5.0 mg kg^{-1} bodyweight as 2 doses 24 hr apart; group 4 was given LEV @ 15 mg kg^{-1} bodyweight as 2 doses 24 hr apart; group 5 was given RFX @ 15 mg kg^{-1} bodyweight orally as 2 doses 24 hr apart; groups 6 and 7 were given both ABZ plus LEV, and LEV plus RFX at the same dose as and when used individually in groups 3, 4, and 5, respectively.

LEV and RFX dosages were twice the dose rates recommended for sheep and divided into 2 administrations 24 hr apart to take into account differences in bioavailability and efficacy between the 2 species. The same procedure was applied for ABZ but dosage was atleast 1½ times the dose rates recommended for sheep (3.8 mg/kg). IVM was used at the standard dose and for all anthelmintics, the dosage was determined on the basis of the heaviest animal of each group.

Parasitological techniques

Faecal samples were collected per rectum at the time of treatment (day 0) and then 10 and 21 days after treatment. Faecal egg counts were done by the McMaster method sensitive to 100 eggs per g of faeces (epg). Bulked faecal samples from each group at days 0,10 and 21 were cultured for 10 days, infective larvae extracted following Baerman's method and identified (MAFF 1986).

Efficacy calculations

The faecal egg count reduction (FECR) was calculated as $1-(T_2/T_1) \times (C_1/C_2) \times 100$ where T and C are, respectively, the geometric (egg count +1) means for treated and control animals and 1 and 2 designated the counts before and after treatment (Presidente 1985). FECR was also calculated by the same formula using arithmetic means instead of geometric means (Dash *et al.* 1988). The World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines' formula was also used: $(1-T/C) \times 100$ where T and C are the arithmetic means for treated and control groups after treatment (Coles *et al.* 1992).

RESULTS AND DISCUSSION

Arithmetic mean epg counts are given in Table 1. Values of the control group were moderate and had little variation between day 0 and day 21 after treatment. IVM was 100% effective while decreasing efficacies were recorded at day 21 for ABZ and ABZ +LEV (>99%), LEV (<54%), RFX (<60%) and LEV+RFX (<65%) according to the formula of Presidente (1985). Similar classification was obtained with the formulae of Dash *et al.* (1988) and Coles (1992) at day 21 but, except for IVM, efficacy values were lower (Table 2). Moreover, the calculation of the 95% confidence limits indicated that resistance was present in the LEV and RFX groups and in the LEV+RFX group.

Pre-treatment faecal cultures of each group identified *H. contortus* as the predominant parasite with an occurrence rates of 90% in the herd. The occurrence rates of other species were: *Trichostrongylus* (5%), *Cooperia* (2%), *Nematodirus* (1%) and *Oesophagostomum* spp. (2%). Post-treatment faecal cultures of the LEV+RFX group had only *H. concortus* larvae showing they were resistant to the anthelmintics.

Table 1. Comparison of mean nematode egg per gram (epg) counts in faeces of control goats and those given different anthelmintic treatments

Treatment	No. of animals	Arithmetic epg (Mean ±SD)		
		Day 0	Day 10	Day 21
Control	15	1180±694	1380±453	1260±546
Ivermectin (0.2 mg kg^{-1})	16	1167±1463	0	0
Albendazole (5.0 mg kg^{-1})	16	1017±441	17±37	33±47
Levamisole (15.0 mg kg^{-1})	16	1350±1069	435±335	750±457
Rafoxanide (15.0 mg kg^{-1})	15	1300±540	500±453	760±476
Albendazole[a] +levamisole	15	920±147	0	20±40
Levamisole[a] +rafoxanide	14	1667±1285	500±224	725±390

[a]At the same dose rate as when used individually.

The calculated efficacy of anthelmintics was higher when geometric means, rather than arithmetic means were used. This is in agreement with findings obtained in sheep (Pandey and Sivaraj 1994) and supports the views of others (Dash *et al.* 1988, Chartier and Pors 1994) in that geometric means may overestimate the real efficacy of an anthelmintic and therefore may not be able to detect lower levels of anthelmintic resistance. The dose rate of the anthelmintics applied to goats in the present study can be considered adequate. Thus, the reduced efficacy recorded for LEV and RFX indicated that resistance to these drugs occurred on this farm. LEV is at present the most widely used anthelmintic in Kenya, and it is sold under at least 12 trade names by different manufacturers, which leads farmers to think that they are switching anthelmintics (Wanyangu *et al.* 1996). In the farm investigated here, the predominant use of LEV for a long time at dose rates recommended for sheep may have contributed to the development of resistance, despite the moderate number of drenchs per year (McKenna 1984). In a national survey, resistance to LEV was 50% in goat farms surveyed, and all farms that dosed more than 5-times annually had anthelmintic resistance (Wanyangu *et al.* 1996). The occurrence of an additional resistance of *H. contortus* to RFX was rather surprising and may be attributed to the combined use of BZs plus salicylanilides in the previous years (Waruiru *et al.* 1998). All the products used in this study were well tolerated. Although the therapeutic index of LEV is lower than that of

Table 2. Comparative efficacy of different anthelmintics expressed as faecal egg count reduction per cent (FECR%) in goats, calculated by 3 different methods using geometric mean (gm) or arithmetic mean (am) from the same data of egg counts

	FECR% according to tne formulae of					
	Presidente (1985) (g)		Dash *et al.* 1988 (g)		WAVVP (Coles *et al.* 1992) (am)	
Treatment	Day 10	Day 21	Day 10	Day 21	Day 10	Day 21
Ivermectin (0.2 mg kg^{-1})	100	100	100	100	100 (-)	100 (-)
Albendazole (5.0 mg kg^{-1})	99.8	99.6	98.6	96.9	98.8 (96;100)[a]	97.4 (95;99)
Levamisole (15.0 mg kg^{-1})	75.4	53.8	72.5	47.9	68.5 (-2;89)	40.5 (-61;70)
Rafoxanide (15.0 mg kg^{-1})	66.8	59.6	67.1	45.2	63.8 (-12;86)	39.7 (-54;70)
Albendazole[b] *+levamisole*	100	99.8	100	97.9	100 (-)	98.4 (93;100)
Levamisole[b] + rafoxanide	74.2	65.0	74.4	59.2	63.8 (-15;85)	42.5 (-47;77)

[a]95 % confidence limits. [b]At the same dose rate as and when used individually.

many other anthelmintics, toxicity is rarely seen. When it does occur it is due to a stimulant effect on ganglia and is manifested by salivation, bradycardia, muscular tremors and, in the extreme case, death from respiratory failure (Bogan and Armour 1987).

The results indicated a high efficacy of IVM given orally at the standard dose as reported by Benz *et al.* (1989). However, goats producing milk for human consumption should not be treated with IVM during lactation or within 28 days of the commencement of lactation. ABZ was also highly efficacious when given at 1½ dose rates of sheep and, this was in contrast to earlier findings obtained in goats on the same farm (Waruiru *et al.* 1998). The efficacy of a single treatment at the recommended sheep dose rate of 3.8 mg/kg in goats was poor against *H. contortus* in goats in Brazil (Charles *et al.* 1989) but, at double this rate, ABZ was highly effective against common GI nematodes of goats (Pomroy *et al.* 1988). As the pharmacokinetic or efficacy data indicated that little is likely to be gained by giving large doses of BZs to goats as a single treatment, repeated or multiple doses at intervals of 12 to 24 hr appears to be more effective (Sangster *et al.* 1991) and suggested that the use of continuous release devices might be one way of extending the useful life of BZs in goats as in sheep (Sangster *et al.* 1990). However, the efficacy of this mode of administration in goats has not yet been evaluated.

Irrespective of any confusion regarding appropriate dose rates, the choice of anthelmintics for use on goat farms may be complicated by the occurrence of resistance involving LEV, BZs, salicylanicides or IVM (Mwamachi *et al.* 1995, Wanyangu *et al.* 1996). Because of this, it is recommended that the performance of all anthelmintics in goats be routinely evaluated by performing post-treatment egg counts. However, great care should be exercised in the diagnosis of LEV and/or RFX resistance using FECRT as these anthelmintics are not entirely effective against immature stages of *H. contortus* (Grimshaw *et al.* 1996). It may be necessary to take faecal samples less than 10 days post-treatment to avoid false positive results which may arise from the development of immature stages to egg laying adults in the interval between treatment and sampling (Grimshaw *et al.* 1996).

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Serum copper (Cu) and zinc (Zn) concentration in relation to super ovulatory response in embryo donor (Sahiwal) and conception rate in embryo recipient (crossbred) cattle

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ABSTRACT

Serum copper (Cu) and zinc (Zn) levels were measured in embryo donor (Sahiwal) and embryo recipient (crossbred) cows in relation to super ovulatory response and conception rate respectively. Sahiwal cattle (19) were super ovulated with equine and porcine follicle stimulating hormone and they were divided into 3 groups on the basis of super ovulatory response (good, fair and non responders). The levels of copper and zinc in serum were higher in the animals, which had responded well to the gonadotrophin treatment in comparison to those responded fair and did not responded at all. The levels of copper ranged from 1.42±0.17ppm to 2.15±0.11 ppm, 1.23±0.04 to 1.53 ±0.08 ppm and 1.36±0.10 to 1.75±0.13 ppm in good, fair and non responder animals, whereas, the levels of zinc (Zn) ranged between 2.38±0.18 to 2.83±0.22 ppm, 2.00±0.07 to 2.82±0.27 and 1.79±0.24 to 2.82±0.11 ppm in good, fair and non responder respectively. The animals, which conceived, had higher levels of copper in comparison to those, which did not become pregnant. The levels in pregnant animals varied from 1.98±0.16 ppm to 2.35±0.15 ppm, whereas in non-pregnant animals it varied from 1.73±0.13 to 2.23±0.13 ppm. The level of variation in the zinc among different days of pregnancy were not significant but variation in groups (pregnant and non-pregnant) was significant (P<0.01).

Key words: Animal reproduction, Copper, Embryo donor, Embryo recipient, Pregnancy, Superovulation, Zinc

Hypocuprosis in cattle is associated with female reproductive disorders; the most common symptom has been prenatal mortality, particularly embryonic loss. A low copper content in the diet either prevent implantation or induce embryonic loss and fetal death (McChowell 1968; McChowell and Hall 1970). A high incidence of infertility in cattle in Australia (Bennets *et al.* 1948) was associated with cases of falling diseases, anaemia and retarded growth in areas where pasture copper was less than 3 ppm. The pituitary gland and ovaries of cows contained 9.5 and 4.8 µg copper/g dry weight (Stockl and Weiser 1968). The lower copper level in some repeat breeders cows was also observed by Prasad and Rao (1997). Zinc is an essential nutrient required for normal development, growth and tissue synthesis and may be a specific dietary factor modulating fetal growth. Many workers Dufty (1977) and Kumar and Vadhve (1994) have documented low zinc levels in anestrous heifers than normally cycling cattle, as the plasma zinc levels tended to be higher at the time of estrus (1.50±0.27µg/ml) than during the diestrus (1.01±0.16µg/ml). Zinc deficiency in relation to reproduction is documented as being responsible for reproductive infertility and defects in prostaglandin metabolism (Cunnae *et al.* 1983). In the present study the relationship of copper and zinc was evaluated in relation to superovulation in embryo donor cattle and conception in embryo recipient cattle.

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MATERIALS AND METHODS

The study was conducted at Livestock Research Centre of the University. The animals were selected on the basis of reproductive history, clinical and per rectal examination twice at the time of estrus. Embryo donors, Sahiwal (19) were super ovulated by using equine and porcine follicle stimulating hormone given as per following schedule. Donor animals were divided into 3 groups on the basis of super ovulatory response (good, fair and non responders). The blood samples from the embryo donor and embryo recipient cattle were collected as per following schedule.

Super ovulatory treatment with FSH-P and FSH-E

Super ovulatory treatment was initiated on day 10 of estrous cycle and was spread for 4 days. FSH-P was given @400 mg NIH in 8 divided doses I/M in descending dose manner; 130, 110, 90, 70 mg on day 10, 11, 12, 13 respectively. FSH-E was given @50mg in 8 divided doses and also in descending

dose manner 15, 13, 12, 10mg on day 10, 11, 12 and 13 respectively. Injecting prostaglandins on day 12 of estrous cycle @25 mg i/m induced estrus.

Sampling schedule of embryo donor and recipient animals

Embryo donor: Blood serum samples from embryo donor animals were collected on day of I P.G. treatment, day of estrus, second day of estrus, fifth day of estrus, day of initiation of FSH treatment (10), day of II P.G. treatment (12), last day of FSH treatment (13), day of superovulation (S_0), third day of superovulation (S_3) and seventh day of superovulation (S_7) or day of embryo collection.

Embryo recipient: Blood serum samples were collected on day of embryo transfer and on days 4, 7, 14, 21 and 28 post ET. The samples were preserved at -20°C in deep freezer till analysis.

Blood (15ml) was collected from jugular vein of embryo donor and recipient cow and blood serum was separated. The serum was stored at –20°C till analysis.

Before analysis of copper and zinc, the serum samples were first digested by nitric acid and triple acid mixture for the analysis. For digestion, 5ml of concentrated nitric acid was added to test-tube containing 1 ml of serum. It was then kept over a hot plate for 30 min. After cooling, 5 ml of triple acid (nitric acid, perchloric acid and sulphuric acid in the ratio of 10: 4: 1) was added into it. It was again kept on hot plate till the contents reduced to 1 ml. It was then cooled and volume was made to 10ml by adding triple glass distilled water and the estimation was done by atomic absorption spectrophotometer using following instrumental settings.

Elements	Max. lamp current	Wave length (nm)	Slit setting (nm)	Flame
Copper	5.0	324.75	0.4	Air-C_2H_2 (oxd)
Zinc	10.0	213.86	0.4	Air-C_2H_2 (oxd)

Table 1. Serum copper (Cu) levels (ppm; mean±SE) in embryo donor cows

Donor response	Days from estrus: -3 (PG)	0 (DE)	2	5	10 (I-FSH)	12 (III-FSH)	13 (L-FSH)	S_0	S_3	S_7
	Bd	Bd	Bcd	Bbcd	Bbcd	Bd	Bd	Aab	Babc	Aa
Good	2.12 ±0.11 A	2.15 ±0.11 A	1.99 ±0.11 A	1.81 ±0.21 AB	1.83 ±0.09 A	2.06 ±0.27 A	2.12 ±0.33 A	1.57 ±0.19 A	1.64 ±0.21 A	1.42 ±0.17 A
Fair	1.29 ±0.11 Aab	1.53 ±0.08 Aab	1.41 ±0.08 ABb	1.45 ±0.07 Aa	1.36 ±0.04 Aab	1.38 ±0.05 Aab	1.40 ±0.03 Aa	1.36 ±0.20 Aab	1.23 ±0.04 ABab	1.40 ±0.05 Aab
Non-responders	1.41 ±0.06	1.61 ±0.05	1.75 ±0.13	1.37 ±0.04	1.40 ±0.04	1.44 ±0.03	1.36 ±0.10	1.49 ±0.07	1.46 ±0.05	1.52 ±0.07

Cd_3 at 5%=0.375, F value:a=37.01**, b=2.03**, axb=1.45ns.

Means within a row with different small suprscript are significantly different (P<0.05); means within a column with different capital superscript are significantly diffrent (P<0.05); PG=prostaglandin, DE=day of estrus, FSH=follicle stimulating hormone, L=last.

Table 2. Serum copper (Cu) levels (ppm; mean±SE) in embryo recipient cows

Recipients	Day of E.T.	Day 4 post E.T.	Day 7 post E.T.	Day 14 post E.T.	Day 21 post E.T.	Day 28 post E.T.
Pregnant	2.21^{Aa} ±0.11	2.35^{Aa} ±0.15	2.09^{Aa} ±0.10	2.19^{Aa} ±0.16	1.98^{Aa} ±0.16	2.13^{Aa} ±0.14
Non-pregnant	1.73^{aB} ±0.13	1.93^{aB} ±0.16	2.11^{Aa} ±0.13	2.23^{Aa} ±0.07	1.78^{Aa} ±0.09	1.89^{Aa} ±0.14

Cd_3 at 5%=0.373, F value:a=7.45**, b=1.66ns, axb=1.35ns.

Means within a row with different small superscript are significantly different (P<0.05); means within a column with different capital superscript are significantly different (P<0.05).

RESULTS AND DISCUSSION

In the present study the level of copper, in good responder animals ranged from 1.42±0.17 ppm to 2.15±0.11ppm and in non-responders animals it was 1.36±0.10 to 1.75±0.13 ppm (Table 1). The higher level of copper in the animals which responded well to the gonadotrophin treatment are in agreement with Pradhan *et al.* (1995) and Vhora *et al.* (1995) who have reported higher values of copper in normal cyclic animal than post-partum anestrous animal.

Present findings agree well with the findings of Kalita *et al.* (1999). They have reported variations in the levels of copper in normal cycling, repeat breeder and anestrous cows though the levels were statistically non-significant (1.69±0.18 ppm, 1.48±0.19 ppm and 1.46±0.16 ppm respectively). The

Table 3. Serum zinc (Zn) levels (ppm; mean±SE) in embryo donor cows

Donor response	Days from estrus									
	-3 (PG)	0 (DE)	2	5	10 (I-FSH)	12 (III-FSH)	13 (L-FSH)	S_0	S_3	S_7
	Ba	Aa	Aa	Ba	Ba	Aa	Aa	Aa	Ba	Aa
Good	2.83 ±0.22	2.39 ±0.21	2.46 ±0.17	2.40 ±0.13	2.79 ±0.31	2.48 ±0.16	2.38 ±0.18	2.49 ±0.22	2.77 ±0.15	2.58 ±0.19
	Aa	Abc	Aa	Aba	ABab	Aa	Aa	Ac	Aa	Aab
Fair	2.00 ±0.07	2.60 ±0.12	2.09 ±0.09	2.09 ±0.15	2.31 ±0.18	2.01 ±0.22	2.04 ±0.22	2.82 ±0.27	2.01 ±0.20	2.24 ±0.17
	Aa	Ab	Aa	Aa	Aa	Aa	Aa	Ab	Aa	Aa
Non-respondrs	2.07 ±0.59	2.80 ±0.53	2.28 ±0.17	1.79 ±0.24	1.95 ±0.10	2.02 ±0.04	2.07 ±0.12	2.82 ±0.11	2.20 ±2.20	2.24 ±0.17

Cd_3 at 5%=0.491, F value:a=12.74**, b=3.59**, axb=1.91*.

Means within a row with different small suprscript are significantly different (P<0.05); means within a column with different capital superscript are significantly diffrent (P<0.05); PG=prostaglandin, DE=day of estrus, FSH=follicle stimulating hormone, L=last.

animals, which conceived, had higher levels of copper in comparison to those, which did not become pregnant (Table 2). Present findings of copper in pregnant and non-pregnant animals are also fitted well with the findings of Sarmah *et al.* (1999). They found significant increase in pregnant groups of non-descript cattle.

The values of zinc (Zn) differ significantly among different groups and they were significantly lower in non-responder animals to the super ovulatory treatments (Table 3). The findings are in accordance to many workers like Dufty (1977) and Kumar and Vadhve (1994). They have documented low zinc levels in anestrous heifer than normal cycling cattle. Kumar and Vadhve (1994) have documented higher levels of zinc at the time of estrus (1.50±0.27µg/ml) than that during the diestrus (1.01±0.16µg/ml). The higher values of zinc on the day of estrus or around estrus could be due to increased level of estrogen on these days.

In the present study, the level of zinc variations during pregnancy was significant (<0.05) in comparison to non-pregnant (Table 4). The variation on account of days between the days in pregnant and non-pregnant cows was nonsignificant (Table 4). The levels were higher during the early pregnancy in comparison to non-pregnant animals.

Table 4. Serum zinc (Zn) levels (ppm; mean±SE) in embryo recipient cows

Recipients	Day of ET	Day 4 post ET	Day 7 post ET	Day 14 post ET	Day 21 post ET	Day 28 post ET
Pregnant	2.95^{Aa} ±0.24	2.89^{Aa} ±0.15	2.52^{Aa} ±0.12	3.10^{Aa} ±0.07	2.85^{Aa} ±0.16	2.85^{Aa} ±0.06
Non-pregnant	2.25^{aB} ±0.18	2.74^{Aa} ±0.04	2.09^{Aa} ±0.10	2.06^{aB} ±0.14	2.07^{aB} ±0.15	2.20^{aB} ±0.21

Cd_3 at 5%=0.448, F value:a=47.40**, b=2.2ns, axb=1.97ns.

Means within a row with different small superscript are significantly different (P<0.05); means within a column with different capital superscript are significantly different (P<0.05).

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Bovine infertility – a field oriented categorisation based on investigation among crossbred cattle in a district of Kerala

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ABSTRACT

Animals (500) were examined for gynaecological reasons and the infertility conditions were suitably categorised for easier description. Out of these animals , 374 (75%) were diagnosed to be infertile cases, which included 148 cows and 226 heifers. Infertility cases mainly were of 2 types based on manifestation such as anoestrum (65%) and repeat breeding (35%). Out of the 242 anoestrum cases, 178 (74%) were true anoestrum and rest (26%) were cases of false anoestrum. Among 132 repeat breeding animals 26 (20%) had ovulatory disturbances, 70 (53%) had reproductive tract infections (RTI) and in 33 (25%) both ovulatory disturbances and RTI together were seen. Major reasons for the conditions were nutritional deficiencies, reproductive tract infections and functional disturbances suspected out of elimination of males. It is concluded that most infertility conditions among crossbred cattle are arising out of management defects either of the farmers or technician, which in turn form indirect consequences of intensive crossbreeding and widespread AI programme.

Key words: Animal reproduction, Anoestrum, Bovine, Crossbred, Infertility, Repeat breeding

Through the intensive crossbreeding efforts Kerala state has converted more than 70% cattle heads into exotic crossbreds and consequently achieved a major hike in milk production (1996 AH census). However, there are lot of accompanying problems faced by dairy farmers, which are of great economic significance, infertility being one of the major ones (Callaghan *et al.* 2000). Over the years there has occurred lot of changes in the incidence, prevalence, type and causes of infertility depending upon the genetic changes of the herd and management situations (Gunasekharan *et al.* 2000, Kutty and Ramachandran 2000). But timely change has not occurred in the approach and management of infertility conditions having relevance to the existing field realities and husbandry situations.

There are many reports on the incidence and nature of infertility conditions among crossbred cattle from different states under various management situations. However, investigation of infertility conditions presently prevalent in a state much advanced with respect to the generation of crossbred cattle will be highly informative and useful. Hence, this study was carried out as part of an ongoing infertility investigation programme. More over, attempt has been made to categorise infertility conditions in a simple, field oriented and more practical way for easy understanding and management under the prevailing husbandry situations.

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MATERIALS AND METHODS

The study was carried out at Regional Cattle Infertility Research Centre of Kerala Agricultural University at Kozhikode, which is earmarked for infertility investigation in Malabar districts of Kerala. Crossbred cattle (500) were subjected to gynaecological examination either at the centre or at field level camps organised during the year 1999-2000 and suitable corrective measures were adopted. Data were properly recorded using 'Infertility Investigation Sheet' designed for the purpose. Tentative diagnosis of the cases was done based on the history and clinico-gynaecological findings. Infertility conditions were categorised based on the history, manifestations and tentative diagnosis of underlying causes for easier description and management.

RESULTS AND DISCUSSION

Details regarding place of examination, type of animals examined and infertility cases diagnosed are shown in Table 1. Out of 500 animals examined 374 (75%) were cases of actual infertility and rest of them were brought for assessment of reproductive status, pregnancy diagnosis and so on. Of this 84% of the animals were examined at infertility campus

Table 1. Details regarding examination of animals

Criteria	Divisions	Number	Percentage
Location	At the centre	82	16
	Field level camps	418	84
Type of animal	Cow	148	40
	Heifer	226	60
Details of cases	Infertility cases	374	75
	Pregnancy diagnosis and others	126	25

organised in villages and the rest were brought for investigation at the centre. Sixty per cent infertility conditions were observed in heifers and the rest in cows.

Based on history and manifestation infertility cases were mainly 2 types such as anoestrum - 65% (not showed heat signs at the expected time period) and repeat breeding – 35% (repeated heat signs even after more than 3 services). Major categories and proportions of infertility conditions under each type are shown in Table 2.

Of anoestrum cases 74% were true anoestrum cases characterised by non-functional (smooth) ovaries, forming 48% of total cases. Rest (26%) anoestrum cases were false

Table 2. Clinical categorisation and proportions of infertility conditions

Reproductive history	Clinical manifestation	Distribution of cases in categories	Percentage of infertility cases
Anoestrum	242	100%	65%
True anoestrum	178	74%	48%
False anoestrum	64	26%	17%
Repeat Breeding	132	100%	35%
R. Tract infections	67	51%	18%
Ovulatory defects	23	17%	6%
Both together	36	27%	10%
Others	6	5%	1%
Infertility Total	374		100

anoestrum, wherein the ovaries were functional with mature follicles or luteal structures, but the owner or attendant did not observe heat signs. Sub categories of anoestrum conditions encountered based on the stage of occurrence and attributed causative factors are shown in Table 3

Out of 178 true anoestrum cases examined, 123 (69%) were in heifers above 2 years of age (prepartum anoestrum) and 55 (31%) were in cows (postpartum anoestrum). In 144 (81%) cases anoestrum was pre service (before doing any service), while in the rest 34 (19%) cases, anoestrum was post service (after doing service, but were found to be non pregnant upon examination). Distribution of pre service and post service anoestrum among cows and heifers are included in Table 3. Altogether in 28 cases of true anoestrum, there

Table 3. Distribution of true and false anoestrum cases

Type of infertility	Cases	Type of infertilty	Cases
True anoestrum	178	False anoestrum	64
Prepartum	123	Undetected oestrum	5
Preservice	98	Suboestrum	41
Post service	25	Luteal inhibition	18
Post partum	55	Luteal cyst	2
Preservice	46	Persistent C.L	16
Post service	9	Foreign mass	1
With R.T.infection	28	R.T.infections	15

were signs of non-specific reproductive tract infections (RTI), majority being post service cases.

Out of 64 cases diagnosed as false anoestrum, 41 (64%) were suboestrum cases, where there were palpable evidences of regular cycling, but was not detected by the owner due to the absence of visible external signs. In 5 animals even though there was regular cycling with certain external signs, owners had not realised them as proper heat and not taken for service. In rest of the 18 (28%) animals anoestrum was accompanied by luteal inhibition either by persistent corpus luteum (16) or luteal cyst on the ovary (2 cases). In 15 cases RTI was the reason attributed for the persistence of corpus luteum while in 1 case there was mummified mass inside the uterus.

Based on clinical manifestation and history, repeat breeding cases were attributable to either RTI (51%) or ovulatory disturbances (17%) alone in 68% cases while both together was attributable in 27% cases. Distribution of repeat breeding cases based on causation and manifestation is shown in Table 4.

Out of the 59 animals diagnosed to have ovulatory defects, 41 (69%) were cases of delayed ovulation, wherein the heat signs were prolonged beyond second day of heat. In 11 (19%)

Table 4. Distribution of repeat breeding cases

Cases	Distribution	% of each sub-categories	% of total repeat breeding cases
Repeat Breeding	132		100%
Ovulatory disturbances	59	100%	45%
Delayed ovulation	41	69%	
Anovulation	11	19%	
Follicular cyst	7	12%	
R.T.infections	103	100%	78%
Vaginitis	23	22%	
Cervicitis	21	20%	
Endometritis	101	98%	
Salpingitis	5	5%	
Ovulatory defects and RTI	33		25%
Others	3		2%
Hormonal imbalances	3		
Miscellaneous			

cases, there was total failure of ovulation following heat as indicated by absence of any functional luteal structures (anovulation) and in remaining 7 (12%) cases follicle was transformed into follicular cyst following anovulation and was accompanied by characteristic tubular changes and history of intermittent heat signs.

Out of 103 repeat breeding animals with RTI s, 101 (98%) had manifestations of endometritis, and there were vaginitis and cervicitis in 23 and 21 of them respectively. In 5 animals salpinx were thickened either unilaterally or bilaterally and with or without ovaro-bursal adhesions suggestive of salpingitis. Out of 103 animals, 33 had ovulatory disturbances also. Other causation for repeat breeding was detected only in 3 animals, wherein hormonal imbalance was suspected since regular consumption of plants containing high levels of phyto-oestrogens and association of short cycles was reported.

Farmers often fail to satisfy the high nutrient demand for crossbred animals and reproductive performance is adversely affected (Srivastava *et al.* 2000, Pillai 1980)). Even though some of the farmers offer adequate quantity of concentrate to their animals, often fail to ensure adequate quality. Kerala being a state with scarcity of concentrates, most of the farmers depend on marketed cattle feeds and quality of the feed is a major factor contributing to infertility (George and Nair 1995).

High proportion of pre service anoestrum and sub-oestrum cases are indicative of overall deficiency of nutrients (Agarwal and Umashankar 2000), while post service anoestrum and postpartum anoestrum are reflections of energy deficiency and/or poor service and obstetrical management (Rao and Naidu 2000). False anoestrum with PCL and repeat breeding cases are mainly caused by RTI, which is often consequence of poor and unscientific service management (Arthur *et al.* 1989). Source of infection was believed to be natural service in few cases, while untimely or unscientific AI in most of the cases attributable to management failures of farmers' and technicians.

Ovulatory disturbances contributing to repeat breeding can be attributed to micro-nutrient deficiencies and resultant hormonal imbalances (Singh *et al.* 2000). Moreover possibility of functional imbalances consequent to widespread acceptance of artificial insemination, elimination of males from the herd, abolition of male effect, and absence of mating stimulus cannot be ruled out. High incidence of prolonged oestrus lasting 3-4 days in herds bred through AI is supportive to such thoughts (Gunasekharan *et al.* 2000). Besides disappearance of most of the behavioural signs of oestrus in such herds points to alterations in physiological mechanisms following elimination of males, male effect and mating process.

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A comparative study on belt-loop pyloric gastropexy with circumcostal pyloric gastropexy in dogs

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ABSTRACT

A comparative study on belt-loop pyloric gastropexy and circumcostal pyloric gastropexy was done in 12 apparently healthy mongrel dogs of either sex. The animals were divided into groups I and 2 of 6 animals. Each group was then sub-divided into A and B sub groups of 3 animals each. Belt-loop pyloric gastropexy and circumcostal pyloric gastropexy was performed in groups 1 and 2 respectively. Animals of sub group A and B of either group were maintained for 20 to 40 days, respectively, after surgery. Sub-group 'A' animals were sacrificed on 20th and sub-group 'B' on 40th day post operation. Based on clinical, radiographical, gross and histopathological examinations, the efficacy of the 2 techniques was compared and concluded that belt-loop pyloric gastropexy is simpler and easier of the 2 techniques in treatment and prevention of relapse of gastric dilatation voluvlus (GDV) in dogs.

Key words: Belt-loop, Dilatation-voluvlus, Dogs, Gastropexy, Surgery

Gastric dilation voluvlus (GDV) is a life threatening syndrome characterized by cranial abdominal distension with tympany, spleenomegaly and retching with inability to vomit. It is most commonly seen in the native, large and deep chested breeds of dogs. GDV occurs when a source of gas, food and/ or fluid is present in the stomach along with compromised gastric out-flow. Postprandial exercise or excitement is also seen to precipitate the onset of gastric dilatation and volvulus. When stomach is not emptied promptly, it may lead to dilatation and rotation of the stomach on its mesenteric axis, interfering with the blood supply to the stomach and pathologic changes affecting nearly every organ system of the animal, which may lead quietly to death. The condition is a medical and surgical emergency with high mortality rate (Betts *et al.* 1974, Dann 1976, Wingfield 1983, Flanders and Harvery 1984 and Schulman *et al.* 1986).

Risk of recurrence, following an attack of GDV is very high and recurrence rates between 5 and 29% have been reported with tube gastropexy. Fallah *et al.* (1982) opined that circumcostal pyloric gastropexy is superior to tube gastrostomy, gastrocolopexy, gastropexy and fundicpexy techniques because it avoids many problems encountered with them as it does not require surgical entry into stomach in treatment of GDV.

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MATERIALS AND METHODS

Mongrel dogs (12) were randomly divided into groups 1 and 2 of 6 animals each, and each group was sub-divided into A and B of 3 animals. Belt-loop pyloric gastropexy was performed in group 1 and circumcostal pyloric gastropexy in group 2. All the animals were premedicated with triflupromazine hydrochloride @ of 1 mg/kg body weight given intravenously and general anesthesia was induced by using 5% thiopentone sodium @ 25 mg/kg body weight, intravenously.

In belt-loop pyloric gastropexy the dog was placed in dorsal recumbency and the abdomen was opened by a ventral midline incision extending from the xiphoid cartilage to midway between the umbilicus and pelvic inlet. Fibres of the transversus abdominis muscle and costal arch on the right side were identified. A belt-loop of transversus abdominis muscle was created by making 2 small (3mm) transverse stab incisions through parietal peritoneum and transversus abdominis fascia, 2.5 cm apart. Belt-loop was centered approximately one-third of the distance ventral to the dorsal midline. A 2.5 cm by 4 cm tongue shaped seromuscular stomach flap was conceived in the pyloric antrum, by centering a branch of the right gastroepiploic artery in the base of the flap. The abdomen was lavaged and closed routinely.

In circumcostal pyloric gastropexy, the abdomen was opened by a cranial midline incision extending from xiphoid

towards umbilicus. An 'I' shaped incision of about 5 cm long and 3 cm wide was made at the ventral pyloric antrum, to create two 1.5×1.5 cm flaps of serosa and muscle. The site of costal fixation was 12th rib. Two stay sutures were placed at the base of flap. The suture ends were passed through the appropriate inter costal tunnel, and the stomach was pulled against the right abdominal wall, and were sutured to each other lateral to the rib, ventral to the costo-chondral junction with simple interrupted sutures using 2-0 monofilament polypropylene. The stay sutures were removed and the abdominal incision was closed in a routine manner.

The clinical signs such as for vomiting and other gastrointestinal abnormalities were observed for 7 post-operative days. Radiographic examination, gross and histopathological studies were studied.

RESULTS AND DISCUSSION

Clinical observation revealed that none of the dogs had vomition and gastrointestinal complications following any of the 2 techniques i.e., belt-loop pyloric gastropexy and circumcostal pyloric gastropexy. The above findings are in agreement with those of Fallah *et al.* (1982).

Radiographic examination of both groups revealed that the techniques do not interfere with the normal physiological function of the stomach except slightly altering the position of the stomach. The pyloric surgery did not affect the normal gastric emptying time in any of the dogs. And in agreement with the findings of Betts *et al.* (1976) and Fallah *et al.* (1982).

Close necropsy examination of both groups revealed that there was no infection. In both the techniques the stomach was firmly attached to the right abdominal wall. No other adhesion were observed between and other abdominal organs, indicating that both the gastropexy procedures were free from such type of complications. These findings supports the observation of Rao *et al.* (1989).

Histopathological examination revealed no significant difference between the 2 groups at post-operative day 20. Both groups showed diffused fibrocollageneous bands consisting of both immature and mature fibroblasts alongwith many budding capillaries and mononuclear cell infiltration. Mononuclear cell infiltration was evident in the muscle and to some extent in pyloric mucosa. In group 2, a slight amount of fibrous tissue appeared around the periphery of the rib. Degenerative changes were not evident in the pyloric mucosa.

At post-operative day 40 in both groups thick fibrous tissue was noticed between mucosa and muscle, which consisted of mainly matured fibroblasts and matured capillary buds. Thick and extensive fibrous tissue between muscle and mucosa suggests the formation for adhesions. Mononuclear cell infiltration in the connective tissue, muscle and pyloric mucosa is very less. These findings are in agreement with those of Fallah *et al.* (1982) and Rao *et al.* (1989).

Circumcostal pyloric gastropexy is technically more difficult to perform than most gastropexy techniques and requires more anesthesia time. Latrogenic rib fracture and inadvertent penetration of the diaphragm can occur while isolating the rib for passing the gastric flap. These complications may further lengthen the surgical time. On an average circumcostal gastropexy technique took 40 min to complete the intra-operative procedure.

Circumcostal pyloric gastropexy is technically more difficult to perform than belt-loop gastropexy technique and requires more anesthesia time. Intra-operative time of Circumcostal pyloric gastropexy and belt-loop gastropexy is 40 and 20 min respectively. It was concluded that the belt-loop pyloric gastropexy is superior and easier to perform and between of the 2 techniques studied.

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Resurfacing femoral trochleoplasties with free autogenous fascia lata and periosteal grafts in dogs*

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ABSTRACT

An experimental study was carried out in 18 dogs by resurfacing femoral trochleoplasties with free autogenous fascia lata and periosteal grafts. Experimental recession of femoral trochlea was performed in all dogs through lateral parapatellar arthrotomy. The animals were divided into 3 groups of 6 animals each. In groups 1 and 2, the trochleoplasties were resurfaced with free autogenous fascia lata and periosteal grafts, respectively, and group 3 served as controls. Three animals in each group were observed for 4 weeks and the remaining 3 for 8 weeks post-surgery. Both the grafts induced neochondrogenesis as early as 8 weeks post-surgery. In fascia lata resurfaced group, chondrogenesis was from subchondral bone and in periosteum resurfaced joints, it was both from progenitor cells of periosteum and mesenchymal cells of subchondral bone.

Key words: Fascia lata, Femoral Neochondrogenesis, Periosteum, Resurfacing trochleoplasty, Surgery

Trochleoplasty is the most common surgical method indicated in correcting patellar luxation caused either due to shallow or absent trochlear groove in dogs. But trochleoplasty leaves a rough surface for patella to glide over it and causes damage to patellar articular surface (Hulse *et al.* 1986). To prevent this damage trochlear resurfacing is done to induce neochondrogenesis, and also to provide a smooth surface for patella to glide over. Fascia lata which possesses condrogenic potential (Krol *et al.*(1992) and osteogenic character (Rubak 1982) has been used experimentally to resurface damaged articular cartilage defects of femoral trochlea. In the present study the ability of both these grafts to induce neochondrogenesis in trochleoplasty was investigated.

MATERIALS AND METHODS

Healthy mongrel dogs (18) of either sex were kept off feed and water for 12 hr prior to surgery. After sedation with triflupromazine hydrochloride @ 1 mg/kg body weight, general anesthesia was induced by intravenous injection 2.5% thiopentone sodium @ 25 mg/kg body weight. The stifle joint was approached through a lateral para-patellar cutaneous incision. A stab incision through fascia and joint capsule was extended to invade the joint. Flexion of the joint and medial luxation of patella exposed the articular surface of femoral trochlea completely. The articular cartilage of the femoral trochlea was resected down to bleeding of cancellous bone using a periosteotome. Then the joint cavity was flushed with sterile normal saline. In group I animals, trochleoplasty is resurfaced with a rectangular piece of fascia lata graft measuring 1"×5" harvested from the lateral aspect of thigh. The graft was fixed on resected femoral trochlea by making 4 drill-holes to both femoral condyles as suggested by Engkvist and Wilander (1979) and Chandra Sekharam *et al.* (1991) using No. 'o' silk.

The area was flushed with normal saline and patella was replaced into the trochlear groove. The joint capsule was closed with continuous interlocking suture using No. 2/0 chromic catgut and cutaneous incision was sutured using No. 1 silk in horizontal mattress fashion.

In animals of group 2, the trochleoplasty was resurfaced with periosteal graft. A rectangular piece of periosteum (1cm×5cm) was harvested from upper two-thirds of medial metaphysis of the tibia and collected by the help of periostcal elevator. The graft was kept in normal saline until transplantation. The graft fixation over resected area and closure of arthrotomy wound was done as in group 1. In group 3 animals, trocheloplasty alone was performed. The animals were kept on streptopenicillin 0.5 g daily for 5 post operative days, and diclofenac sodium 50 mg for 3 post operative days.

In each group 3 animals were sacrificed at the end of 4 weeks, and the remaining 3 at 8 weeks post surgery to note

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gross and histopathological findings.

RESULTS AND DISCUSSION

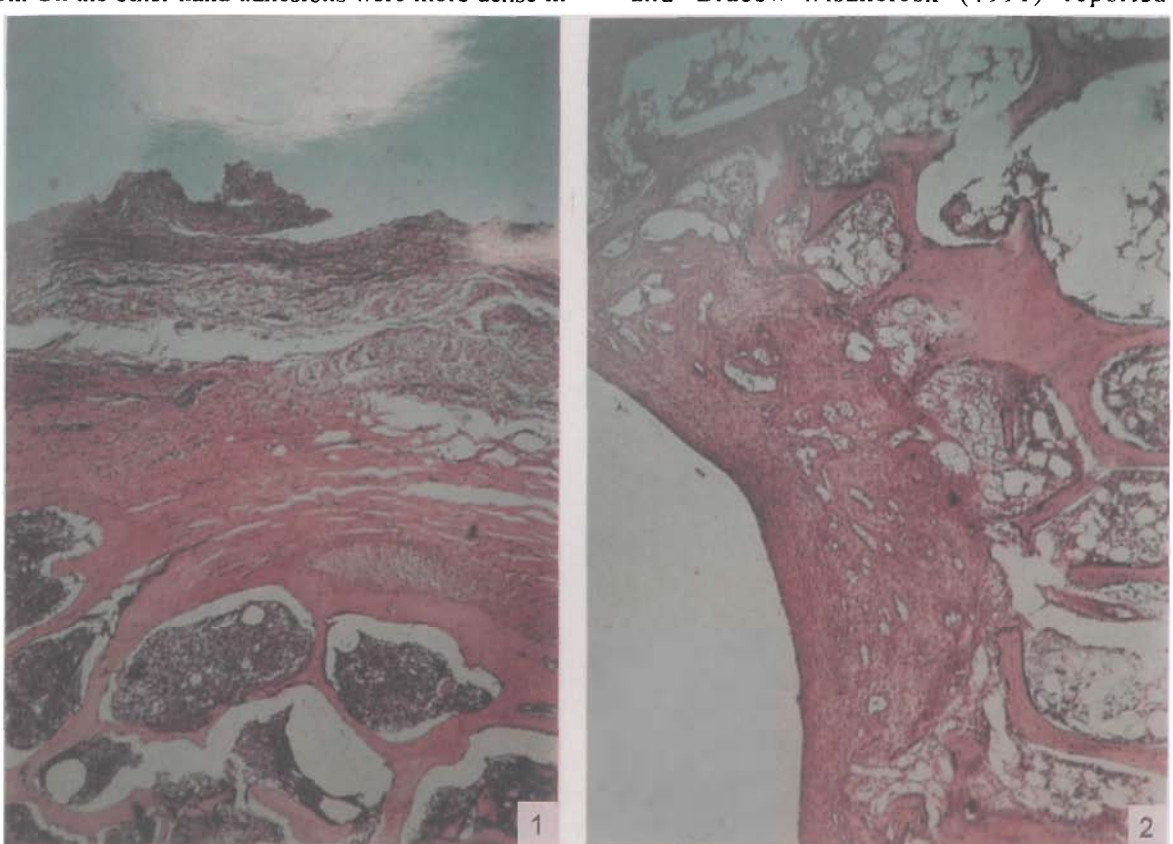
Autologous fascia lata and periosteal grafts used for resurfacing femoral trochleoplasty were readily accepted. The fascia lata graft displacement from the recipient bed occurred in 1 animal. This was due to poor fixing of the graft as suggested by Ohlsen and Widenfolk (1983) and also due to back of immobilisation of the limb. Patellar dislocation occurred in 1 animal in periosteal grafted group in early post operative days (POD).

Lameness gradually waned off as early as eighth post operative day in grafted groups whereas it continued to persist in animals of control group. Joint motion and freedom from pain was more in animals with grafts than animals of control group. Joint movements in control animals were characterized by crepitus, due to degenerative changes in the patellar articular cartilage.

Fibrinous adhesions were less dense in grafted groups than that in control group. Resurfacing the trochleoplasty provided smooth surface and hence aided in continuous passive joint motion. This in turn might have helped in less adhesion formation. On the other hand adhesions were more dense in control groups, where joint motion was totally restricted due to erroded trochlear surface.

There was not much abnormality in synovial fluid either quantitatively or qualitatively in grafted groups unlike control group. This is an essential feature for graft survival, since synovial fluid provided proper nutrition in the early stages. At the end of the fourth post operative week, both the grafts were accepted well and smooth glistening trochlear groove was noticed in both the groups 1 and 2. In control animals, the trochlear groove showed irregular, rough surface. By eighth week chondrogenesis was noticed in both the grafted groups. The deepened trochlear groove was covered entirely by white, smooth, cartilaginous growth covering the entire groove. But in control animals, the groove showed irregular fibrous tissue leaving few uncovered rough areas. The delayed chondrogenesis in control animals might be due to restricted joint motion, severe adhesions and less amount of synovial fluid in joint cavity.

Histological observations revealed regeneration of cartilage in the fascia lata grafted joints from deeper most layers of subchondral bone (Fig. 1). The linearly arranged, dividing chondrocytes were observed developing from the basal subchondral bone, by eighth post operative week. But Krol and Bracew-Wiszneiosk (1991) reported foci of



Figs 1-2. **1.** Fascia lata group: 4th post operative week-micrograph showing increased vascularity in superficial layers and few osteoclasts in the trabecular lacunae deeper layers of subchondral bone. **2.** Periosteal group: 4th post operative week-micrograph showing granulation tissue below the surface with ingrowth of blood vessels.

neochondrogenesis only after 12 weeks. The development of cartilage from periosteum was not only form the progenitor cells of periosteum but also from the pleuripotent mesonchymal cells in the subchondral bone as reported by Zarnett and Salter 91989).

In control animals, fibrovascular granulation tissue arising from subchondral bone filled the cartilage defect and many uncovered rough areas were noticed. It is concluded that resurfacing trochleoplasty is a must, to avoid patellar articular cartilage damage and to restore early joint function. Both fascia lata and periosteum showed granulation tissue below the surface with ingrowth of blood vessel (Fig.2) and chondrogenesis by eight post-operative week. Fascia lata provided as best alternative graft to the conventionally used periosteum graft.

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Yersinia pseudotuberculosis infection in lion *(Panthera leo)* cubs

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Key words: Animal health, Cubs, Septicaemic disease, Wild life, *Yersinia pseudotuberculosis*

Pseudotuberculosis due to *Y. pseudotuberculosis* has historically been associated with an acute to chronic zoonotic disease. The present communication records the isolation of *Y. pseudotuberculosis* from 2 lion cubs aged about 3 weeks. They developed sudden illness, stopped eating, quantity of faeces diminishing coupled with profound listlessness or depression and which succumbed to acute septicaemia. Heart blood, spleen, lung and liver tissues were collected under sterile condition and processed for bacteriological investigations. Intestinal contents were also collected and processed separately. The isolation of the organisms was attempted in selective and enrichment media. Impression smears from spleen, lung and liver were stained with Gram's and Giemsa stains following standard techniques. The morbid tissues were also collected in 10% buffered formalin for histopathological examination.

Based on cultural and morphological characteristics, the organisms indistinguishable from *Y. pseudotuberculosis* were isolated and characterized. A generalized hyperemia with haemorrhages in parenchymatous organs were seen. The lungs were oedematous and the gastrointestinal tract was almost empty. Impression smears showed large number of gram negative coccobacilli. Histopathlogically, tissue sections of lung, liver, spleen, kidney, heart, brain and intestine revealed extensive congestive and haemorrhagic changes which were more severe in liver and kidney. In addition, liver exhibited discrete microscopic areas of focal necrosis with many individual hepatocytes in variable stages of degeneration with clumps of the invading organism.

The kidney had massive glomerular haemorrhages besides tubular degeneration. Most of the necropsy and histopathological findings are in agreement with the earlier conclusions of Mair *et al.* (1967) and Obwolo (1976).

Y. pseudotuberculosis is considered to be a common inhabitant of intestine in a wide variety of domestic and wild animals that are often asymptomatic carriers. Wild birds and rodents are the reservoirs of the organisms. The transmission of the organisms of pseudotuberculosis under natural environment is confined apparently to oral-faecal routes either by direct contact with sick or asymptomatic carrier animals or through food contaminated by the excretions of these animals (Bercovier and Mollaret 1984). These observations on the transmission of the bacterium among lion cubs could be the possible reasons of infection.

The confirmation of association of this organism leading to death of cubs due to fatal septicaemia is alarming and calls for attention in routine diagnostic investigations. Therefore, efforts are warranted to control the transmission of this organism in zoological and national parks as well as in research institutes.

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SUMMARY

Nicropsy of 2 male lion cubs revealed an acute septicaemia disease. Morbid samples in the form of heart blood, spleen, lung and liver tissues were collected and processed for bacteriological investigation. Pure cultures of *Yersinia pseudotuberculosis* were observed separately from all the samples from both the cubs. Histopathologically tissue sections of lung, liver, spleen, kidney, heart, brain and intestine revealed extensive congestive and haemorrhagic changes suggestive of an acute septicaemia disease.

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Apoptosis induced by avian reovirus

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Key words: Avian reovirus, Apoptosis, Chicken embryo fibroblasts, Lymphocytes, Chicken

Apoptosis is a mode of programmed cell death that occurs under normal physiological conditions and may be induced by various biochemical or pathological stimuli (Teodoro and Branton 1997). Many viruses are capable of inducing programmed cell death pathways that lead to apoptosis of infected cells (Everett and McFaden 1999). Reoviruses are important models for studies on pathogenesis. Prototype mammalian reovirus strains type 1 Lang (TIL) and type 3 Dearing (T3D) are known to induce apoptosis in murine L929 (L) cells (Tyler *et al.* 1995). Apoptosis also occurs following reovirus infection *in vivo* (Oberhaus *et al.* 1997, Oberhaus *et al.* 1998) and studies show that apoptosis is an important mechanism of injury in reovirus infection (LaHair *et al.* 1998).

The following experiment was designed to determine avian reovirus (ARV) induced apoptosis in chicken embryo fibroblasts (CEF) and peripheral blood mononuclear cells (PBMC). ARV strain VA-1 isolated from a case of arthritis (Kataria *et al.* 1982) obtained from Avian Diseases Division, Indian Veterinary Research Institute, Izatnagar was titrated in CEF cell culture. CEF cell culture was prepared from 10-11 day-old chicken embryos as described by Merchant *et al.* (1960). CEF monolayers were infected with 0.1 ml of stock ARV (10^4 $TCID_{50}$ ml^{-1}) or plain medium M-199 and incubated at 37°C. At 24, 48 and 72 hr of infection, 1 each of infected and control flask was removed and cells were scraped and collected for cellular DNA extraction. To investigate apoptosis in PBMC, blood was collected from healthy control birds and PBMC were separated by density gradient centrifugation. After 3 washes in RPMI- 1640 medium containing 10% newborn calf serum, the cells were resuspended at a concentration of 5×10^6 cells ml^{-1} and 2 ml aliquots of cell suspension were incubated with 0.1 ml of live or heat inactivated stock ARV (10^4 $TCID_{50}$ ml^{-1}) or plain RPMI-1640 medium. At 12 hr of incubation, cells were pelleted for cellular DNA extraction.

Qualitative analysis of internucleosomal DNA fragmentation was done by agarose gel electrophoresis. The cell pellet of CEF or PBMC containing approximately 5×10^6 cells was lysed with 500 µl TTE solution (10mM Tris, 0.15% triton X-100, 1mM EDTA) and vortexed vigorously. The supernatant containing fragmented DNA was collected in a separate tube and DNA was precipitated from it using ice cold isopropanol. The precipitated DNA was pelleted by micro centrifuging the tube, air dried and dissolved in 20-50 µl TE (10mM Tris, 1mM EDTA) buffer.

The DNA extracted from ARV infected CEF showed a nucleosomal laddering pattern indicating DNA fragmentation (Fig. 1) which was most intense at 72hr post infection. The corresponding non-infected control CEF cultures did not undergo DNA fragmentation even at 72hr infection. The virus produced visible cytopathic effects (CPE) in CEF culture by 48hr of infection. The CPE was characterized by rounding of cells in isolated areas, which subsequently coalesced on further incubation. The cells detached from the glass surface by 48hr leaving round empty spaces in the monolayer giving it a cobweb appearance. The CPE was similar to that reported by Kataria *et al.* (1982) but the mechanism of induction of CPE by ARV is still unknown. This study provides evidence that ARV causes cellular DNA fragmentation in CEF cell culture, which is one of the characteristics of cells undergoing apoptosis. The results indicate that apoptosis could be an important mechanism of reovirus induced CPE.

The present study also showed that *in vitro* infection of chicken PBMC with ARV causes the cells to undergo apoptosis. The DNA extracted from PBMC exposed to live or inactivated ARV at 12hr post incubation showed a clear nucleosomal laddering pattern (Fig. 2) while the control culture did not exhibit DNA fragmentation. There was complete unresponsiveness of PBMC to *in vitro* antigen specific stimulation using ARV while performing lymphoproliferation assay. The *in vitro* stimulation with viral antigen resulted in activation of monocytes/ macrophages and depletion of lymphocytes in older cultures (72hr) (unpublished data). The activating effect of reovirus on cultured chicken bone marrow derived macrophages has been reported earlier by Bulow and Klasen (1983). The depletion of lymphocytes from *in vitro* cultures can be attributed to apoptosis induced by ARV.

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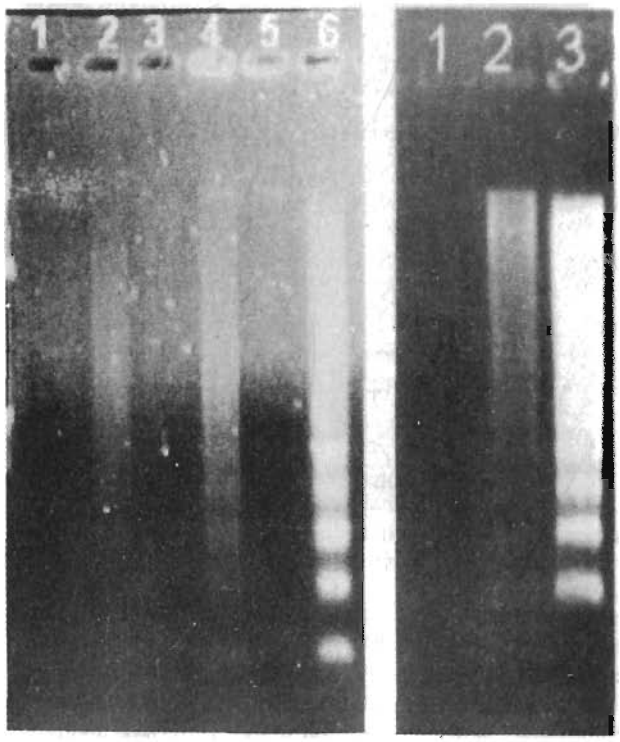

Figs 1-2. **1.**Avian reovirus strain VA-1 induced apoptosis in chicken embryofibroblast culture. Lanes 1,3 and 5 control 24, 48 and 72 hr cultures respectively. Lanes 2,4 and 6 infected 24, 48 and 72 hr cultures respectively. **2.** Avian reovirus strain VA-1 induced apoptosis in peripheral blood mononuclear cells on 12hr post inoculation. Lane1- control, Lane2- incubated with live virus VA-1, Lane3- incubated with heat inactivated VA-1.

Chickens infected with ARV are known to exhibit a transient immunosuppression during early stages of infection (Springer *et al.* 1983 and Montgomery *et al.* 1986), the mechanism of immunosuppression is not well understood. This study provides evidence that *in vitro* infection of CEF and PBMC with ARV causes cellular DNA fragmentation and causes cells to undergo apoptosis. The findings suggest that ARV induced immunosupression and accompanying pathological changes in lymphoid organs *in vivo* may be caused at least in part by the activation of programmed cell death in lymphocytes.

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Evaluation of polymerase chain reaction (PCR) for identification of virulent *Pasteurella multocida* and its comparison with animal inoculation test

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Key words: Animal health, Haemorrhagic septicemia, PCR, Pasteurella, Virulence

Haemorrhagic septicemia (H S) is an acute fatal septicaemic disease of cattle and buffalo caused by *Pasteurella multocida*. Dutta *et al.* (1990) estimated that in India, during the past 4 decades, this disease has accounted for 46- 55% of all bovine mortality resulting in heavy economic losses in India, during the past 4 decades.

Diagnosis of the disease is dependent on culture isolation and serologically using somatic antigen characterization. A combination of clinical signs, ;gross pathological lesions and epidemiological features coupled with isolation of the organism and identification of serotype will help in arriving at a definitive diagnosis (De Alwis 1990). PCR, nucleic acid probes and sequencing, it is possible to understand and identify the genes responsible for causing virulence. Brickell *et al.* (1998) developed a PCR assay which can detect pathogenic *P. multocida* serotype B: 2. Townsend *et al.* (1998) has developed species and type specific PCR identification of *P. multocida*. Rapid diagnosis of H S by PCR assay was also carried by Shivshankara *et al.* (2001) using different set of primers. Keeping these in view the present study was conducted to evaluate PCR for identification of virulent strains and compared with that of mice inoculation studies.

Bacteria

Field isolates (3) of *P. multocida* along with vaccinal strain P^{52} were collected from Diagnostic Microbiology Laboratory. These isolations were made from different places during outbreaks. Two field isolations were also made from cattle and buffaloes in an outbreak of HS during this work (Table 1). All the isolates were confirmed by biochemical tests (Table 2). DNA from vaccine (P^{52}) strain of *P. multocida* and an isolate of fowl cholera i.e. avian strain of *P. multocida* were used of PCR purposes.

PCR kit and HS specific primers as per Brickell *et al.* (1998) were procured commercially and the sequence were as follows:

IPFWD 5' - CGA AAG AAA CCC AAG GCG AA - 3'
IPREV 5' - ACA ATC GAA TAA TAA CCG TGA GAC - 3'

Preparation of culture lysate

Bacterial culture of 24 hr in 0.5ml volume was centrifuged the supernatant discarded. The pellet was resuspended in 20 µl distilled water and boiled for 10 min the tubes were immediately kept in an icebox.

PCR was conducted on 2 µl of culture lysate using culture lysate with 0.25µM of each primers, 1X PCR reaction buffer 10mM dNTP mix., 0.5 units of Taq DNA polymerase in 20µl.

Table 1. Details of clinical samples collected from outbreaks

Place	Source	Specimens
T. Dasarahalli,	Cattle	Blood, oedma
Bangalore urban	Buffaloe	fluid, long bone
District		
Karehalli,	Buffalo	Blood.
Kolar District		Nasal swab
Military dairy farm,	calf	Nasal swab
Bangalore		
Alapanahalli	Buffaloe	Nasal swab
Bangalore rural district		Blood
Davanagere	Buffaloe	Blood

Table 2. Results of biochemical tests

Isolate No.	Gram's recation	Catalase	Indole	Oxidase	Mice inoculation test	PCR
1	-ve	+ve	+ve	+ve	Avirulent	-ve
2	-ve	+ve	+ve	+ve	Avirulent	-ve
3	-ve	+ve	+ve	+ve	Virulent	+ve
4	-ve	+ve	+ve	+ve	Virulent	+ve
5	-ve	+ve	+ve	+ve	Virulent	+ve

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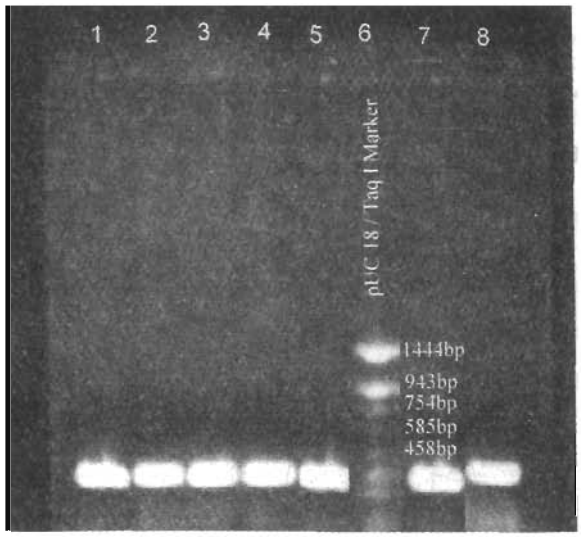

Fig. 1. Gel showing specific amplification in samples suspected for haemorrhagic septicemia by PCR.

The programme of amplification was denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 30 sec for 35 cycles performed on MJ thermal cycler. Amplified products were separated by agarose gel electrophoresis using 1.5% agarose on tris, boric acid and EDTA (TBE) buffer, stained with ethidium bromide. The DNA fragments were viewed by UV transilluminator and documented using gel documentation system.

Mice inoculation test

Broth culture (0.2ml, 18hr old was inoculated subcutaneously to adult mice weighing about 300g. The inoculated mice were kept under observation for 1 week. Virulent cultures killed the mice with in 24 hr (Table 2).

All the 5 isolates confirmed to *Pasteurella* species by biochemical tests (Table 2). On TSI media all isolates gave slow acid reaction with no gas and no detectable hydrogen sulfide.

The oligos did not amplify the isolates that had been maintained on culture and had undergone passage over a period of several months (Fig.1) but the same isolates had shown amplification when they were freshly isolated. These results clearly indicated that the organism have lost their virulence when kept for several months and undergone several passages, and is incapable of causing the disease but had all biochemical characters intact. This was confirmed by mice inoculation test in which the mice did not die. Thus it confirms that the PCR technique using the above said primers can be used as a tool to differentiate virulent and avirulent *Pasteurella* and it also confirms that the organism loses its virulence over a period of time when maintained under laboratory conditions.

It was also observed that the DNA from vaccine strain p^{52} strain of *P. multocida* gave amplification and fowl cholera isolate i.e. avian strain of *P. multocida* did not gave amplification. These results are on par with observations made by Brickell *et al.* (1998) who reported that these primers would specifically amplify *P. multocida* serotype B: 2.

SUMMARY

Polymerase chain reaction (PCR) was performed to identify virulent isolates of *P. multocida* using IPFWD and IPREV primers and compared with that of mice inoculation tests. Out of 5 isolates tested, 3 isolates showed amplification and killed mice where as 2 isolates which have undergone several passages did not show amplification and nonpathogenic to mice. All the 5 isolates were confirmed to *Pasteurella* species by cultural and biochemical tests.

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Isolation and *in-vitro* sensitivity pattern of pathogenic *Escherichia coli* from diarrhoeic lambs and calves

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Escherichia coli is the predominant gram-negative aerobic organism in the intestinal tract in man, animals and poultry. While *E.coli* confined to intestinal tract are commensals, but in certain circumstances may produce disease (Carter *et al.* 1995). From standpoint of pathogenic mechanisms and disease 4 major categories of *E. coli* are recognized: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) and enterohaemorrhagic *E. coli* (ETEC). In addition, 2 less-well-defined *E. coli* categories recognized in animals and humans are enteroaggregative and cytotoxin necrotizing factor-positive. The aforementioned categories are represented by different serotypes. Serotypes are combinations of O (cell-wall lipopolysaccharide), H (flagellar protein) and K (capsular polysaccharide or envelope) antigens (Collee *et al.* 1996). The isolation and distribution of various *E. coli* serotypes was studied in India by Sarma *et al.* (1987) and Dubey *et al.* (2000). Similarly, the prevalence of *E. coli* serotypes in various pathological samples in Kashmir valley was studied by Wani *et al.* (1989) and in normal and repeat breading cows by Seh *et al.* (2000). The present study reports the prevalence of various serotypes, especially pathogenic ones, in diarrhoeic calves and lambs, their antimicrobial susceptibility and pathogenic public health significance.

Faecal samples (40) collected from Sheep Breeding Farm, Keva; Sheep Breeding Farm, Goabal; Cattle Breeding Farm, Manasbal and Military Dairy Farm, Srinagar, were processed. The samples were collected in sterile plastic tubes and carried on ice to laboratory. Out of these 10 samples were intestinal contents from 10 dead lambs, which showed the symptoms of septicaemia and haemorrhagic enteritis and 10 samples were from live diarrhoeic lambs as well as 20 were from live diarrhoeic calves. The samples were directly cultured on MacConkey's agar at 37°C overnight. The rose pink colonies were picked up and subcultured on eosin methylene blue (EMB) agar plates to observe the characteristic metallic sheen. The isolated colonies were picked up in nutrient agar cultural slants as pure, culture and subjected to standard morphological, biochemical tests (Edwards and Ewing 1972). All the confirmed *E. coli* isolates were got serotyped from National Salmonella and Escherichia Centre, Central Research Institute, Kasauli. The *in-vitro* susceptibility of isolates to antimicrobial agents was determined by single disc diffusion method described by Bauer *et al.* (1966). The antimicrobial discs used for the study were gentamicin, tetracycline, erythromycin, cefadroxil, ciprofloxacin, chloramphenicol, ampicillin/cloxacillin and norfloxacin, marketed by HiMedia, Mumbai (India). Interpretation of isolates as sensitive and resistant was made as per the interpretation chart of the supplier.

Out of 40 diarrhoeic faecal samples, 30 isolates of *E. coli* were recovered. Out of these 30, 2 isolates were untypable and 3 were rough. The remaining 25 *E. coli* isolates belonged to 15 different serotypes. The prevalence of various serotypes and their relative distribution in diarrhoeic lambs and calves in Kashmir valley is given is Table 1. These isolates were subjected to antimicrobial sensitivity test against 8 antibiotics. The *in-vitro* sensitivity showed that all isolates were sensitive to chloramphenicol, 90% to gentamicin, 86.6% to ciprofloxacin and norfloxacin and 26% to tetracycline while as all isolates showed resistance to erythromycin, cefadroxil and ampicillin/cloxacillin.

Infection with enterotoxigenic *E. coli* (ETEC) in calves and lambs takes the form of diarrhoea and dehydration induced by enterotoxins (Acres 1985). Two main classes of enterotoxins are heat labile (LT) and heat resistant (ST). Of the latter, there are 2 subtypes – Sta and STb. The ETEC group is represented by various serotypes. In the present study,

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Table 1. Prevalence of *E. coli* serotypes in faecal samples of diarrhoeic lambs and calves in Kashmir valley

Serotype	No. of isolates from			Percentage	Category / public health status
	Calves (20)	Lambs (20)	Total (40)		
O3	1	0	1	3.33	Enteroaggregative / AEEC
O8	0	4	4	13.33	ETEC/ zoonotic
O9	0	1	1	3.33	-
O15	1	0	1	3.33	ETEC/ zoonotic
O26	1	0	1	3.33	EPEC/ zoonotic
O38	0	3	3	10.00	-
O55	2	0	2	6.66	EPEC
O59	0	1	1	3.33	-
O75	2	0	2	6.66	-
O78	1	0	1	3.33	ETEC/ zoonotic
O88	1	0	1	3.33	-
O102	0	1	1	3.33	-
O107	2	0	2	6.66	-
O114	0	2	2	6.66	EPEC
O170	0	2	2	6.66	-
Rough	1	2	3	10.00	-
UT	1	1	2	6.66	-
Total	13	17	30	100.00	-

Figures in parenthesis indicate total number of samples. ETEC= Enterotoxigenic *E. coli*, EPEC=enteropathogenic *E. coli*, AEEC = attaching and effacing *E. coli*.

O8 was the most predominant serotype isolated from 13.33% of the samples followed by O38 (10.00%) and O170, O114, O75, O107and O55 (6.66% each). The remaining serotypes i.e. O3, O9, O15, O59, O78, O88 and O102 had the prevalence rate of 3.33%. These findings are similar to that by Blanco *et al.* (1996) and Wray *et al.* (1991) they also reported that out of bovine and ovine isolates O8 was the most frequent serotype. However, in this study O8 and O114 were isolated from lambs, while as O15, O26, O55, and O78 from calves only. The strains O3, O15, O26, O38, O55, O59, O102, O107, O114 and O170 are being reported for the first time from Kashmir valley. Serotypes O8, O15 and O78 belong to ETEC group. Levine (1987) and Dubey *et al.* (2000) reported that O8 serotype produce LT. Serotypes O26, O55 and O114 are enteropathogenic and are known for production of diarrhoea in infants and adults (Crichton 1996). O3 serotype has been found attaching and effacing *E. coli* strain and containing beta intimin gene identical to those of rabbit/ human EPEC, thus may be associated with enteric disease in small ruminants (Cid *et al.* 2001). Similarly, Kang *et al.* (2001) have reported the gut colonization by oral infection with O3: H2 strain of *E. coli* in adult rabbits.

Isolation of serotypes O3, O8, O15, O26, O78 and O114 from diarrhoeic calves and lambs is of major public health concern as their zoonotic significance is established, and these have been isolated very frequently from infantile diarrhoea suffering from gastroenteritis and travellers sickness (Levine 1987; Crichton 1996 and Kang *et al.* 2001). Serotype O75 was incriminated with pyelonephritis and had contributed to discovery of P fimbriae, which adhere to specific receptor molecules on urinary tract epithelial cells (Crichton 1996). Hence, the isolation of these pathogenic *E. coli* serotypes from these animals indicates a potential threat to human health from animals and their products.

The *in-vitro* sensitivity of *E. coli* isolates showed that 90% isolates were sensitive to gentamicin and 86.6% to ciprofloxacin and norfloxacin. These findings are in agreement with the findings of Seh *et al.* (2000) they also reported their 90.90% sensitivity to gentamicin, ciprofloxacin and norfloxacin and complete resistance to erythromycin, cefadroxil and ampicillin/cloxacillin. Blanco *et al.* (1996) also recorded the highest percentage of resistance to ampicillin. In present study, all the isolates were sensitive to chloramphenicol, which is contrary to the findings of Blanco *et al.* (1996) as they observed the highest percentage of resistance to chloramphenicol. Only 26% of strains were sensitive to tetracycline, and this is the most commonly used antibiotic by Kashmir veterinary clinicians. Thus these findings have the therapeutic implications in treatment of neonatal diarrhoea of domestic livestock.

SUMMARY

Escherichia coli was isolated from diarrhoic calves and lambs. Out of 40 samples, 40 isolates of *E. coli* were recovered, which belonged to 15 sero types, 2 were untypable and were

rough. These isolates were subjected to 8 antibiotics for testing their antimicrobial sensitivity. These tests revealed that 90% isolates were sensitive to gentamicin and 56.6% to ciprofloxacin and norfloxacin.

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Warble (*Hypoderma diana*) infestation in Indian gazelle (*Gazella gazella bennetti*)

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Infestation of deer with the larvae of *Hypoderma diana* causes serious damage to hides. Occasional deaths due to anaphylactic shock or toxaemia and damage to the central nervous system by *Hypoderma diana* occurs primarily in deer, whereas sporadic cases in sheep of heavy infestation causing severe ill health with mortality have been reported (*Brauer* 1858). *Hypoderma diana* may also cause human myiasis (Beaver *et al.*1984) A male deer of 4 years was brought to Government Polyclinic, Bikaner, from local zoo with history of eruptions at the dorsum and gluteal regions for the last 20 days. The eruption was soft and fluctuating measuring about 2-3 cm in diameter. There may be 60-70 such lesions on the animal. The animal felt pain on touch. The *Hypoderma* larvae was removed by slowly injecting 0.5ml of 3% of hydrogen peroxide solution into the breathing hole using a blunt needle shank of the syringe and taking care not to pierce the grub. Most grubs will emerge within 15 sec after foaming action of hydrogen peroxide begins and leaving behind cleansed cavity (Scholl and Barrett 1986) some larvae were removed by squeezing through gloved forefinger and thumb. The cavity is then painted with povidone iodine. Injection of ivermectin 0.5ml subcutaneously (@ 0.3mg/kg body weight) was given to kill remaining larvae, which couldn't be removed manually. Injection of phenylbutazone 2ml intramuscularly (@ 20mg/kg body weight) was given to combat any toxins likely to be released from dead larva which can cause systemic shock and local inflammatory reactions (Eyre *et al.* 1981) The length of larvae was 6-20mm and each segment of larva beared number of flat tubercles with spines in a row as revealed by hand lens examination. The colour of larvae varied from whitish to light brown. The posterior spiracle of larva was removed and treated with 10% KOH for several hours and mounted on slide and examined under dissecting microscope. A pair of kidney shaped posterior spiracles with numerous pores were observed which is pathognomonic to the larvae of *Hypoderma diana*.

Haematological values of blood sample taken from infected animal before treatment reveals haemoglobin-8.0g%, PCV-28%, TLC-12.0x10^3/_µl, DLC: - Neutrophil-42%, eosinophil-8%, lymphocyte-44%, monocyte-0.5%, total protein-2.9g%. Against the normal values reported by Fowler (1986) were haemoglobin contents-10.9 – 14.1g%, PCV-35.0 – 39.66%, TLC4.9 – 13.5x10^3/_µl, neutrophil-20.7- 69.8%, eosinophil-0.25-0.75%, total protein-3 – 7.5g%.

The animal responded very well with ivermectin administration and eruption started decreasing after 20th day of medication although most of larvae were removed manually.

The adult female flies glue their several hundreds of eggs on hairs on legs of host during warm weather of May or June, hatching occurs after 4 to 6 days and larva penetrate the skin and migrate to spinal canal (Blood and Radostits 1994) & rest there for 2 to 4 months. Finally they migrate to subcutaneous tissues of the back, cut breathing holes in the skin to which they appose their spiracles, and, moulting twice, grow larger, forming warbles. The diagnosis of *Hypoderma* infestation is generally based on the presence of these warbles in the back of animal (McCurnin 1998). If animal kept untreated then these larvae will fall to ground within 1 month and pupate on ground, adult flies emerges some 3 – 5 weeks later.

SUMMARY

A male deer from zoo having eruptions at the dorsum and gluteal region was studied. the larvae were removed and found to be of *Hypoderma diana*.

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Gross and histopathological studies on biocasings for second intension urethral healing in dogs*

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A prescrotal urethrotomy is usually made in dogs to remove caliculi lodged caudal to ospenis. Controversy exists over the closure of urethrotomy incision. Some surgeons prefer to allow urethrotomy incision to heal by second intension healing (Brown 1975, Wingfield and Rawlings 1979 and Gahring 1983). Stricture formation is a common complication after primary or secondary closure healing of urethral incisions (Brown 1975 and Stone 1984). Vikram Mohindra *et al.* (1996) reported the advantage of wrapping amniotic membrane around the urethrotomy incision to avoid stricture formation. An attempt was made to evaluate the efficacy of biocasings, viz. gelatin, fibrin and amniotic membrane for second intention healing in dogs.

Mongrel dogs (32) weighing 12-26 kg were premedicated with triflupromazine HCl @ of 2mg/kg b wt intramuscularly 10 min prior to the general anaesthesia. 2.5% W/V solution of thiopentone sodium @ of 25 mg/kg b wt intravenously was administered to induce and maintain general anaesthesia. The dogs were placed in dorsal recumbency and the urinary bladder was catheterized by using suitable size of the polyethylene tube and the catheter was left *in situ* by fixing it at preputial orifice. A prescrotal urethrotomy was performed in all the animals and randomly divided in to 4 groups of 8 animals each. The urethrotomy incision was left unsutured in control animals (group 1) and wrapped with gelatin, fibrin and amniotic membrane in animals of groups 2, 3 and 4 respectively. The cutaneous incision was sutured after leaving the retractor penis muscle over the urethrotomy incision with horizontal mattress pattern. The catheter was removed on third postoperative day. The biological materials were processed at the Central Leather Research Institute, Chennai, and supplied in a sealed ampoules/polyethylene packs in a required size.

Animals (2) from each group were euthanized on 7, 14, 30 and 60th postoperative day. Detailed autopsy was conducted and gross lesions at the operative site were observed. The tissue from urethrotomy site was collected and preserved in buffered normal saline. The tissues were then processed, 5μ thickness sections were cut and stained with a haematoxyline and eosin.

Gross examination of the operative site on day 7, revealed congestion and edema in all the groups. Congestion was severe in animals of group 1 (control) and group 2 (gelatin), whereas fibrin and amnion showed adherence to the operative site. The adhesions were thick in control and gelatin groups, filamentous in fibrin and thin in amnion groups. Animals of gelatin group showed presence of haematomas. Varying degrees of adhesions were noticed with the surrounding tissue at the operative site, indicating severity of inflammation. Poogrid and Wood (1986) and Vikram Mohindra *et al.* (1996) reported that inflammation might be due to a snugly fitting catheter, which allowed urine leakage. As the time advanced, the congestion and edema decreased gradually. On 14th day, the operative site was completely covered with fibrous tissue. Mild to moderate congestion and edema were noticed in subcutaneous tissue around the operative site. Two dogs from control and gelatin groups showed haemorrhagic foci on the urethral mucosa while, fibrin and amnion groups showed normal urethral mucosa.

On day 30 necropsy revealed mild changes at the operative site when compared to previous observation and healing at the operated site was complete. Urethral mucosa was clear without any haemorrhages in all the groups. No remnants of gelatin, fibrin and amnion material were observed. At the operative site there was mild swelling in gelatin group, whereas urethra in fibrin and amnion groups showed smooth mucosal surface. On 60th day in control and gelatin groups the urethral lumen was stenosed while fibrin and amnion groups showed patent urethral lumen. The incision site was completely sealed by the casings without any inflammatory

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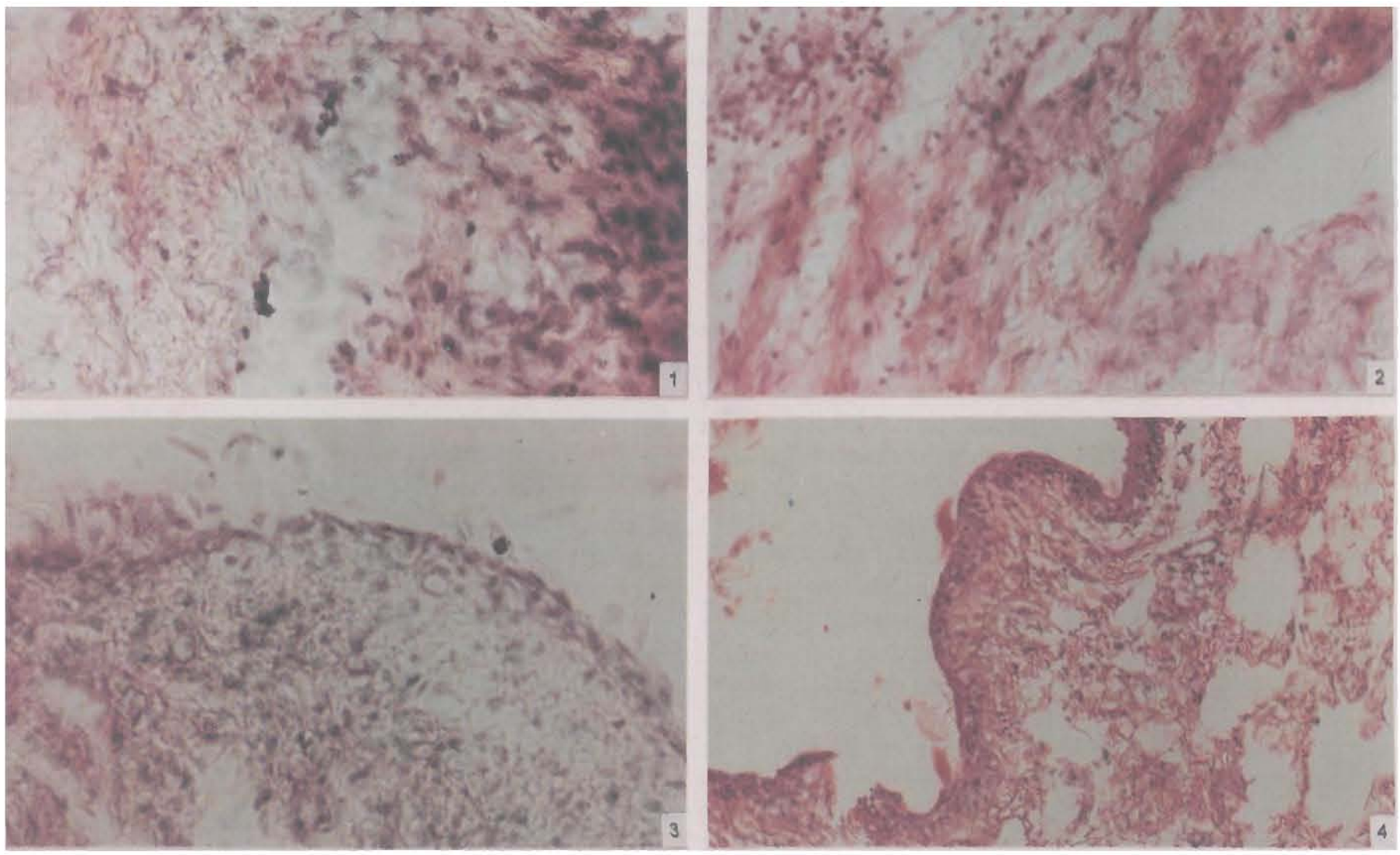
reaction in all the groups. Brown (1975), Wingfield and Rawlings (1979) observed stricture formation in both sutured and unsutured urethral incisions. On the contrary, Bellah (1989) and Vikram Mohindra *et al.* (1996) reported stricture formation in sutured urethra. Haller *et al.* (1987) did not observe stricture following perineal urethrotomy. Waldron *et al.* (1985) and Weber *et al.* (1985) observed slight widening of the urethral lumen following urethrotomy.

There are well defined stages through which wound healing of urethra progresses. Substrate, proliferative and maturation phases result in epithelialization, synthesis of fibrous protein and scar tissue contraction respectively (Peacock 1984). Accurate alignment of incised urethral edges is important since excessive granulation tissue formation occurs when uroepithelium does not cover the urethral wound (Bellah 1989). Inaccurate uroepithelial apposition often results in stricture and may cause complete obstruction. Scar tissue remodeling in corpus spongiosum leads to luminal stenosis over a period of months (Peacock 1984).

Histopathological studies

On seventh day, histologically there was infiltration of lymphocytes in the mucosa and submucosa of the urethra in all the groups (Fig.1). Urethra in control and gelatin treated groups showed severe inflammatory reaction when compared to fibrin and amnion groups, while these groups showed angioblastic and fibroblastic proliferation and mild infiltration of neutrophils and macrophages (Fig. 2). Remnants of biocasings were observed histologically which indicated incomplete healing process. The urethral lumen was patent. Fibroblasts oriented horizontally with collagen fibres at the site of application in fibrin encased urethra whereas amnion wrapped urethra showed urethral lumen without indication of capillary bed formation. Denudation of the epithelium at the incision site was evident in gelatin wrapped urethra.

The sections from 14 th day revealed abundant fibrous tissue below the urethral mucosa at place of incision in control group whereas the fibrous tissue formation was moderate in gelatin and mild in fibrin and amnion groups. Inflammatory cells were more in control and gelatin and less in fibrin and amnion groups with severe angioblastic activity for new capillary bed formation. The epithelialization was incomplete in all the groups however signs of epithelialization were evident (Fig.3). The urethral lumen was patent Colocho *et al.* (1974) observed fibrous and inflammatory action with amnion whereas Thomson and Parks (1984) reported stimulation of epithelialization by amnion. Jadon *et al.* (1985) reported marked angioblastic and fibroblastic activity with mild



Figs 1-4. **1.** Infiltration of Lymphocytes in the mucosa and submucosa of the urethra. control group on day 7 (H&E100×); **2.** Angioblastic and fibroblastic proliferation and mild infiltration of neutrophils and macrophages. amnion group on day 7 (H&E 200×); 3. Signs of epithelialization-fibrin group on day 14 (H&E 630×); **4.** Complete epithelialization with neovascularization zone. Amnion group on day 30 (H&E 200×)

infiltration with amnion. Control and gelatin treated animals showed more inflammatory cells compared to fibrin and amnion treated groups.

Most of the sections on 30th day showed complete epitheliazation with neovascularized zones (Fig. 4). Fibrous tissue formation was abundant in control group moderate in gelatin and amnion groups and less in fibrin group. Mild inflammatory reaction with accumulation of neutrophils and lymphoid cells in the mucosal and sub mucosal layers was evident in control and gelatin groups. Fibrin groups showed horizontal infiltration of collagen fibers.The sections from 60th day revealed more fibrosis in control and gelatin group and less fibrosis in fibrin and amnion groups around incision site. The epithelialization was complete in all groups and there was no evidence of any inflammatory reaction and hemorrhage. The urethral lumen in control and gelatin groups was stenosed while the other groups showed patent lumen. Few sections showed focal nodular fibrosis in control and gelatin treated urethra with stenosis. Marked fibrosis and severe epithelial necrosis was noticed in urethra where urethral wall was not sutured (Weber *et al.* 1985, Vikram Mohindra *et al.* 1996). Animals of groups 3 and 4 showed less fibrosis with patent urethra. Peacock (1984) mentioned that scar tissue remodeling in peri urethral space leads to urethral stenosis.

In the present study, among the 3 biocasings used fibrin treated group showed comparatively better results followed by amnion and gelatin groups. Teh (1979) observed that, fibrin application stimulates the production of fibroblasts with increased vascularization at the grafted site. Enhanced phagocytosis, haemostatic effect and increased vascularization using fibrin were also reported (Schumacher *et al.* 1996). Wheaton *et al.* (1994) mentioned the haemostatic effect without inflammatory process and adhesions at the grafted site with fibrin application. Troensegaard and Henson (1950), Trelford and Trelford (1979) and Robson (1981) reported rapid epithelialization, pain reduction and increased vascularization properties of amnion during healing process. Though the results are in agreement with the previous reports the efficacy was lower than the fibrin groups. Orikasa and Tsuji (1970) observed early epithelialization histologically while Ganesh *et al.* (1994) observed faster healing process with gelatin sheet. However this group showed leakage, fistula formation and strictures indicating the ineffectiveness of the material for its usage at operative site. The biocasings proved to be satisfactory in controlling postoperative complications and to accelerate healing of urethrotomy wound in experimental dogs. Hence these can be used safely for clinical cases.

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Effects of polyherbal antistress and adaptogen drugs and levamisole on egg laying, hatching rates, weights and growth of White Leghorn chicken

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Key words: Adaptogen, Antistress drugs, Gradable egg, Growth,Hatching rate, Hatching weight, Poultry, White Leghorn

The intensive system of management and breeding inevitably expose the birds to various stresses like transportation, vaccination, medication and handling for regular checking (Shrivastava 1994). In stress conditions, there is excessive release of catecholamines, which stimulate the activity of muscles and glycogenolysis and results in the loss of productivity of birds. Now a days, use of antistress, adaptogen and immunostimulating drugs has become a common practice in the poultry industry. A polyherbal antistress and adaptogen, had been reported to stimulate growth in broiler chicken (Pradhan 1995). Therefore, the present study was undertaken to assess the effects of these antistress drugs (immuno stimulation), the extract of *Withania, Somnifera, Oamum sanctum* and *Manjifera indica*. Levamisole hydrochloride of yecemic 2, 3, 5, 6 phenol midozo (2, 1–6) thiazole on the body weight (growth), feed efficiency, egg weight, grading and hatching rate of eggs of White Leghorn chicken.

Unvaccinated day-old White Leghorn chicks (120) from the same hatch were randomly divided into 3 equal groups (1, 2 and 3) and maintained under deep litter system of management with *ad lib.* supply of feed and water and were routinely vaccinated with Newcastle disease, fowl pox and infectious bursal disease vaccines. The male to female ratio was maintained at 1: 10. Group 1 was administered with AD1 @ 10 ml/100 chicks per day from day-1 up to day-21 and @ 20 ml/100 chicks per day from day-35 up to day-56 in drinking water. Group 2 was administered with livamisole @ 1 mg/50 g body weight per day from day-1 up to day-21 and at the same dose from day-42 up to day-60 in drinking water. Group 3 was kept as untreated control. From each group 10 birds were weighed randomly at weekly intervals up to 20 weeks of age. The average feed consumption and feed conversion ratio (FCR) were calculated at 8, 12, 16 and 20 weeks of age. Eggs (30) from each group were randomly collected at second, third and fourth week of laying. The eggs were graded (grade A, grade B and nongradable) on the basis of egg weight and various quality factors (Singh 1990). The exterior quality factors include shape, soundness and cleanliness of shell and the interior quality factors, determined by candling, include condition of air cells (regular and clean outline, depth up to 4 mm), albumen (firm and thick) and yolk (well centred with indistinct outline). The gradable eggs were incubated to hatch and the progeny chicks (groups S_1, L_1 and C_1 from breeder groups 1, 2 and 3 respectively) were weighed on 0-day and then up to 4 weeks of age at weekly intervals. The statistical

Table 1. Average feed consumption (g), body weight (g) and FCR of breeder groups (1, 2 and 3) at 8, 12, 16 and 20 weeks of age and body weight of the progeny groups (S_1, L_1 and C_1) at 0-day and 4 weeks of age

Breeder	8th week			12th week			16th week			20th week				Body weight (g)	
group	Feed consumption (g)	Body weight (g)	FCR	Feed consumption (g)	Body weight (g)	FCR	Feed consumption (g)	Body weight (g)	FCR	Feed consumption (g)	Body weight (g)	FCR	Progeny group	0-day	4th week
1	1517 ±6.98	532 ±0.80	2.85	2774 ±7.51	920 ±1.79	3.02	4307 ±9.61	1195 ±2.66	3.60	6314 ±8.84	1510 ±2.84	4.18	S_1	41.55 ±0.16	150.40 ±0.60
2	1526 ±8.99	538 ±1.64	2.84	2866 ±6.13	956 ±1.70	3.00	4321 ±9.98	1240 ±1.78	3.48	6423 ±8.83	1465 ±2.78	4.35	L_1	40.00 ±0.18	152.50 ±0.82
3	1600 ±6.70	513 ±1.33	3.12	3001 ±8.06	902 ±1.68	3.32	4407 ±7.64	1142 ±1.75	3.86	6509 ±9.77	1410 ±2.59	4.62	C_1	39.45 ±0.15	128.00 ±0.68

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Table 2. Average egg weights and percentages of gradable eggs at different weeks of laying from three different breeder groups

Weeks at laying	Group 1		Group 2		Group 3	
	Average egg weight (g)	Gradable eggs (%)	Average egg weight (g)	Gradable eggs (%)	Average egg weight (g)	Gradable eggs (%)
Second	46.13±0.12	65 (32+33) *	46.02±0.54	60 (30+30)	44.10±0.12	54 (24+30)
Third	48.56±0.56	82 (38+44)	47.42±0.19	80 (35+45)	45.51±0.14	70 (35+35)
Fourth	49.83±0.19	88 (48+40)	48.30±0.18	86 (42+44)	46.23±0.16	75 (40+35)

*The first and second figures within parentheses indicate the gradable egg percentages obtained from grade A and grade B, respectively.

analysis was carried out as per Snedecor and Cochran (1980).

The data showing the effect of AD_1 and levamisole on feed consumption, body weight and feed efficiency (Table 1) showed that AD_1 and AD_2 had no significant effect on feed consumption of birds at 8, 12, 16 and 20 weeks of age. Similar findings were also reported by Behl *et al.* (1995) and Prasad *et al.* (1998) who reported that feeding of ascorbic acid (antistress agent) did not have significant effect on feed consumption. The groups 1 and 2 gained higher body weight and better feed efficiency at the above ages than the untreated control which was statistically nonsignificant. The result is in accordance with Pradhan (1995) who reported higher body weight and feed efficiency in AD_1 treated broiler chicken.

The first egg was laid by a hen of the groups 1, 2 and 3 at 135 days, 127 days and 150 days, respectively, which indicated that $levamisol_2$ treated group 2 attained the sexual maturity earliest followed by 1 and the control group 3. This might be due to the growth promoting, adaptogenic and immunostimulating effects of the drugs.

The mean egg weights (Table 2) revealed that the group 1 and group 2 laid noticeably larger eggs than the untreated control group 3 at second, third and fourth week of laying which was statistically nonsignificant. An increase in egg weight on supplementing ascorbic acid, an antistress agent, in diet was reported by Orban *et al.* (1993). Table 2 showed that the group 1 produced higher percentages of gradable eggs followed by group 2, while group 3 produced much lower percentage of gradable eggs. This clearly indicated that AD_1 had positive effect on egg quality. Maiti *et al.* (1994) reported that egg production was more pronounced in a herbal antistress and adaptogen treated layers and average egg weight was significantly higher than the untreated birds even in cold stress.

The hatching percentages were 74%, 70% and 68% in groups 1, 2 and 3 respectively. The size of the egg is a trait of economic importance in poultry which affects hatchability. Asusquo and Okon (1994) found that hatchability of medium (45-52 g) and large (53-59 g) size eggs were higher than the small (38-44 g). The average hatching weights (0-day) in the progeny groups S_1, L_1 and C_1 were 41.55 g, 40.00g and 39.45 g respectively. Sharma and Bora (1960) observed that the hatching weight of chicks increased with the increase in egg weight. The average weekly body weight of the progeny groups increased in similar trend up to 4 weeks of age (Table 1). The body weight of the progeny chicks of group S_1 and L_1 were noticeably higher than the progeny chicks of group C_1.

SUMMARY

The study of antistress drugs and levamisole on feed consumption, body weight, FCR, age at sexual maturity, egg production, hatching rate and body weight of progeny chicks revealed that it had positive effects upon these traits.

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Restoration of phagocytic function of the reticuloendothelial system by levamisole in dexamethasone-treated rabbits*

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Key words: Parasitology, Phagocytic function, Rabbit, Reticulo endothelial system

The survival and capacity of production of an animal depends on the successful body defence against microbial invasions. Dexamethasone, a commonly used drug in veterinary medicine causes immuno suppression (Koptopoulos *et al.* 1992). Levamisole, having anthelmintic and immunomodulatory property, restored defective immune function under certain clinical and laboratory conditions (Renoux 1978). In this work, an attempt was made to know whether levamisole could restore the immunosuppression in dexa methasone – treated rabbits.

Four groups of 6 New Zealand White crossbred male rabbits weighing between 800 g and 1200 g obtained from Department of Laboratory Animal Medicine, TANUVAS, Madhavaran, Chennai were used for the study. All the animals were maintained under identical environmental conditions and were given a balanced diet. Dexamethasone Na and levamisole HCl were used as pure salts. The following experimental design was used.

Group 1: Untreated control
Group 2: Treated with dexamethasone Na @ 2 mg/kg sc for 7 days
Group 3: Treated with levamisole HCl @ 3 mg/kg sc thrice a week,
Group 4: Treated with dexamethasone Na @ 2 mg/kg sc for 7 days + levamisole HCl @ 3 mg/kg sc thrice a week.

On day 10, 0.5 ml of colloidal carbon was given intra venously to all the groups of rabbits. Blood samples were collected (25 µl) from ear veins after 5, 10, 15, 30, 60 and 90 min and lysed in 3 ml of distilled water. The optical density was measured spectrophotometrically at 650 nm. The graph of absorbance against time was plotted and phagocytic index calculated (Pallabi De *et al.* 1998) using the formula,

Phagocytic index = R_{ct}/R_{cc}

where R_{ct} = Regression coefficient (treatment)

R_{cc} = Regression coefficient (control)

On day 21, the animals were slaughtered and the spleen weight was recorded.

The slopes and phagocytic indices of the 4 groups are shown in Table 1. Dexamethasone group showed a value of 0.54 which implies that the phagocytic function of the reticulo endothelial system have decreased. Levamisole group were active in normal (1.52) and more active in immunosuppressed animals (2.12) thus restoring the suppressed phagocytic function. When proper colloidal suspension of particle sizes which do not cross the capillary barriers were injected intravenously, the particles were phagocytosed by the kupffer cells of the liver and the reticular cells of the spleen (Biozzi *et al.* 1958). The suppressive effect on immune system by

Table 1. Effect of drugs on phagocytic function (R_{ct}/R_{cc}) and spleen weight (mean±SE)

Group	Slope (regression cofficient)	Phagocytic index (R_{ct}/R_c)	Spleen weight (mg/100gBW)
I (control)	–0.0058	1	30.35±1.61^{ab}
II (dexamethasone)	–0.027	0.54	22.17±0.95^{a}
III (levamisole)	–0.0076	1.52	34.43±1.14^{ab}
IV (dexamethasone + levamisole)	–0.0106	2.12	35.99±1.52^{ab}

Values of R_{ct}/T_{cc} between 1 and 1.5 are active; values of R_{ct}/R_{cc}>1.5 are more active; means bearing superscripts (a, b) differ significantly between groups (P<0.01).

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dexamethasone is indicated by its less activity in carbon clearance, in part functionally impairing cells of mononuclear phagocytic system (Jain 1986). Similar increased rate of carbon clearance from the blood by levamisole was reported by Van Ginckel and Hoebeke (1975). The decrease in spleen size in dexamethasone group observed in the present study (Table 1) is in agreement with the reports of Davison *et al.* (1983) which support the concept that spleen weight decreases in immunosuppressed animals (Spencer and Oliver 1996). These effects were reversed by levamisole. Wooles and Di Luzio (1963) reported that reticuloendothelial stimulants induced hypertrophy of the reticuloendothelial system organs and showed an increase in the spleen weight as percentage of body weight. Therefore levamisole was able to restore the phagocytic function of reticuloendothelial system suppressed by dexamethasone in rabbits.

SUMMARY

Th efficacy of levamisole in restoring the immunosuppression in dexamethasone-treated rabbits. Our results revealed that levamisole was able to restore the phagocytic function of reticuloendothelial system.

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Adhesions following cystotomy in dogs

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Cystotomy is indicated for removal of cystic caliculi, neoplasia, diverticulum, unresponsive haemorrhagic cystitis and for exploratory purposes. Different sites for bladder incisions have been suggested, viz dorsal (Archibald 1965) and ventral. Noordry and Trotter (1963) and Hickman and Walker (1980) preferred dorsal cystotomy because of less chance for urine leakage and avoidance of post-operative adhesions of the urinary bladder to the ventral abdominal wall. Hence, the present experimental study was undertaken to investigate the extent of adhesions that develop following dorsal and ventral cystotomy and evaluate the usefulness of poly-vinyl pyrrolidone in dogs in reducing abdominal adhesions following cystotomy.

The study was conducted on 32 healthy mongrel bitches divided into 4 groups of 8 animals each. Dorsal and ventral cystotomies were performed in groups 1 and 2 respectively. In group 3, 2% solution of polyvinyl pyrrolidone was introduced, intraperitoneally, in an attempt, to study its efficacy in reducing the adhesions. The dogs of group 4 served as controls. All the animals in 4 groups were premedicated with triflupromazine hydrochloride @ 1 mg/kg bwt given intramuscularly about 30 min before general anesthesia. The dogs were anaesthetized with thiopentone sodium 2.5% solution @ 25 mg/kg bwt, intravenously 2.5% solution. These animals where placed in dorsal recumbency and the operation site at ventral abdominal region was prepared in the routine manner for aseptic surgery. In group 1 following laparotomy urinary bladder was exteriorized and gently pressed to evacuate the urine. After full evacuation the vascular area on the ventral aspect of urinary bladder was exposed. An incision of 2-4 cm was made on the dorsal surface of fundus in the least vascular area. The bladder incision was closed by a single row of continuous Lembert's suture using 3/0 chromic catgut. The urinary bladder was replaced in its normal position. The omentum was drawn back and replaced over the urinary bladder. Before closing the abdomen, 60ml of sterilised 2% solution of polyvinyl pyrrolodine was poured in to the peritoneal cavity. The skin incision was sutured with monofilament nylon using horizontal mattress sutures. In group 2, an incision of about 2-4 cm was made on ventral aspect of bladder. In group 4 the urinary bladder was exteriorized and replaced at its original position. Clinical signs, gross pathological changes and histopathological examinations were studied in all groups.

The temperature, pulse and respiratory rates in all animals of all the 4 groups remained within the normal range throughout the observation period indicating that all the animals tolerated the operation well. However, haematurea was noticed in the animals of groups 1, 2 and 3 during the first 1 or 2 micturitions in the immediate post operative period. This was considered to be a normal sequelae of urinary bladder surgery. Observations of the bladder at postmortem examination in dogs of group 1 on post operative day seventh indicated that healing progressed well. Wound healing on both the mucosal and serosal aspects seemed to be adequate with the suture line on both the surfaces barely discernable. The suture line on the serosal aspect could be identified only by the presence of adhesions between the bladder suture line and other tissues. This was because of the inversion type of sutures employed bringing the serosal surfaces into proximity. Fusion on mucosal surface was also equally good and it would not have been possible to identify the suture line but for the presence of small submucosal haemorrhagic spots around. Similar submucosal haemorrhagic spots were also noticed in all animals sacrificed on post operative day 15 but to a lesser degree, indicating that they were not of much significance and get resorted as healing progresses.

Almost similar gross pathological changes were noticed in all the dogs of groups 2 and 3 suggesting that neither a change in the site of bladder incision nor addition of a 2% solution of polyvinyl pyrolidone altered the gross pathological changes produced by surgical encroachment of the urinary bladder in dogs. Evidence of urine leakage was no observed in any animal of groups 1, 2 and 3.

In group 1, the adhesions between abdominal suture line and the omentum was a constant finding. Adhesions were

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also formed between the broad ligament of uterus and bladder suture line in 7 dogs. In the animals of group 2 adhesion of the omentum both to the abdominal was and bladder suture line was noticed in all the animals. Almost similar observation regarding formation of adhesions following dorsal and ventral cystotomies was also reported by Desch and Wagner (1986). Polyvinyl pyrolidoine as used for supplementing blood transfusion and is stated to be a safe and efficient plasma substitute (David *et al.* 1968 and Brander and Pugh 1977). The intra peritoneal use of 60ml of a 2% solution of polyvinyl pyrrolidone was well tolerated by all the animals of group 3. Postmortem examinations made in groups 1 and 3 showed that polyvinyl pyrolidone neither interfered with the progression of healing nor did it cause changes in other abdominal organs in any of the animals. It is evident that PVP is safe for use in dogs at the said concentration and it also, to some extent, reduces the occurrence of adhesions. Similar observation following caesarean section in cattle was made by Bostedt and Brummer (1969).

Microscopic examination of the sections obtained from the bladder wall at the suture line from the animals of group1, 2 and 3 indicated that the healing progressed adequately by post operative day 7 and almost complete with serosal and muscular union and complete mucosal regeneration in all the animals by post-operative day 15. However, in the sections of group 2, epithelial regeneration under the scare tissue and subsequent sloughing of the scare tissue was noticed. Since the histopathological picture was similar in the 3 groups, the said changes cannot be attributed to the presence of PVP in the animals of group 3. It may therefore, be safely assumed that PVP has some useful application and not adverse effects. The use of PVP was safe and helpful to some extent in reducing the occurrence of adhesions.

SUMMARY

The extent on adhesion developed following dorsal and ventral cystotomy was studied. The usefulness of polyvinyl pyrolidone in reducing this adhesion was also studied in dogs. The PVP was found useful in reducing the occurrence of adhesions.

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Neutrophilic response of uterus to intrauterine administration of immunomodulator, *E.coli* lipopolysaccharide in buffaloes (*Bubalus Bubalis*) suffering from dystocia

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Key words: Animal reproduction, Neutrophilic response, Uterus

Optimal defense mechanism of the reproductive tract such as normality of the epithelium, uterine involution, mucus secretion, antibodies and non-specific phagocytosis are essential for the recovery from infectious endometritis (Hussain 1989). Phagocytosis (80%) by polymorphonuclear lymphocytes (PMNL) cells in the uterine exudates is the basic factor in antibacterial defenses in the postpartum uterus (Vandeplassche and Bouters 1981). The incidence of postpartum uterine infections has not decreased despite the wide spread use of antibiotics (Hussain 1989). Antibacterial drugs markedly inhibit or delay phagocytic activity of uterine $PMNL_s$ thereby, decreasing the uterine defenses (Jayappa and Loken 1983). This harmful effect was enhanced in drug resistant bacteria (Massera *et al.* 1980). Indiscriminate and inadequate use of antibacterials has also led to the emergence of partial to complete microbial resistance. Uterine defense stimulation using LPS of *E. coli* has been shown to effectively increase influx of neutrophils into uterine lumen of normal mares (Williamson *et al.* 1987. Hughes and Couto 1988) and normal cows (Klucenski *et al.* 1990) The experimentally introduced uterine infection disappeared in *E. coli* endotoxin treated group (Hussain and Daniel 1992). However, the studies on the use of *E. coli* LPS to treat dystocia associated bacterial endometritis in dairy animals especially buffaloes are not available. The present investigation was thus conducted to evaluate histo-pathologically the status of uterine neutrophilic reaction to intra-uterine administration of *E. coli* Lps and to study the uterine bacterial load.

Dystocia affected buffaloes (10) in which per-vaginal delivery was effected by traction after correction or by partial to complete per-cutaneous fetotomy were included in the study. These were divided into 2 groups of 5 animals each and were treated as below:

Group 1: E. coli LPS (from *E. coli* serotype 026: B6 contains 10,000 endotoxin units per mg of LPS, L 8274), 200µg intra-uterine dissolved in 50 ml distilled water as a single dose.

Group 2: E coli. LPS, 300µg intrauterine dissolved in 50 ml distilled water as a single dose. The uterine fluid and uterine swabs were collected before delivery, immediately after fetal delivery and on days 1,2 and 7 postpartum. The uterine biopsy was collected at 0 hr and 48 hr post-calving. An additional biopsy at 8 hr post-calving was also collected in both the LPS treated groups.

After restraining the animal and washing the perineum, the vulva was disinfected with spirit swabs (95% ethyl alcohol) and finally with povidine-iodine solution. With an assistant parting the vulvur lips, a sterilized intrauterine catheter attached with an adapter and disposable 20 ml syringe was advanced into the body of uterus and the uterine secretions were collected for immediate processing.

The uterine biopsy was collected from the medial uterine wall with a uterine biopsy punch. The biopsy sample was fixed in 10% buffered formaline solution. Using pour plate method, 1 ml each of serial 10-fold dilutions of uterine fluid with sterile normal saline was mixed with to molten nutrient agar in petri plates. Duplicate plates were prepared for each dilution and the plates were incubated at 37°C for 48hr. The colonies were counted using counter and the total bacterial load was calculated by multiplying the No. of colonies in a given dilution by the dilution factor. The results were expressed as CFU ml of the sample.

Sections, 6µm thick, were stained with haematoxyline and eosin and evaluated without knowledge of the sample identity (Gridley 1960) for the PMNL infiltration and tissue alterations. Each section was coded and processed randomly so that

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ratings could be unbiased.

Data were derived for mean values of total bacterial load. To assess the significance of variation between the 2 groups, the data was subjected to one-way analysis of variance using a completely randomized design (Singh *et al.* 1991).

The uterine bacterial isolates up to 7 days postpartum revealed the presence of *E coli, Stapphylococcus aureus, B. hemolytic, Streptococci, Proteus, Actinomyces pyogenes, Pseudomonas aeruginosa, Klebsiella, Staphylococcus epidermidus* and *Bacillus* spp. As compared to the initial levels ($185.60 \pm 64.62 \times 10^3$ CFU/ml), the bacterial load was significantly lower at day 1 post treatment ($61.00 \pm 21.16 \times 10^3$ CFU/ml) and remained so in animals treated with LPS 300 µg while in group treated with LPS 200 µg, gradual decline was seen up to day 7 post treatment.

The epithelium congestion and oedema, presence of haemorrhage, mononuclear cells and overall inflammatory score showed little variation between the groups except size of the uterine glands which increased in group 2 because increase in exudates constituting mainly the $PMNL_s$.

PMNL infiltration increase in the uterine tissue at 8 hr post-treatment. The infiltration was dose dependant and neutrophil reaction sustained up to 48 hr in group 2 as compared to group 1 in which the neutrophil reaction had decreased at 48 hr post-treatment after an initial rise. Enhancement of non-specific uterine defense mechanism with use of immuno-modulator drugs was reported in equines (Williamsom *et al.* 1987, Hughes and Couto 1988) and cows (Klucenski *et al.* 1990. Hussain and Daniel 1992) suffering from endometritis. LPS through stimulation of IL-1 and IL-8 causes extravasations of PMNL at site of infection (Tizzard 1987).

As dystocia is a highly stressful event (Prabhakar 1995), higher cortisol level in buffaloes further suppress the functional activity of neutrophils and hence the host defense mechanisms, which predispose the uterus to bacterial infections (Kerhil *et al.* 1985, Cai *et al.* 1994). The significant decline in the bacterial load in the group treated with *E.coli* LPS (300 µg) by day 1 might be due to phagocytosis by the PMNL; the fact was confirmed histopathologically by the dose dependent infiltration of $PMNL_s$ into the uterine tissues. Non-specific uterine defences play a significant part in clearing uterine bacterial infections (Vandeplassche and Bouters 1981, Hussain 1989). Within the LPS treated groups, LPS 300µg appeared to be more effective in reducing the bacterial load by promoting the uterine defense mechanism. Hussain and Daniel (1992) and Saini *et al.* (1995) too have reported significant reduction in bacterial counts following *E. coli* LPS administration in cattle. As evident from the findings, infusion of 300 µg *E.coli* LPS lead to maximum infiltration of PMNL into the uterus and significant reduction in the bacterial load. It thus, appears that uterine LPS infusion is advantageous to stimulate the uterine defences post-partum to combat the bacterial infections.

SUMMARY

Buffaloes (10) having dystocia were administered 200 or 300 µg *E. coli* lipopolysaccharide (PPS) intrauterine after delivery of fetus. Treatment induced neutrophillic infiltration which was high and sustained up to 48hr uterine tissue of animals treated with 300 µ LPS. There was significant reduction in the uterine bacterial load by day 1 postpartum in animals of this group.

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Macromorphology of kidney of one-horned Rhino calf

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Key words: Kidney, Morphology, Rhino

The great one-horned rhinoceros is the largest land mammal next to the elephant having a blackish grey body coat. It is a denizen of the grass-jungles and prefers swampy ground. However, study on the macromorphology of kidney of one-horned rhino calf is scarce. Therefore, an attempt was to elucidate some gross anatomical features of the kidney of a rhino calf.

The right and left kidneys of a 2-year old male Indian one-horned rhino calf were collected during postmortem examination. The kidneys were cleaned off their fat and the different biometry and numbers of lobes were recorded.

The right kidney was heavier (200 g), longer (13.1 cm) and wider (7.6 cm) than left (150g, 10.75 cm, 6.06 cm respectively) while the left kidney was triangular and thicker (3.73cm) than the right (3.65 cm).

The right kidney of rhino calf has 2 surfaces - dorsal and ventral while the left had 3 surfaces-dorsal, ventral and cranial. The dorsal surface of right kidney was slightly convex than the flattened ventral surface. The lateral border of both the kidneys was long, convex and thin, whereas medial border was short, straight and thick (Fig. 1). The gastric surface present only on the left kidney was flattened.

Both the kidneys were lobulated. The lobes were of variable shape and size. The right kidney had 63 lobes altogether in contrast to the left which had 47 lobes. The number of lobes were more in one horned rhino calf than ox having only 20 lobes (Raghavan 1964, Sisson 1975) and buffalo (Malik *et al.* 1978). The hilus of each kidney (Fig. 1) was oriented craniocaudally and situated near the medial border. Renal pelvis was absent in both the kidneys as in ox (Nickel 1979).

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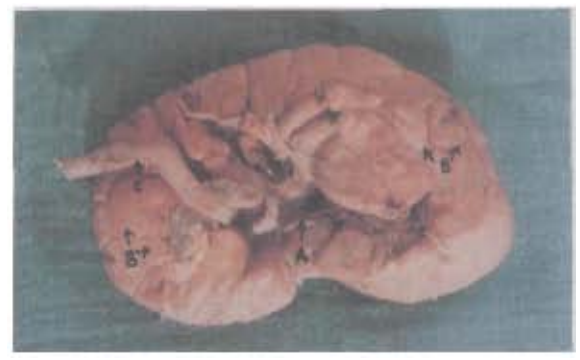

Fig. 1. Ventral surface of right kidney of one horned rhino calf showing the hilus (A), lobes (B) and ureter (C).

SUMMARY

The morphology of kidney of one-horned rhino calf was studied. The right kidney was heavier, longer and under them right kidney. Right kidney had 2 surfaces while left had 3 surfaces. Both the kidneys were lobulated.

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Concentration of 5-HT (serotonin) in placenta and uterus of buffaloes during pregnancy

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Key words: Buffalo, Neurotransmitter, Placenta, Reproduction, 5-HT, Serotonin

5-HT is a monoamine neurotransmitter has been implicated in the control of LH secretion by the pituitary gland (Sirotkin and Schaeffer 1997, Vitale and Chiocchio 1993). Furthermore, *in vitro* experiments demonstrate that 5-HT has direct effects on reproductive organs by influencing steroidogenesis (Tanaka *et al.* 1993, Battista *et al.* 1987). In view of the important role of 5-HT in the reproductive process, the study was made to measure the concentrations of this indolamine in uterine and placental tissues of buffaloes during pregnancy.

Uteri of pregnant buffaloes collected from local abattoir, were dissected into fetal cotyledons (FC), allantochorion (AC), maternal intercaruncular area (MICA) and maternal caruncles (MC). Stage of pregnancy was ascertained by measuring crown rump length of the fetus (Soliman 1975) and classified into early (50-120 days, n=5) and mid (121-170 days, n=5) pregnancy. Few samples were also collected from buffaloes after relieving dystocia by caesarian section and from cow term placenta. Tissue 5-HT was extracted in acetone (5ml/g wet weight) according to Parrat and West (1957). Tissue 5-HT was bioassayed on isolated rat fundus strip. The minimum tissue sensitivity was in the range of 1-10 ng per 10-ml bath fluid. The 5-HT contents in the samples were calculated in terms of 5-HT base. The data were analyzed by applying student 't' test (Snedecor and Cochran 1989).

Our results revealed that 5-HT content of buffalo FC increased with advancement of pregnancy (early, 5.12±1.05 vs mid, 36.85±9.33 ng/g). The concentration was also significantly higher ($P<0.01$) in FC (86.99±12.61 ng/g) of the cow term placenta (n=6). In term placenta of buffaloes suffering from dystocia (n=6) concentration of 5-HT in FC was 32.66±6.03 ng/g. On the other hand, 5-HT content of MC was low and not affected with the stage of pregnancy. The concentration in 3 samples collected from buffaloes suffering from dystocia was 6.00±1.08 ng/g. In marked contrast to FC and MC, this amine was not present in measurable amount in AC and MICA during pregnancy. An increased concentration of placental 5-HT was also reported in goats with the advancement of pregnancy (Raviprakash 1986).

The significance of rise in 5-HT concentration in fetal cotyledons of buffalo during pregnancy is not known. Evidence has been gathered in a number of invertebrates and vertebrates that 5-HT might involve in morphogenetic process during embryogenesis (Lauder 1988). More importantly, 5-HT is also reported to be involved in pregnancy maintenance and termination of pregnancy by influencing steroidogenesis. It is well known that placenta produces a number of hormones including oestrogen and progesterone. 5-HT is reported to stimulate steroid production (Tanaka *et al* 1993) and progesterone production (Battista *et al.* 1987). A similar mechanism of estrogen and progesterone stimulation by 5-HT may be speculated in placenta. Furthermore, Sirotkin (1995) reported that 5-HT stimulated oxytocin output by porcine granulosa cells. Sharma and Pant (2000) reported that buffalo placenta also has oxytocin or oxytocin like factor and highest concentration was detected in FC . Therefore increasing level of 5-HT and oxytocin towards advancing pregnancy may be responsible for uterine contraction and pregnancy termination. It was also proposed that 5-HT together with other autocoids could make substantial contribution to the contraction of both circular and longitudinal smooth muscle component essential for complete postpartum closure of the umbilical vessels (Roach 1976).

SUMMARY

The concentration of 5-HT in uterine and placental tissues of buffaloes during pregnancy, was estimated. The concentration of 5-HT increased with advancement of pregnancy. Further studies are therefore warranted to ascertain its physiological role in pregnancy maintenance or its termination.

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Histomorphology of testis of goat (*Capra hircus*)

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Key words: Goat, Histomorphology, Testis

The spermatogenesis involves complicated series of changes before the spermatozoa are released for fertilization from epididymis, hence, contemplating the importance of this organ, the present work was undertaken.

Testis (15) of local goat, 1 to 2 years of age, were collected from the slaughterhouse. The tissue samples of testis were fixed in 10% neutral buffered formalin for 48 hr and then dehydrated in ascending grades of alcohol. The dehydrated tissues were cleared in touline, processed for paraffin technique of light microscopy (Singh and Sulochana 1996). The paraffin sections of 5 μ thickness were stained by haematoxylin and eosin (Bancroft and Steven 1982), VanGieson's stain for collagen fibres and Verhoeff's stain for elastic fibres (Singh and Sulochana 1996).

The testis was covered externally by connective tissue capsule called as tunica albuginea, which was consisted of numerous collagen fibres and a few elastic fibres. These observations were similar to those reported by Dellmann and Wrobell (1981) in ruminants, and Sudhakar and Sharma (1993) in spitti ponies.

The thickness of tunica albuginea was observed at different locations in same gland. The average thickness of tunica albuginea in goat was recorded as 33.02 ± 2.34 μm.

Beneath the tunica albuginea, there was a layer of loose connective tissue, tunica vasculosa, which was rich in blood vessels. In continuation with the tunica albuginea, there were many strands of connective tissue, septula testis, which were converging towards the mediastinum and separating the lobules. Each lobule consisted of numerous convoluted seminiferous tubules. These seminiferous tubules decreased in number and increased in size with the advancement of age. The average number of seminiferous tubules per sq mm was 22.0 ± 0.49.

The maximum vertical diameter of seminiferous tubules was recorded as 24.25 ± 1.02 μm and maximum horizontal diameter of seminiferous tubules of goat was recorded as 16.16 ± 0.75 μm.

Each convoluted seminiferous tubule was surrounded by a dense connective tissue layer of basal lamina. The seminiferous tubule was lined by stratified epithelium and consisting of sertoli cells and spermatogenic cells.

The spermatogenic cells were in different phases of development and were located between sertoli cell. The outermost layer of seminiferous tubules was formed by stratified cuboidal epithelial cells, which were arranged at the basal lamina. These cells were large and oval in shape and had oval nuclei and designated as spermatogonial cells (Fig. 1). Some of the spermatogonial cells were seen undergoing a phase of multiplication and developing in to primary spermatocytes.

The primary spermatocytes were rounded in shape with the spherical nuclei. The cytoplasm was lightly stained. The primary spermatocytes were larger than spermatogonial cells. The secondary spermatocytes were smaller in size than that of primary spermatocytes. These cells were rounded in shape and had centrally located spherical nuclei and scanty cytoplasm (Fig. 1). The secondary spermatocytes got transformed in to spermatids. Round, elongating, elongated types of spermatids were observed.

The round spermatids were the smallest cells, which had spherical nuclei at the cytoplasmic rim. The nuclei had a prominent nuclear membrane. The elongating spermatids were characterized by presence of elongating nuclei and eosinophilic cytoplasm. The elongated spermatids contained elongating nuclei and they were usually found attached to the sertoli cells. Some cells were observed with detached cytoplasmic masses and were acidophilic in nature. These spermatids were found toward the lumen of the tubules (Fig. 1). These findings were similar to those reported by Rao *et al.* (1970) in buffalo, Bernstone and Desjardins (1974) in bovines, Dellmann and Wrobell (1981) in ruminants and Bordoloi and Dhingra (1990) in goat.

In between the spermatogenic cells, some elongated pyramidal shaped cells rested on basement membrane and had spherical or oval shaped nuclei. These cells were

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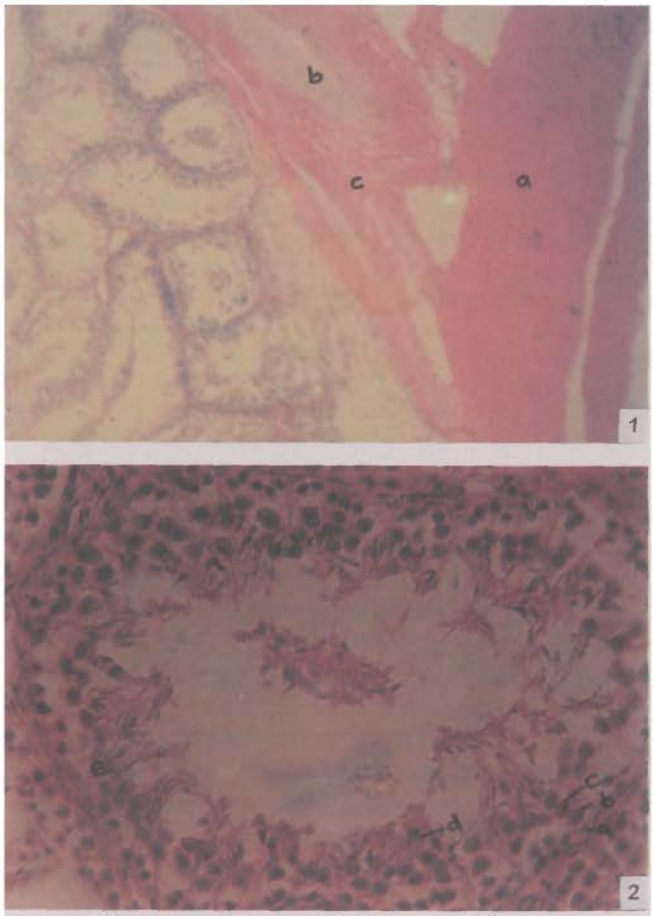

Figs 1-2. **1.** Microphotograph showing seminiferous tubules. a. basal lamina, b. spermatogonial cells, c. primary spermatocytes, d. secondary spermatocytes, e. sertoli cells. H&E × 250 **2.** Microphotograph showing types of leydig cells. a. Type-I leydig cells b. type-II leydig cells. c. typ-III leydig cell, d. type IV leydig cell, (H&E × 400).

designated as sertoli cells or nurse cells (Fig. 1). These observations corroborated with those reported by Dellmann and Wrobell (1981) in ruminants and Bordoloi and Dhingra (1990) in goat. The average number of sertoli cells per sq mm area in goat in present study was 12.3 ± 0.59.

Leydig cells formed a well vascularised group of polyhedral cells, which occupied the space between seminiferous tubules. Similar observations were reported by Fawcett *et al.* (1973) in mammals and Dellmann and Wrobell (1981) in ruminants. The average number of leydig cells per sq mm area in goat testis was 14.0 ± 0.79. This was in accordance with the fact that the leydig cells secrete gonadal hormone, estrogen, which play an important role when animal reaches puberty and sexual maturity.

During the present work 4 types of leydig cells were identified as type -I, type -II, type- III and type- IV on the basis of shape of cells, shape and position of nuclei and staining characteristic of cytoplasm. Bordoloi and Dhingra (1983), however, reported only 3 types of leydig cells in testis of goat. The variation in the cellular morphology of the leydig cells during the present study may be because of the functional status of the cell at the time of slaughter.

Type- I leydig cells were seen elongated in shape with scanty and lightly eosinophilic cytoplasmic granules. The nuclei were elongated to oval shaped (Fig. 2). Type-II leydig cells were observed as irregular triangular in shape. The cytoplasmic granules were moderate and shape of nuclei of cells was spherical and centrally placed (Fig. 2). Type –III leydig cells were noted to be large rounded shaped cells with scanty cytoplasmic granules. Their nuclei were rounded, vacuolated and eccentrically placed (Fig. 2). Type – IV leydig cells had indistinct boundaries with scanty cytoplasmic granules. The nuclei of the cells were vacuolated and rounded in shape (Fig. 2).

SUMMARY

The histomorphological study conducted on testis of local goat revealed that the tunica albuginea was made up of mainly collagen fibres. From tunica albuginea, many septula testes originated forming lobules. Each lobule consisted of many seminiferous tubules, and their size and shape of seminiferous tubules increased with the advancement of age. The seminiferous tubules of testis were lined by stratified epithelium consisting of spermatogenic cells and sertoli cells. The spermatogenic cells were found in different phases of development and they were located between sertoli cells. These development stages included spermatogonial cells, primary spermatocytes, secondary spermatocytes and 3 types of spermatids. The number of sertoli cells and leydig cells per sq mm area was 13.2 ± 0.59 and 14.0 ± 0.79 respectively. Four types of leydig cells were identified as type - I, type – II, type – III and type – IV.

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Exfoliative vaginal cytology of different phases of oestrus in bitches*

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Key words: Bitches, Oestrus phases, Vaginal cytology

Exfoliative vaginal cytology provides useful information about the degree of vaginal mucosal proliferation in bitches (Hewitt and England 2000), and a simple method to monitor the stage of the oestrous cycle in canines (England 1998). The alterations that occur in the vaginal mucosa and the vaginal vault as a result of an increase in serum oestrogen concentration during pro-oestrus and oestrus will reflect in the appearance of exfoliated vaginal epithelial cells. Thus, the vaginal cytology can serve as a reliable indirect oestrogen assay (Feldman and Nelson 1996). The methods of preparing vaginal smears are simple and can be used in veterinary practice with minimal effort and expense. In the present study, the vaginal cytology of different phases of oestrus was studied.

Bitches (12) irrespective of breed and parity and with known history of oestrus were utilized. The bitches were kept in standing position and the vulval lips were separated gently. The sterile cotton swab dipped in normal saline solution was pressed against the caudodorsal surface of the vaginal vault initially while inserting to avoid the clitoral fossa. Thereafter the swab was advanced in a craniodorsal direction, towards the vertebral column until it passed over the arch and rotated to a complete revolution in each direction and withdrawn.

The cotton swab was rolled gently from one end of the glass slide to the other end to prepare a smear. Care was taken not press firmly nor rub the swab against the slide. The smears were air-dried and stained with Leishman's stain in standard manner. After drying of smear, The slide was observed under oil immersion objective of the microscope.

The smears collected from all the bitches were examined and the results were given in Table 1. In the present study, at pro-oestrus, an average of 63.33 ± 0.75, 32.50 ± 0.96 and 4.17 ± 0.58% of superficial, intermediate and parabasal cells were observed which almost concurred with the results of Christie *et al.* (1972) who observed 64.00 and 36.00% of superficial and intermediate cells respectively. Whereas Bugalia *et al.* (1998) observed 50.00, 40.00 and 10.00% of intermediate, superficial and parabasal cells respectively. During pro-oestrus, there will be increased peripheral plasma concentration of oestrogen which cause thickening of the vaginal mucosa and increase in the number of cell layers. The mucosa changes from cuboidal epithelium to keratinized squamous epithelium. As the mucosa thickens, the surface

Table 1. Exfoliative vaginal cytology of different phases of oestrus in bitches (mean±SE)

Phase	Superficial cells	Intermediate cells	Parabasal cells	Neutrophils %	RBC %	Background %
Pro-oestrus	63.33±0.75	32.50±0.96	4.17±0.58	8.33(++) 16.67 (-)	83.33 (+++) 16.67 (+++)	Dirty (100)
Oestrus	96.08±0.50	2.92±0.32	1.00±0.16	83.33 (-) 16.67 (+)	66.67 (-) 33.33 (+)	Clear (100)
Dioestrus	18.17±0.88	28.67±0.77	53.16±0.89	66.67 (+++) 33.33 (++++)	66.67 (-) 33.33 (+)	(66.67) Dirty (33.33)
Anoestrus	52.50±0.91	37.42±0.83	10.08±0.69	100 (++)	91.67 (+) 8.33 (-)	Dirty (100)

Absent (-) Very few (+) Few (++); Moderate (+++); Abundant (++++).

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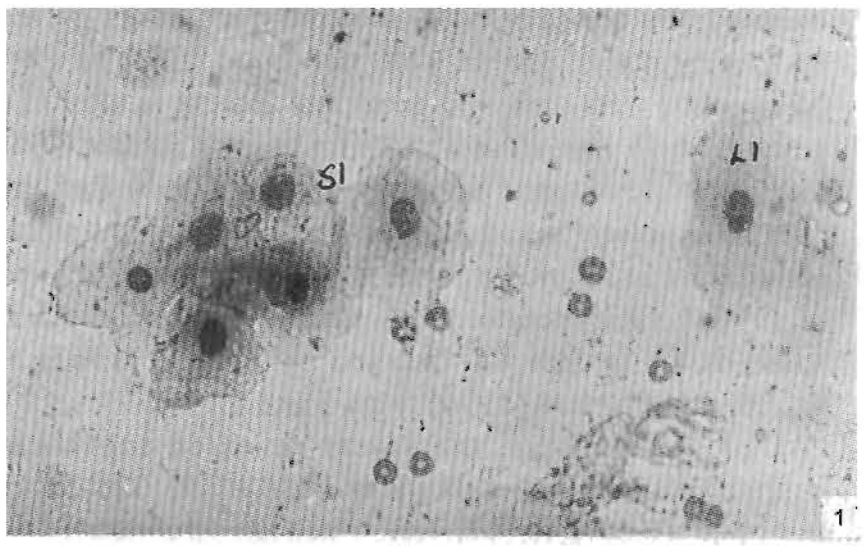
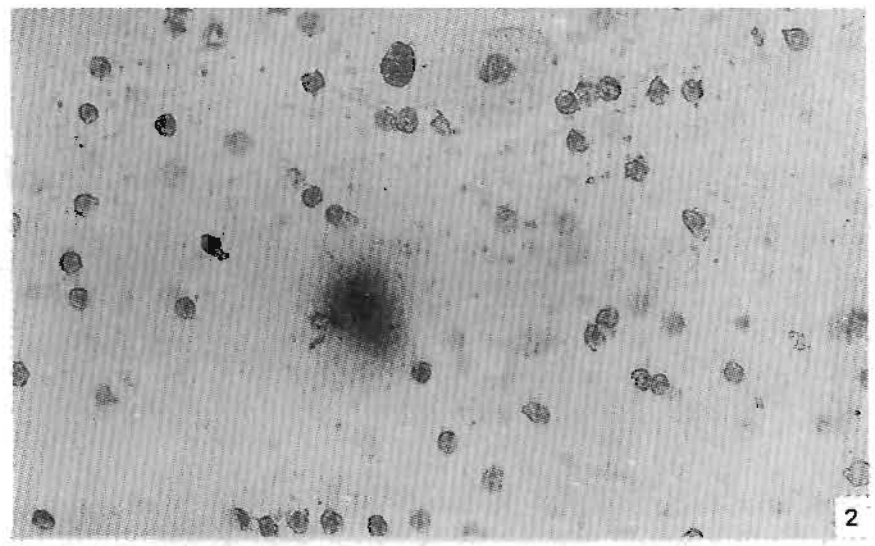
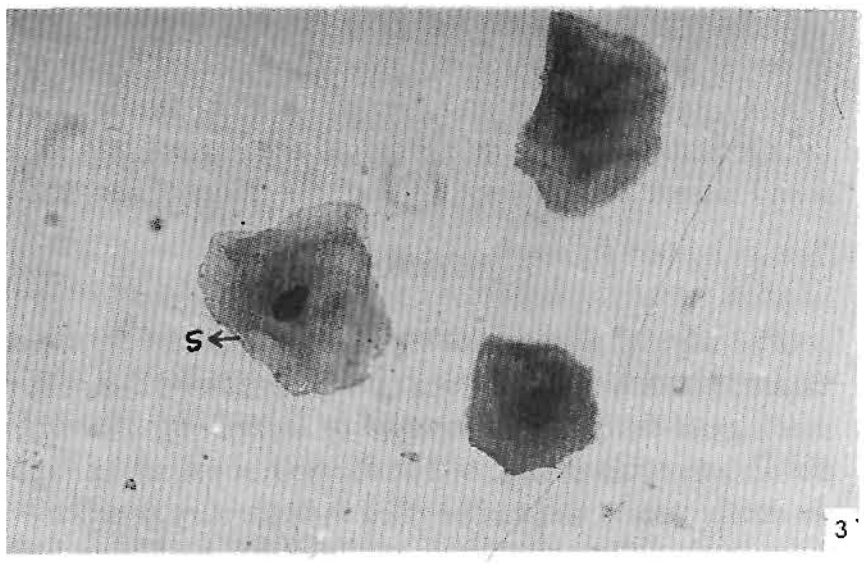
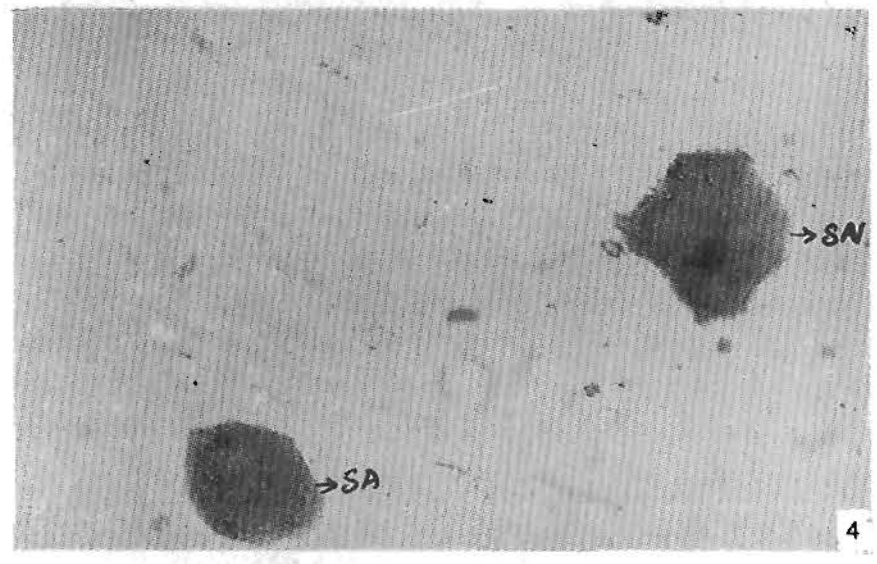
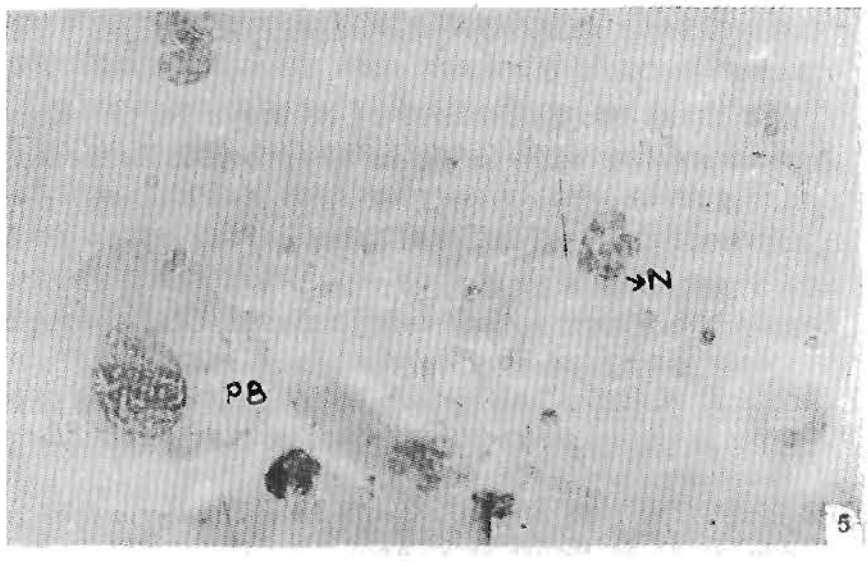
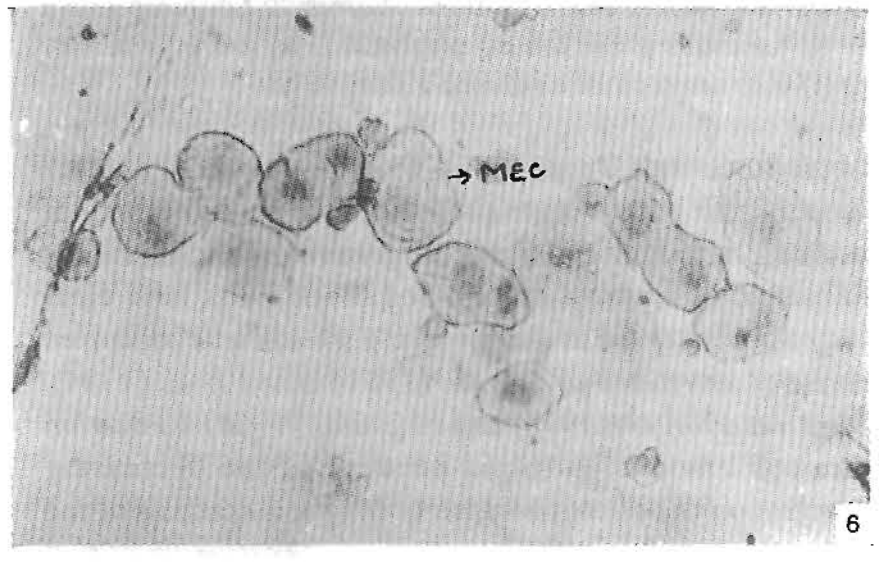










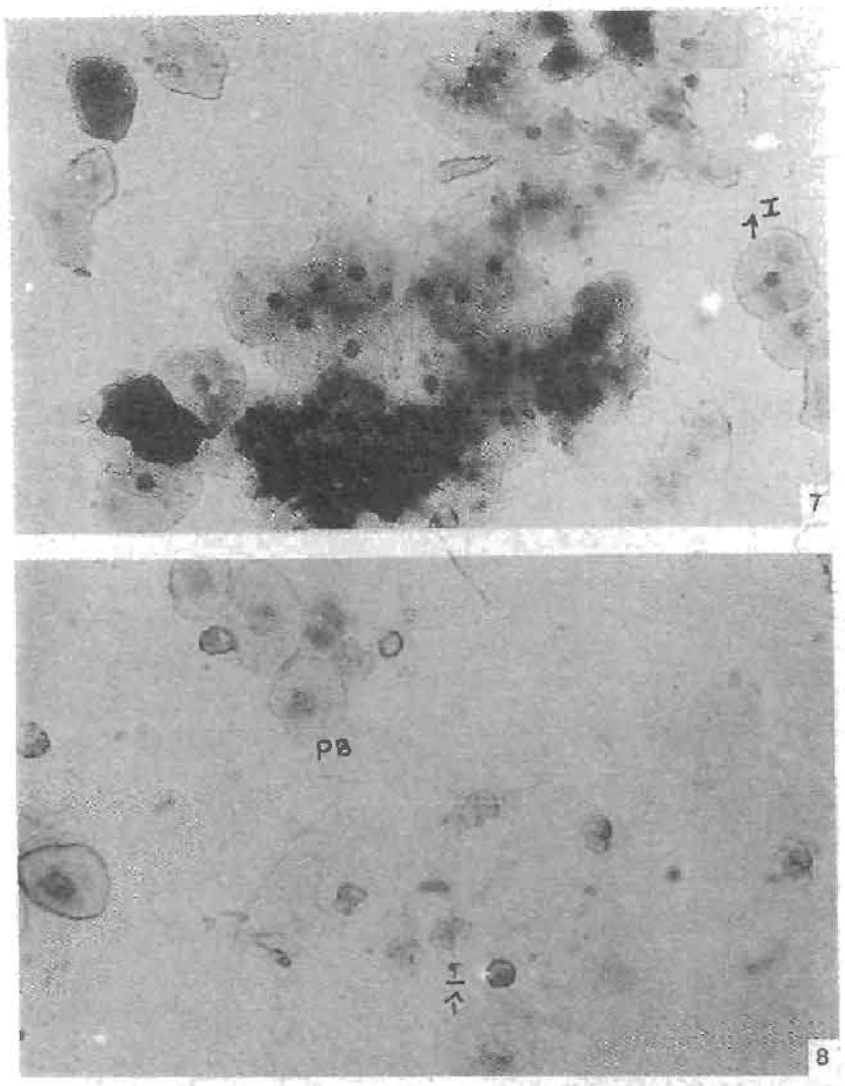


Figs 1-6. **1.** Pro-oestrus phase showing large intermediate (LI) and superficial intermediate cells (SI). Note more red blood cells and dirty background. Leishman's stain 40×. **2.** Pro-oestrus phase showing superficial cells with pyknotic nuclei. Note red blood cells and dirty background. Leishman's stain 40×. **3.** Oestrus phase showing superficial cells with pyknotic nuclei and without nucleus, absence of red blood cells and with clear background. Leishman's stain 40×. **4.** Oestrus phase showing clear background, superficial nucleated (SN) and a nucleated (SA) cells and without red blood cells. Leishman's stain 40×. **5.** Metoestrus phase showing parabasal cells (PB) with neutrophils and clear background. Leishman's stain 40×. **6.** Metoestrus phase showing parabasal cells (PB) intermediate cells (I) and characteristic "Metoestrum" (MEC). Leishman's stain 40×.

cells change in their size, shape and staining character, becoming larger, irregularly shaped and ultimately anuclear (England 1998).

Neutrophils at this phase were very few in number, might be due to thickening of the wall under oestrogen's effect and neutrophils could not enter the vaginal lumen (Feldman and Nelson 1996). Red blood cells were more because of diapedesis through uterine capillaries due to oestrogens effect (Olsen *et al.* 1984) and the background was dirty because of cellular debris and mucin threads as reported by Schutte (1967) and Feldman and Nelson (196) (Figs 1 and 2).

During oestrus, the smears in present study revealed more of superficial cells (96.08 ± 0.50) with very few intermediate and parabasal cell (2.92 ± 0.32 and 1.00 ± 0.16), which was



Figs 7-8. 7. Anoestrus phase showing predominant parabasal cells (PB) and intermediate cells (I) without neutrophils. Leishman's stain 40×. 8. Anoestrus phase sowing parabasal (PB) and intermediate cells (I) with neutrophils. Leishman's stain 40×.

in accordance with Bugalia *et al.* (1998). The more number of superficial cells was mainly because of the effect of decreasing oestrogen and rising progesterone concentrations.

Absence of neutrophils and red blood cells with clear background observed in almost all the smears was concurred with the observations of Schutte (1967) Olsen *et al.* (1984), Feldman and Nelson (1996) and England (19968). Reduction in the red blood cells might because of the decreasing concentration of oestrogen which limits the diapedesis of red blood cells (Figs 3 and 4).

The superficial, intermediate and parabasal cells during dioestrus were around 18.17 ± 0.88, 28.67 ± 0.77 and 53.16 ± 0.89%, respectively, which were almost similar to the observations of Bugalia *et al.* (1998) who has reported 20.00, 20.00 and 60.00% respectively. On the contrary, Christie *et al.* (1972) reported 28.00, 69.00 and 3.00% cells respectively. At the end of fertilization period, the plasma progesterone remains at high concentration resulting in sloughing of much of the vaginal epithelium. The number of cell layers decreases, deeper cells are uncovered and the percentage of large irregularly shaped annular cells decreased (England 1998). Neutrophils were present in moderate amount in most of the smears, which were similar to the findings of Schutte (1967), Olsen *et al.* (1984) and Feldman and Nelson (1996). Red blood cells were present in some and absent in some of the smears, which were in accordance with the observations of Olsen *et al.* (1984). Characteristic metoestrum cells were noticed in some of the smears as observed by Schutte (1967) and Feldman and Nelson (1996) (Figs 5 and 6).

During anoestrus, the smear revealed superficial, intermediate and parabasal cells as 52.50 ± 0.91; 37.42 ± 0.83 and 10.08 ± 0.69%, respectively, which almost concurred with the results of Bugalia *et al.* (1998). Neutrophils were present in fewer numbers, which are in accordance of Olsen *et al.* (1984) and Feldman and Nelson (1996) findings. The background was dirty in almost all the smears and in line with Feldman and Nelson (1996) observations (Figs 7 and 8).

SUMMARY

The vaginal cytology of different stages of oestrus were studied in bitches. The Present study confirmed that, vaginal cytology is fairly simple method of identifying the stage of the oestrus cycle in canine. The method of preparing vaginal smears is simple and can be used in veterinary practice.

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Poisoning in dairy animals

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Key words: Animal health, Poisoning

Sporadic cases of malicious poisoning of companion and farm animals are not uncommon. These cases involved the use of rodenticides/ insecticides (Sidhu *et al.* 1995). The present report deals with a rare outbreak of malicious toxicity in farm animals using aluminium phosphide.

The present outbreaks causing death of 320 cows and buffaloes in Bharpurgarh and Gardhiwal, District Patiala (Punjab) occurred in July and September. The outbreaks were attended and blood and tissue samples of affected/dead animals were collected. The fodder and water given to animals were also brought to laboratory for toxicological analyses. There was no change in feeding or in the management of animals during the investigation. There was no history of entry of new animals in the villages of affected herds. The treatments tried by local veterinarians were: atropine sulphate, antibiotics, calcium borogluconate and chloral hydrate; but of no use.

Affected animals suddenly stopped feeding and drinking, developed tympany, respiratory difficulty, restlessness, hypersalivation, convulsions and fell on the ground and died. The body temperature was normal or subnormal. Five buffaloes and 6 cows were found dead without showing any signs of illness. The deaths occurred sporadically killing 2-3 animals daily. Blood, rumen contents and tissues of affected/ dead animals, fodder and water given to animals were tested for the presence of insecticides, strychnine, lead, cyanide, nitrate and nitrite but nothing could be detected. The bacteriological and parasitological examination of blood of affected animals was also negative. To rule out the possibility of enterotoxaemia, 5 brown mice were inoculated with filtrate of rumen and intestinal contents of dead animals @ 0.2 ml/ mice by intra-peritoneum route. Although there was no mortality, on the very next day the mice were weak and shrivelled. This may be due to low residue levels of toxic material in inocculum prepared from rumen and intestinal contents of affected animals.

Laboratory confirmation of toxicity was done by detection of aluminium phosphide in rumen contents, liver and kidney of dead animals using qualitative test (Niyogi 1971). Postmortem of dead animals showed no gross abnormality. Histopathological examination showed congestion and haemorrhages in viscera. In experimental rats, aluminium phosphide administered by oral route induced similar changes in visceral organs after 24-48hr exposure (Kumar *et al.* 1998). Three experimental healthy buffalo calves were administered 6 g of aluminium phosphide (56%). One calf died 4 hr after aluminium phosphide administration without showing any symptom except hypersalivation. Two calves showed hypersalivation, difficult respiration, convulsions and died within 6-10 hr of aluminium phosphide administration. Post-mortem of calves revealed severe damage to lungs. No significant lesions were seen on histopathological studies except haemorrhages in different tissues.

The poisoning may be the result of phosphine gas release from aluminium phosphide. Phosphine may be produced in rumen or gastrointestinal tract of ruminants. It is reported that phosphine gas is liberated when aluminium phosphide comes in contact with moisture (Kumar *et al.* 1998). The mechanism of aluminium phosphide toxicity is not clear. There is no specific antidote for its toxicity but supportive therapy and care could save some animals. It is concluded that reviewing of this outbreak should be used to prevent future unfortunate incidents. Sudden deaths of animals should raise the suspicion for such an episode.

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SUMMARY

Poisoning outbreak in dairy animals was studied. The deaths in animals were found mainly due to aluminium phosphide administration in fodder.

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Growth performance on different feeding and rearing practices in pigs

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ABSTRACT

The study was carried out on 'T & D' (Tamworth and *desi*) breed of pigs on different feeding practices to evaluate growth performances. The pigs maintained on concentrate feed had higher weight gain than those maintained on hotel and rice fermented wastes during preweaning period. However, higher weight gain during postweaning periods from 3rd to 10th month of age was observed in piglets maintained on hotel waste with 25% concentrate feed. The crude protein content was significantly higher (25.01 to 26.23%) in hotel wastes rations in comparison to concentrate (18.60%) and rice fermented (18.76%) groups. Significantly highest calcium (1.15 to 1.31%) and phosphorus (0.52 to 0.61%) values were obtained in concentrate and hotel waste feeds.

Key words: Body weight, Chemical composition, Feed, Hotel wastes, Pigs

Swine husbandry is important in solving animal protein requirement at comparatively cheaper rate of production due to better feed conversion efficiency than other liverstock. However, economical feeding is important for profitable swine production, since 75 to 80% of expenditure incurred on feeding of animals. Hence, the present study was undertaken to evaluate growth performance of pigs on different feed resources and feeding practices.

MATERIALS AND METHODS

The present investigation was carried out on "T & D" (Tamworth and *desi*) breed of pigs under following 5 managements:

i) A1: *Ad lib.* concentration feeding without grazing - 30 pig
ii) A2: *Ad lib.* hotel waste without grazing - 20 pigs
iii) A3: 75% hotel + 25% rice fermented wastes with 1-2 hr grazing - 23 pigs
iv) A4: 75% hotel waste + 25% concentrate without grazing - 27 pigs
v) A5: 70% rice fermented waste + paddy husk + green grass with 3-4 hr grazing 73 pig.

All experimental pigs were given identification mark either with 2% silver nitrate solution on their body or with ear notching. Monthly body weights were taken with the help of spring balance. The birth weight was recorded within 16 hr of farrowing. The chemical composition of feed were analysed as per procedure of AOAC (1980). Feed samples were collected at fortnightly interval. Statistical analysis of data were done according to Snedecor and Cochran (1968).

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RESULTS AND DISCUSSION

Growth rate

The body weight of pigs are presented in Table 1. Management conditions had significant ($P < 0.01$) effect at all the ages from 1st to 10 month of age. However, it had nonsignificant influence on birth weight.

At second month of age, significantly highest body weight was recorded in A1 with lowest (6.54 ± 0.13 kg) in A5. The body weight of piglets belonging to group 2 was significantly lowest than that of A3 possibly due to poor utilization of nutrients by sow on hotel waste alone than 25% supplemented farm diets or rice fermented waste diets. The supplementation of concentrate feeds or rice fermented waste had improved the better balance of nutrients for the sow leading to increase in their milk production for the piglets. Singh *et al.* (1996) observed higher value (8.50 ± 0.18 kg) in comparison to present one under village conditions in semi-intensive system with grazing for 2-3 hr daily.

At 6 month, significantly highest body weight was observed in A4 than A1 followed by A2, A5 and A3 (Table 1). The lower body weight of piglets of A5 in comparison to A1, A2 and A4 was probably because of insufficient nutrients provided by rice fermented waste with poor grazing capacity during growing stage.

Table 1. Growth rate of "T & D" pigs reared under diffrent mangemental conditions

Age	A1	A2	A3	A4	A5
Weight at different ages (kg)					
At birth	1.09[a]	0.91[a]	0.83[a]	1.16[a]	0.91[a]
	±0.03	±0.02	±0.03	±0.24	±0.01
	(30)	(20)	(23)	(27)	(73)
1st month	5.52[a]	4.35[b]	4.35[b]	4.36[b]	3.68[c]
	±0.19	±0.18	±0.18	±0.14	±0.09
	(30)	(20)	(26)	(25)	(73)
2nd month	10.13[a]	7.64[b]	8.63[c]	8.60[c]	6.54[d]
	±0.31	±0.27	±0.28	±0.31	±0.15
	(30)	(17)	(26)	(24)	(111)
6th month	34.84[a]	31.20[b]	22.27[c]	38.33[d]	25.49[e]
	±1.25	±0.61	±0.56	±0.80	±0.66
	(25)	(15)	(20)	(23)	(42)
10th month	67.83[a]	67.26[a]	51.87[b]	78.02[c]	61.90[d]
	±2.99	±1.05	±1.63	±1.17	±1.16
	(6)	(15)	(8)	(23)	(42)
Daily weight gain (g)					
Pre-weaning	150.67	112.17	130.00	124.00	93.83
Post-weaning	239.67	248.42	180.17	289.25	230.67
Over all	222.47	221.17	170.13	256.20	200.60

Figures in parentheses indicate number of observarions. Means under the same superscript did not differ significantly.

Chemical composition of feeds

Significant ($P < 0.01$) effect of different types of feed on all the chemical composition under study was observed (Table 2).

Significantly higher value of DM was observed in concentrate feed. On the other hand, significantly lowest DM was recorded in hotel waste because of large quantity of water along with left-over foods. However, DM value in feeds of rice fermented waste mixed with crushed maize and wheat bran was 77.27 ± 1.82%.

Crude protein value of concentrate feed and rice fermented waste was estimated to be 18.60 and 18.76%, respectively, and almost similar as formulated on book values for grower pig rations. However, the CP value of hotel waste, used in different proportion was significantly higher (25.01 to 26.23%) might be due to higher CP value in human food of vegetable and non-vegetable composition. Tirkey *et al.* (1999) reported CP value of grower ration between 19.47 and 20.44% in various rations of concentrate + rice fermented waste in different proportions.

Significantly higher EE, varying from 7.13 to 7.63%, was recorded in hotel waste rations compared to concentrate and rice fermented waste ration (Table 2). CF of various rations varied between 4.20 and 7.73%.

Significantly higher NFE value was recorded in concentrate feed, whereas lowest was found in hotel waste and hotel + rice fermented waste. Lowest total ash was recorded in feeds in which concentrate was mixed whereas highest value was found in hotel waste feed. Highest AIA was found in A3, which did not differ significantly from A2 and A4. On the other hand, significantly lower value was observed in A5.

Table 2. Average chemical composition of different feed (%) on DM basis

Particulars	A1	A2	A3	A4	A5
DM	92.27[a]	35.41[b]	49.25[c]	58.50[d]	77.27[e]
	±0.42	±0.75	±1.57	±1.81	±1.82
	(10)	(20)	(30)	(10)	(20)
CP	18.60[a]	26.23[b]	26.02[b]	25.01[b]	18.76[a]
	±0.20	±0.90	±0.74	±0.52	±0.58
	(10)	(20)	(30)	(10)	(20)
Ether extract	5.28[a]	7.63[b]	7.54[b]	7.13[b]	6.12[a]
	±0.20	±0.29	±0.27	±0.03	±0.27
	(10)	(20)	(30)	(10)	(20)
Crude fibre	4.20[a]	6.73[b]	5.54[c]	4.41[a]	7.62[d]
	±0.03	±0.37	±0.24	±0.19	±0.32
	(10)	(20)	(30)	(10)	(20)
NFE	64.76[a]	49.07[b]	50.23[b]	56.47[c]	56.47[c]
	±0.25	±1.26	±0.83	±0.69	±0.63
	(10)	(20)	(30)	(10)	(20)
Total ash	7.15[a]	10.33[b]	10.67[b]	6.98[a]	11.02[b]
	±0.04	±0.29	±0.54	±0.27	±0.64
	(10)	(20)	(30)	(10)	(20)
AIA	2.81[a]	4.14[b]	4.38[b]	3.92[b]	2.12[c]
	±0.07	±0.22	±0.19	±0.17	±0.17
	(10)	(20)	(30)	(10)	(20)
Calcium	1.15[a]	1.31[a]	1.21[a]	1.28[a]	0.76[b]
	±0.09	±0.08	±0.18	±0.11	±0.02
	(10)	(20)	(30)	(10)	(20)
Phosphorus	0.61[a]	0.52[a]	0.58[a]	0.55[a]	0.32[b]
	±0.03	±0.03	±0.02	±0.04	±0.02
	(10)	(20)	(30)	(10)	(20)

Means under the superscripts did not differ significantly.
Figures in parentheses indicate number of observations.

Significantly lowest calcium value was found in A5. Tirkey *et al.* (1999) reported 1.03 to 1.10% in 4 different types of rations.

The phosphorus contents (Table 2) in 4 types of feeds (A1 to A4) were satisfactory for growth of pigs as recommeded by various workers. Our findings are in confirmity with the findings of Tirkey *et al.* (1999) who obtained 0.53 to 0.55% by replacing wheat bran of standard feed with different proportions of rice fermented waste.

Pigs can be reared on hotel waste with faster growth rate for profitable swine production.

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Chemical detoxification of aflatoxins in contaminated poultry feed

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ABSTRACT

Detoxification of aflatoxins in the poultry mixed feed naturally contaminated at the level of 775±25 ppb was done using chemicals, viz. sodium bisulfite ($NaHSO_3$) and sodium hydroxide (NaOH). These animals at different levels (0.25, 0.5, 1.0 and 2.0%) were used to treat the contaminated poultry feed at 3 moisture levels (10, 15 and 20%). The treated feed samples were screened for aflatoxins after 24 hr, 10 days and 1 month by pressure mini column. Sodium bisulfite at 0.5% and sodium hydroxide at 2.0% concentration, at 20% moisture level could detoxify the aflatoxins to bring the level below the tolerance level of 30 ppb set by Government of India under the Prevention of Food Adulteration Act.

Key words: Aflatoxins, Chemical detoxification, Poultry feed

Aflatoxins are the secondary metabolites produced by the widely distributed fungi, *Aspergillus flavus* and *A. pasasiticus*. Aflatoxins are acutely toxic, teratogenic, carcinogenic and mutagenic (Van Rensburg *et al.* 1985, IARC 1987). Aflatoxins cause enormous economic losses to the nation directly or indirectly every year because of the large number of commodities affected. The situation has motivated research in the prevention, elimination and detoxification of aflatoxins in these commodities. Undoubtedly prevention is the best method for controlling mycotoxins in feeds (Goldblatt and Dollear 1979) but in spite of all efforts, contamination is unavoidable at the present time. Several investigators have studied various chemicals to see if they could degrade and detoxify aflatoxins (Fischbach and Campbell 1965, Sreenivasamurthy *et al.* 1967, Trager and Stoloff 1967, Mann *et al.* 1970). However, many chemicals which effectively detoxify aflatoxins are not suitable for use in feeds as they may reduce the nutritional quality of the feed, produce off flavours and off odours in the feed, or leave toxic residues in the feed. Hence, it would be desirable to find a chemical, which is an acceptable additive that will also degrade aflatoxins.

This study was conducted on *in vitro* degradation of aflatoxins using sodium bisulfite and sodium hydroxide at different concentrations and at different moisture levels.

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MATERIALS AND METHODS

Feed sample

Poultry mixed feed naturally contaminated with aflatoxin (775±25 ppb) was procured from the affected farms. Extraction of the aflatoxins was done by acetone: water (85: 15) and detection and estimation was done by employing pressure minicolumn (Kathuria *et al.* 1993). Dry matter content of the feed was estimated as per AACC (1990).

Treatment of feed with chemicals

Sodium bisulfite ($NaHSO_3$) and sodium hydroxide (NaOH) were used as the detoxifying agents. Naturally contaminated feed samples (200g) were treated with sodium bisulfite and sodium hydroxide separately, at concentration of 0.25, 0.50, 1.00 and 2.0% based on the dry matter of the feed samples. There treatment levels were studied at 3 moisture levels i.e. 10, 15 and 20%. The desired moisture level was achieved using the following formula (AACC 1990).

Water (ml) to be added = sample taken g x [(100-OM/ 100-DM) – 1]

Where

OM, Original moisture level in sample; DM, desired moisture level in sample.

The feed samples were kept in polythene bags for maintaining the moisture levels and stored at room temperature. Aflatoxin contaminated feed samples without chemical treatment at 3 moisture levels were kept as control. The treated feed samples were screened for aflatoxins after 24 hr, 10 days and 1 month by pressure mini column and also observed visually for any fungal growth.

Table 1. Aflatoxoin concentration (ppb) in poultry mixed feed after detoxification process with different level of sodium bisulfite and sodium hydroxide (Mean+25ppb)

Chemical	Treatment Concentration (%)	Storage period								
		1 day			10 day			30 day		
		Moisture level (%)								
		10	15	20	10	15	20	10	15	20
Control	-	775	775	775	775	775*	775*	775	975*	975*
Sodium	0.25	575	275	75	575	275*	75*	575	375*	175*
bisulfite	0.50	575	175	<25	575	175*	<25*	575	175*	75*
($NaHSO_3$)	1.00	375	75	<25	375	75	<25*	375	175	75*
	2.00	275	<25	<25	175	<25	<25	275	75	<25
Sodium	0.25	775	375	275	775	375*	275*	775	575*	275*
hydroxide	0.50	575	375	175	575	375*	175*	575	375*	275*
(NaOH)	1.00	375	275	75	375	275*	75*	375	375*	175*
	2.00	275	175	<25	275	175	<25	275	175	75

*Mold growth occurred at these levels.

RESULTS AND DISCUSSION

The aflatoxin levels in naturally contaminated mixed poultry feed after treatment kept for 24 hr, 10 days and 30 days with sodium bisulfite and sodium hydroxide at 3 moisture levels are presented in Table 1. The results showed that all the treatments were effective in reducing the aflatoxin concentration. Sodium bisulfite was more effective than sodium hydroxide at low concentration. At 10% moisture level both sodium bisulfite and sodium hydroxide at 2% concentration detoxified the feed to bring the aflatoxin level to 275±25 ppb, a reduction of 64.52%. When subjected to 15% moisture level, both sodium bisulfite and sodium hydroxide at 2% concentration reduced the aflatoxin concentration to 25 ppb and 175±25 ppb, a reduction of 96.77 and 77.42% respectively. Further, at 20% moisture level, the aflatoxin level was reduced below 25 ppb by sodium bisulfite (0.5%) and sodium hydroxide (2%). This limit is below the tolerance limit of 30 ppb set by Government of India under the prevention of Food Adulteration Act (Sashidhar *et al.* 1989 and Goto 1990). Moerck *et al.* (1980) also reported that 0.5 and 1.0%of sodium bisulfite and 2% of sodium hydroxide were effective in reducing the level of aflatoxin below the FDA guidelines of 20 ppb.

Several bisulfite salts were found safe for use in agricultural foods and are acceptable food additives. Bisulfite inhibits enzymatic and non-enzymatic browning, acts as an antioxidant and reducing agent and effectively controls growth of microorganisms (Roberts and McWeeny 1972). Different mechanisms of aflatoxin degradation by these salts were reported in the literature (Cucullu *et al.* 1976, Doyle and Marth 1978, Moerck *et al.* 1980, Hagler *et al.* 1983). But many times detoxification procedures lead to change in colour of the feed and apparent surface stickiness, which may further affect the palatability and intake of the feed. Moerck *et al.* (1980) reported surface stickiness of corn when treated with sodium hydroxide. However, in the present study sodium bisulfite and sodium hydroxide treatment did not appear to alter the colour of the feed nor did they cause any apparent surface stickiness. This may be due to the fact that in the present study mixed feed was used.

Detoxification of aflatoxins with these salts at 20% moisture level has its own problems as observed during the study. The visible fungal growth was observed on the detoxified feed after 10 days onwards, which was indicative of the role played by high percentage of moisture levels in the feed. So it is advisable that the feed decontaminated of aflatoxins at high level of moisture should be used immediately or efforts should be made to reduce the moisture levels for storage purposes for longer period of time.

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Growth response of West African dwarf lambs fed yam tuber peel

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***Key words**: Animal nutrition, Growth, Lamb, Yam*

Yam (*Dioscorea* spp.) is among the staple energy food crops of most people in the tropics and one of the important root tuber crops of West African (Nweke *et al.* 1991). About 4.18-4.78 million metric tonnes of the by-product (yam tuber peel) is globally available for feeding ruminants, especially sheep. While in Nigeria the yam production is about 3.14 to 3.59 million metric tonnes (Asiedu 1994). Yam tube peel can serve as an important source of energy in ruminant feeding systems, serving either as the basal diet or as supplement. It follows that, poor nutrition during the long dry season of the year (Larsen and Amaning-kwarteng 1976) is among the main constrain to livestock production in Nigeria, which is a major yam producing country in the world, there is considerable livestock weight loss and sometime death which are caused by the rapid decline in the quality and quantity of forages (Otchere *et al.* 1977), that lead to poor intake and digestibility. In Nigeria there is a need to explore yam tuber peel as an alternative feed for livestock, which industries producing yam floor, yam fufu or chips throws out as waste which is scavenged by sheep on rubbish dumps in villages and urban areas. The by-product is slightly richer in protein, fibre, ash, fat and lower in carbohydrate than the tubers, thus the use of yam tuber peel necessitates a protein supplement to adjust the ration to the animals requirements. Information is lacking on intake and utilization of yam tuber peel as supplement feed when fed to sheep. The present study was therefore conducted to assess the potential of sun-dried yam tuber peels as a component of forage in sheep feeding during the dry period of the year.

The yam tuber (mixed species) peels gathered from homes and industrial establishments were sun-dried for 2-3days (Anigbogu 1997). In the study, 3 yam tuber peel based rations were formulated. Grasses and legumes (2:1) of mixed species were processed into hay and the trial rations were prepared as follows: A (70% hay), B (35% hay + 35% yams tuber peel), and C (70% yam tuber peel). Cotton seed-cake and palm kernal-cake were incorporated at 15% each as protein supplement for the rations. The hay was chopped into bits, mix with yam tuber peel, cotton seed-cake and then given *ad lib.* to the experimental lambs and water provided at free choice. An adjustment period of 14 days was allowed before measurements were taken.

Male lambs (27), average = 4.5 weeks, were reared (average floor area) for 77 days in an empty broiler- little house that was naturally ventilated. Wood shaven were spread in the pens as bedding material for the lambs. Lambs were randomized to 9 pens of 3 lambs each with an average pen weight of 25.2 kg and a maximum differences between pens of 0.21 kg.

The data on feed intake, weight gain, cost of feed input/kg live weight gain and dressing percentage of the labs were subjected to analysis of variance of the complete randomized design (Gomez and Gomez 1984) where the statistical differences between treatment means were established by the use of Duncans Multiple range test (Duncan 1955).

Initial weight was similar in the groups (8.4kg) while the

Table 1. Feed intake, body weight gain, feed efficiency, cost of fed input/kg live weight gain, and dressing percentage of West African dwarf lambs fed yam tuber peel based rations

Traits	*A*	*B*	*C*
Initial weight (kg)	8.4	8.4	8.4
Final weight (kg)	11.7[a*]	16.4[b]	25.6[c]
Total gain (kg)	3.3[a]	8.0[b]	17.2[c]
Average daily gain (g)	43[a]	104[b]	223[c]
Total feed consumed/lamb (kg)	71.29[a]	111.64[c]	88.01[b]
Feed efficiency (kg feed /kg gain)	21.47[a]	13.99[b]	5.12[c]
Dressed carcass weight (kg)	6.09[a]	8.44[b]	13.05[c]
Dressing % on pre slaughter weight	52.05	51.46	50.98
Dressing % on empty live weight	63.50	62.75	62.17
Cost of feed input/kg live weight gain (N)[1]	479.80[a]	274.29[b]	197.27[c]

*Unlike superscripts in a row differ significantly (P<0.01), N=Nigerian currency Naira, Nigerian N140=US $1=Indian Rs 50.

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finishing weight and average daily gain in the experiment were higher in C followed by B and A groups (Table 1). The results are in conformity with the findings of Gohl (1981) as the yam tuber peel is highly degradable source of carbohydrate in the rumen (Smith *et al.* 1992). The feed to gain ratio was significantly, ($P<0.01$) narrower in 70% yam tuber peel fed lambs than the other 2 groups. The feed efficiency of the group was 20% which will be considered optimum for sheep in active phase of growth. The improved feed efficiency of the lambs fed yam tuber peel was due to high content of soluble carbohydrate in the feed source. The findings are in agreement with observations of Gohl (1981) and Smith *et al.* (1992) in cattle, sheep, goats and pigs fed yam or cassava tuber peels.

The dressed carcass weight in the 3 groups was highest in C (13.05kg) followed by B and A groups respectively. This is in agreement with the observation made by Anigbogu (1990), as yam tuber peel is a well utilizable source of carbohydrate in the rumen system of sheep. Hahn *et al.* (1992) also observed increase in dressed carcass weight which is associated with a significant increase in live weight gain. However, the dressing percentage on empty live weight was not significant ($P<0.05$), the dressing percentage on pre slaughter weight also followed the same trend with about 52% which can be regarded high for sheep in practical operation of growth. The lower dressing percentages of the lambs fed yam tuber peels as noted in this study was as a result of high degradable carbohydrate and lower fibre present in the major feed source. The findings are in agreement with the revelations of Smith *et al.* (1988), Anigbogu (1990) and Hahn *et al.* (1992) in cattle, goats and sheep fed tuberous root by-products including yam tuber peel. The cost of feed input/kg gain live weight of the lambs were lower in C followed by B and A groups respectively. This was significantly ($P<0.01$) closely confine in 70% yam tuber peel fed lambs than other 2 groups. The observation is in correspondence with the findings of Little and Said (1986), as yam tuber peel is a relatively cheep feed source with highly degradable carbohydrate source for lambs (Ahmed 1977, Gohl 1981, Smith and Bosman 1987).

SUMMARY

The growth performance of lambs fed yam tuber peel was studied. It was observed that the yam tuber peel can serve as an important feed source in ruminant feeding especially sheep, to improve the low quality nutrition during long drier months.

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Physiological, haematological and growth responses of crossbred male calves under two housing conditions*

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The essential function of the housing system is to moderate the extremes of climatic stress and give an microclimate which is as close to the thermo-neutral zone so that animal can devote maximum energy towards productive purposes. The deviation of different physiological, haematological parameters from their normal ranges cause stress to the animals. Therefore the experiment was planned to find out the changes in physiological, haematological and growth parameters under different housing conditions.

Crossbred (KF) male calves (10) were selected from the NDRI herd and divided into 2 groups of 5 each. The experiment was conducted from February to May. Animals were fed 2.0 kg concentrate mixture at morning as the first feed of the day. High quality green fodder like berseem, oat, maize, jowar etc were fed *ad lib.* depending upon the availability of fodder. Water was available round the clock.

Group 1 (experimental) was housed under modified housing conditions. During February and March animals were protected from cold and chilly wind during night with windscreens and animals were given bedding of sorghum straw. During day time they were kept with direct access to sunshine. During April and May extra protection was given during day time. Animals were protected from direct sunshine and not wind using screens made of gunny bags. During peak hot hours animals were given cold water shower. The screen kept wet throughout the day time. Bedding of fine sand provided to give comfort to the animals in the hot climate. The screens were removed during night time. The group 2 (control) animals were kept under existing conditions at Artificial Breeding Complex. Animals were kept in semi-covered area with half walls and animals were exposed to cement- concrete floor throughout the experimental period.

The environmental variables in terms of daily minimum, maximum temperature (7.1 ± 0.2 to 37 ± 0.97°C) dry and wet bulb temperature (9.8±-.63 to 36.4±074 and 8.9±0.40 to 24.23°C) and relative humidity (28.0±2.2 to 88.0±1.68%) were recorded during the entire period of study. The data were analysed by applying the suitable statistical methods as per Snedecor and Cochran (1968).

The responses of animals were observed through the difference in various physiological parameters like respiration rate (RR), heart rate (HR) rectal temperature (RT) and skin temperature (RT), haematological parameters, viz haemoglobin (HB), packed cells volume (PVC), red blood corpuscles (RBC), total leucocytes (TLC) and differential leucocytes count (DLC) in blood and plasma cortisol level were also estimated. Dry matter intake and body dimensions were also recorded under 2 housing conditions.

The overall mean value of RR, HR, RT, ST are given in Table 1. All the physiological values were slightly higher in group 2 animals. The corresponding values during evening hours showed that there were considerable increase in physiological parameters during evening hours, when the microclimatic temperature goes above the thermoneutral zone and also that the animals of group 2 showed more increase in these parameter. Mishra *et al.* (1995) have also reported higher physiological reactions in afternoon than morning values in

Table 1. Mean±SE of different physiological parameters of crossbred males under two housing conditions for overall duration of study

Physiological parameters	Observation time	Group 1	Group 2
Respiration rate	M	31.69±0.46	33.16±0.28
(bpm)	E	46.0±0.41	47.19±0.52
Heart rate	M	61.57±0.55	64.37±0.52
(bpm)	E	76.99±0.39	77.41±0.58
Rctal temperature	M	37.97±0.02	37.95±0.03
(°C)	E	38.84±0.02	38.91±0.02
Skin temperature	M	28.11±0.36	28.42±0.34
(°C)	E	35.98±0.23	36.58±0.18

The values are the means of 140 observations on 5 animals. M-morning, E-evening, THI morning-66.6±0.58, evening–75.5±0.7.

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Table 2. Mean±SE for haematological and hormonal parameters under two housing conditins in crossbred males

Groups/parameters	I	II
PCV (%)	34.13±0.32	34.57±0.29
Hb (g%)	12.65±0.15	12.95±0.15
RBC (10^6/mm^3)	6.22±0.09	6.77±0.09
TLC (/mm^3)	9523.00±126.30	9947.00±145.03
Lymphocyte (%)	71.40±0.61	69.40±0.71
Neutrophils (%)	25.75±0.65	27.85±0.72
Eosinophils (%)	2.18±0.11	2.23±0.13
Monocyte (%)	1.50±0.12	1.59±0.10
MCV (umm^3)	55.61±1.12	51.41±1.22
MCH (pg)	20.51±0.71	19.34±0.48
MCH_2C (%)	36.98±0.52	37.53±0.40
N : L ratio	0.37±0.01	0.41±0.03
Plasma cortisol (ng/ml)	6.03±0.45	6.24±0.54

Hariana cows and crossbred heifers.

Analysis of variance showed that the difference in RR and HR being significant ($P<0.01$) under 2 groups and periods. Correlation analysis showed that all the physiological parameters had got positive and significant correlation with temperature humidity index (THI).

Mansera *et al.* (1940) indicated that blood constituents could be used as an index for assessing the adaptability to tropical climates. Stull and Mc Martin (1992) concluded that neutrophils to lymphocyte (N:L) ratio greater than the range of 0.35 to 1.15 were indicative of stress.

The overall mean values of PVC, Hb RBC and TLC for groups 1 and 2 are given in Table 2. The values obtained during the present study is within the ranges of different haematological parameters reported by Razdan *et al.* (1969) in Tharparkar cattle under different environmental conditions. Analysis of variance revealed the differences in RBC between the 2 groups being significant ($P<0.05$). All these haematological parameters showed negative correlation with THI in both the groups. Hammond *et al.* (1988) also reported negative relationship between some haematological parameters and THI values.

Mean values of percentage of lymphocytes, neutrophils, eosinophils and monocytes for groups 1 and 2 are in general agreement with those reported by Reece and Hotchkiss (1987).

The level of cortisol in plasma was used as stress indicator. In the present study the level of plasma cortisol varied from 0.84 - 19 ng/ml. The cortisol levels were higher during the initial period of experiment when the ambient temperature was low. The similar values were reported by Ingram *et al.* (1979) in different categories of the animals.

The mean dry matter intake (DMI) for group 1 was 5.57±0.66 kg and for group 2 it was 5.22±0.88 kg. In the experiment the average daily gain for groups 1 and 2 were 631.8±93.31 and 636.2±114 g respectively. Karki *et al.* (1993) also found nonsignificant difference in weight gain of calves housed in 3 different types of shelters and groups.

SUMMARY

The changes in physiological, haematological and growth parameters under different housing systems were studied. Results indicate that THI values had the effect on animal system. The exposure of animals to direct solar radiation had an additional adverse effect on physiological responses. Therefore proper shelter with modified management practices is important for avoiding the adverse effect of extremes of climatic conditions of higher productivity.

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Fish polyculture as affected by culture season, stocking density and breed, under subtropical conditions of Egypt

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ABSTRACT

Effects of culture season, stocking density and breed, under Egyptian sub-tropical conditions, were studied on fish polyculture. The body weight, growth rate and fish yield were higher ($P<0.01$ and $P<0.05$) in summer than in winter crop, in Silver carp than in all other species and in 1.0 fish/cubic metre of water than in other stocking densities (fish yield). The feed conversion was higher ($P<0.05$) in winter than that in summer crop and in 3.0 fishes/cubic metre of water than in all other stocking densities. The condition factor was higher ($P<0.05$) in common carp than in all other species. The edible parts were higher ($P<0.05$) in 1.0 fish than in all other stocking densities and in grey mullet breed than in all other species. The non-edible parts were insignificantly higher in summer than in winter crop.

Key words: Carcass traits, Condition factor, Economic efficiency, Fish, Stocking density, Polyculture, Yield

Information on the environmental factors affecting fish farming are considered of great importance for improvement of fish management and productivity.

The normal range of water temperature for tilapia is 19 to 30°C (Wohlfarth and Hulata 1981), 20-31°C for carp (Jhingran and Pullin 1985) and 12-32°C for mullet (Gutherie and James 1972). The optimal salinity is 0.1-10.0 ppm for most fresh water fish as tilapia, carp and Nile catfish (Essa 1989) and 17-30 ppm for marine fish as mullet (Oren 1981). Optimum oxygen concentration for growth of all fish species is between 5.0 and 8.0 ppm oxygen (Piper *et al.* 1982). Optimal concentration of ammonia is 0.001-0.0125 ppm for most fishes and 0.001-0.01 ppm for growing fishes. Optimum pH for fish growth ranges between 6.5 and 9.0 (Piper *et al.* 1982). The optimal stocking density is 10 000-15 000 fish/ha under polyculture conditions (Lovshin *et al.* 1990). The optimal stocking densities are 3000 - 5000 fish/ha for tilapia, 300 to 1000 fish /ha for carp and 700 to 2000 fish ha for mullet under culture conditions (Hepher and Pruginin 1981, 1982). The growth rate of tilapia decreases with the increase in stocking density, under culture conditions (Abdel Wares 1993, Moustafa 1993 and Baker 1994). In the sub-tropical conditions of Egypt, such information are lacking.

In this study, it was aimed to investigate fish polyculture as affected by season of the year, stocking density and breed, under sub-tropical conditions of Egypt.

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MATERIALS AND METHODS

The experimental period extended for 2 seasons (365 days) during the period from 1 June to 31 May, 1993.

Tilapia aurea, common carp grey mullet were raised together with a ratio 10 : 5 : 2 : 3, respectively, in 4 ponds. The densities used were 1, 1.5, 2.0 and 3.0 fishes/cubic metre. Polycultured fingerlings (15 000), were used in summer (June-September) and winter (December-May) seasons, i.e. 7 500 tilapia aurea (*Oreochromis aureus*), 3750 common carp (*Syprinus carpio*), 1 500 silver carp (*Hypophthalmichthys* motitrix) and 2 250 mullet (*Mugil cephalus*) fingerlings were used in each season. Average age for all fishes was 3 months with nearly 50g as average weight. Each number of fishes was divided to 4 stocking densities cultured in earthen ponds in each sea fin of the year (summer and winter). The volume of water in each pond was 1000 cubic metres.

The experimental diets were given as 3% of body weight/ day according to Hanley (1987). The composition and chemical analysis of the experimental diet are presented in Table 1. All ponds were treated similarly. The daily diet was offered 2 times at 9.0 and 13.0 hr. The 4 ponds (40×25×1 cubic metres of water) used in the present study, were new. Each pond was drained and left for complete dryness before being used. The amount of natural food in the ponds was established by adding chicken manure as a source of nitrogen,

Table 1. Ingredients and chemical composition of the experimental diet

Ingredients	%
Yellow corn	47
Soybean-meal	20
Fish-meal	10
Rice bran	20
Mineral mixture*	2
Vitamin mixture**	1
Chemical composition	
Moisture	8.1
Crude protein	22.0
Ether extract	7.5
Crud fibre	9.0
Protein/energy	75.3
Ash	10.3
Metabalisable energy (Me kcal/kg diet)	2793.0

*Each kg of mineral mixture contained: Magnesium 225.0 g, sodium chloride 120.0g, zinc sulphate 11.25g, iron sulphate 7.50 g manganese sulphate 10.0g, copper sulphate 0.75g, potassium iodide 0.075 g and selenium 0.025g.

**Each kg of vitamin mixture contained: Neomycin sulphate 112.5g, vit. A 2200000 IU, vit. K 792.0 mg, vit. D_3 396.0 IU, vit. B_{12} 2.2 mg, riboflavin (B_2) 3.0g, tocopheral acetate 660.0 IU, nicotine amide 13.3g and pantothenic acid 4.8g.

Ismailiya canal through cement inlet. The water of each pond was continuously changed by opening both of outlets and inlets to have a continuous water flow to ensure the suitable water quality required for the experimental fishes. Water temperature was recorded using a thermometer at depth of 25 cm. Dissolved oxygen was determined once daily at 6.00 hr. Water pH was tested once every week. Concentration of ammonia was determined as per Golterman *et al.* (1978). Nitrite, nitrate and salinity were determined every 2 weeks according to Boyd (1979). Transparency of water was measured directly as per Hamza (1985). Records of the meteorological data and water quality are illustrated in Table 2.

Condition factor (K) was estimated according to Faulton (1902) as follows: $K = (W/L^3) \times 100$, where W = body weight (g) and L = body length (cm).

Absolute growth rate (AGR) was calculated (Woods *et al.* 1983) as follows: $AGR = (W_1-W_0)/t$, where W_1 = weight at end of the experimental period, W_0 = weight at the beginning of the experimental period and t = time (growth interval).

Feed conversion (FC) was calculated (Windel 1971) as follows: FC = amount of ration consumed (g)/fish/total gains (g)/fish.

Economic efficiency = (money return per kg gain/feed cost per kg gain)×100.

Table 2. Physico-chemical characteristics of earthen water ponds during the exprimental period

Months	T+	RH++	Nitrite (ppm)	Nitrate (ppm)	Ammonia (ppm)	Salinity (ppm)	Oxygen (ppm)	pH	Temperature (°C)	Transparency
June	26.0	81.8	0.009	0.762	0.018	0.21	6.3	7.4	25.5	13.0
July	29.0	77.9	0.009	0.761	0.019	0.20	6.2	7.5	28.1	13.0
August	30.0	76.9	0.009	0.762	0.018	0.22	5.5	7.6	29.2	13.5
September	28.0	81.0	0.011	0.762	0.017	0.20	5.4	7.6	27.0	14.0
October	25.0	82.0	0.009	0.761	0.020	0.19	5.5	7.4	25.0	14.0
November	20.0	83.0	0.008	0.762	0.019	0.20	5.3	7.3	21.0	13.0
December	15.0	84.4	0.008	0.763	0.019	0.17	6.1	7.1	16.0	13.0
January	11.0	84.6	0.009	0.763	0.018	0.19	5.2	6.9	13.0	13.0
February	10.0	84.7	0.009	0.763	0.019	0.20	5.1	7.0	12.5	13.0
March	15.0	84.4	0.008	0.763	0.019	0.18	6.1	7.1	16.6	14.0
April	19.0	84.3	0.010	0.760	0.018	0.19	6.1	7.1	21.0	14.0
May	23.0	84.3	0.011	0.690	0.018	0.20	6.3	7.2	22.5	*14.0

Salinity was calculated by the relation (1000 micro-mhos=0.78 salinity according to Dewis and Freilas 1970). +Ambient temperature; ++Relative humidity (%).

in addition to phosphate and potassium nitrate fertilizers to ensure the bloom of plankton. At the start of the experiment, such fertilizers were used as 15kg chicken manure, 3.7 kg of potassium nitrate and 7.5 kg of super phosphate per pond then water was added to the depth of 75-125 cm and manure only was added monthly with the mentioned rate. Greater care was undertaken to prevent wild fishes, their eggs and larvae from entering the ponds.

Ponds were supplied with freshwater indirectly from

All data ware statistically analyzed according to Snedecor and Cochran (1982). Significance of the differences among the means were tested by using multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Tilapia aurea, common carp, silver carp and grey mullet were raised together with a ratio of 10: 5: 2: 3, respectively in

Table 3. Fish body weight (g), body length (cm), condition factor and growth rate (g) as affected by season of the year, stocking density and breed

Items	Body weight		Body length		Condition factor +		Growth rate
	0	6m	0	6m	0	6m	(0-6m)
Season							
Summer crop	49.7±6.1^a	474.2±6.0^a	13.4±0.2^a	26.6±3.8^a	1.9±0.3^a	1.9±0.3^a	1.9^a
Winter crop	49.2±1.1^a	211.8±6.3^b	13.0±0.2^a	24.7±3.3^a	1.9±0.1^a	2.2±0.1^a	0.7^b
Significance	NS	**	NS	NS	NS	NS	*
Stocking density							
1.0	50.0±0.2^a	430.2±20.0^a	13.4±0.2^a	28.3±1.0^a	1.9±0.1^a	1.8±1.0^a	1.9^a
1.5	49.0±0.1^a	358.9±11.0^{ab}	13.0±0.1^a	28.8±2.6^a	1.9±0.1^a	2.2±0.2^a	1.5^{ab}
2.0	49.4±0.2^a	286.0±47.0^{ab}	13.6±0.1^a	23.8±1.6^b	1.9±0.1^a	2.3±0.1^a	1.1^b
3.0	48.2±0.2^a	209.6±7.4^b	12.8±0.1^a	21.8±0.7^b	2.0±0.1^a	2.0±0.1^a	1.0^b
Significance	NS	*	NS	*	NS	NS	*
Breed							
Tilapia aurea	51.7±0.5^a	150.5±3.7^c	12.65±0.1^a	19.3±0.3^b	2.2±0.1^a	2.4±0.5^a	0.3^{ab}
Common carp	49.3±0.1^a	337.1±11.4^b	12.9±0.1^a	26.1±0.7^b	2.1±0.2^a	3.1±0.5^a	0.9^{ab}
Silver carp	47.4±2.1^a	645.8±2.5^a	13.7±0.1^a	35.6±1.8^a	1.8±0.1^a	1.4±0.1^b	1.5^a
Grey mullet	49.6±0.2^a	149.6±4.3^c	13.6±0.1^a	21.7±0.8^b	1.7±0.1^a	1.4±0.1^b	0.3^b
Significance	NS	*	NS	*	NS	*	*

**P<0.01, *P0.05, 0=initial weight, M=month, += [Weight of the fish in grams/ (lenght of the fish in centimeters)^3]×100.

4 ponds with different 4 stocking densities (1.0, 1.5, 2.0 and 3.0 fishes/cubic metre of water) in 2 calendar seasons (summer and winter).

Body weight was significantly higher in summer than in winter (P<0.01), in 1.0 fish than in 3.0 fishes/cubic metre density (P<0.05) and in silver carp (P<0.05) than in tilapia aurea and grey mullet (P<0.05, Table 3). These results were in agreement with the findings of Essa *et al.* (1991). The increase in body weight of fish in summer than that in winter is probably due to that the water temperature (Table 3) is optimum for natural food, metabolic activity and growth rate. The increase in body weight values in 1.0 fish than in 3.0 fishes/cubic metre could be attributed to the lower available amounts of natural food and to the increase in competition for the limited food, in the latter case, in addition to the increase in energy- demanding activity levels (Li and Brocksen 1977) and metabolic expenditure (Metcalfe 1986) connected with social interaction. The increase in body weight values in silver carp than in each of common carp, tilapia aurea and grey mullet could be attributed to the differences in genetic potentiality, feeding behaviour and tilapia spawning at early age.

Body length was higher insignificantly in summer than that in winter, significantly (P<0.05) in 1.0 and 1.5 than in 2.0 and 3.0 fishes/cubic metre and significantly (P<0.05) in silver carp compared to grey mullet and tilapia aurea, but the difference between silver carp and common carp was not significant (Table 3). The present results were in agreement with the findings of Hafez (1991) and Moustafa 1993).

The condition factor was higher insignificantly in winter than in summer and in 1.5, 2.0 and 3.0 than in 1.0 fish/cubic metre and significantly (P<0.05) in common carp breed than in silver carp and grey mullet. Tilapia aurea was significantly (P<0.05) higher than in silver carp and grey mullet (Table 3).

The growth rate average was higher (P<.0.05) in summer than in winter, in 1.0 fish than in 2.0 and 3.0 fishes /cubic metre and in silver carp than in tilapia aurea and grey mullet, respectively (Table 3). These results were similar to the findings of Hafez (1991), Abdel-Wares (1993) and Baker (1994). The decrease in absolute growth rate with the increase in density may be due to the increase in competition for food and the increase in energy-demanding activity levels connected with social interaction (Li and Brocksen 1977), and to disturbance during feeding and normal activity. At low stocking density, such disturbances might be absent resulting in increased growth.

Table 4. Feed conversion (kg feed/kg gain) of polycultured fishes as affected by culture season and stocking density

Periods	Fish density (D) per cubic metre of water (fishes)				
	1.0 fish	1.5 fishes	2.0 fishes	3.0 fishes	Significance
Season (S)					
Summer	1.3^a	1.8^a	1.6^a	2.8^b	A
Winter	3.4^b	3.9^a	2.6^b	3.4^b	B
Significance	a	a	A	b	
Interaction					
S×D	*	*	*	*	*

*P<0.05, a,b=P<0.05 between densities, A, B = P<0.05 between seasons.

Feed conversion was significantly (P<0.05) higher in winter than that in summer and in 3.0 fishes than in 1.5 fishes/ cubic metre (Table 4).

Total yield was significantly (P<0.01 or P<0.0-5) higher in summer than in winter and in 2.0 fishes than the other densities (Table 4). The increase in total yield in tilapia aurea, common carp, silver carp and grey mullet under the summer conditions is because their better growth, since such fishes are warm water-breeds and their optimum temperature is between 25-32°C (Boyd and Frank 1979). The present results regarding the carcass traits were in agreement with those of Haez (1991).

Economic efficiency was higher in summer than in winter crop and in 2.0 fishes/cubic metre than in all other stocking densities (Table 5).

Table 5. Effect of culture season and stocking density on economic efficiency of polycultured fishes

Items	Densities (D) per cubic metre of water (fishes)				
	1.0	1.5	2.0	3.0	Significance
Season (S)					
Summer	254.2	245.2	329.6	20.1	A
Winter	88.0	67.2	106.3	7.4	B
Interaction					
S×D	NS	NS	NS	NS	NS

A, B = P<0.05 between seasons.

Percentages of edible and non-edible parts weights were significantly (P<0.01) higher in summer than in winter (61.6 vs 56.9 and 32.5 vs 28.1 respectively). Percentage of the edible parts were higher (P<0.05) with 1.0 fish than with 3.0 fishes (53.5)/cubic metre and in grey mullet (74.1) than in tilapia aurea (56.0), silver carp (53.6) and common carp (53.1), respectively, but tilapia aurea (36.6) was higher (P<0.05) than grey mullet (21.5) in percentage of the non-edible parts.

In conclusion, it could be recommended to raise 2.0 fishes of tilapia aurea, common carp, silver carp and grey mullet/ cubic metre in polyculture with a ratio of 10 : 5 : 2 : 3, respectively, under Egyptian sub-tropical conditions.

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Plankton communities of a subtropical reservoir of Meghalaya (NE India)

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ABSTRACT

Nongmahir reservoir, studied during June, 1995 - May, 1996, shows subtropical nature and soft-alkaline waters characterized by low electrolyte concentration, moderate transparency, unsaturated dissolved oxygen and low nutrient concentrations. Net plankton indicate 17 species of phytoplankton and 28 species of zooplankton, and lower abundance (164 ± 77 n/l) with predominance of phytoplankton (109 ± 94 n/l). The latter exhibit quantitative dominance of chlorophyta (83 ± 99 n/l) and sub-dominant role of cyanophyta > bacillariophyta > chrysophyta > dinophyta. Zooplankton (55 ± 26 n/l) depict copepoda > cladocera and poor densities of rotifera > rhizopoda. Temporal variations of planktonic communities are discussed with special reference to community similarities, abundance, species diversity, dominance and evenness as well as their relationships with abiotic and biotic factors. Remarks are made on trophic status of the reservoir.

Key words: Ecology, Fisheries, Plankton, Reservoir, Trophic status.

Reservoirs comprise important inland aquatic resources of India and exhibit significant biogenic production potential (Sugunan 1997). Limnological studies in India began in early part of the last century and culminated in good fraction of works dealing with reservoir limnology and plankton production in these biotopes. Little is, however, so far known about their limnology and planktonic communities in north-eastern India in general and under its subtropical conditions in particular. Earlier related contributions from this region are confined to limited investigations from Tripura (Bhattacharya and Saha 1986, 1990) and Meghalaya (Sharma 1995) as well as 2 reports on water quality by Sharma (1999) and Sharma and Lyngdoh (1999).

The present study assumes special interest in view of the stated lacuna and deals with limnological investigations in a man-made hydroelectric reservoir of Meghalaya with reference to its water quality and temporal variations in composition and abundance of net plankton and its constituent groups. Comments are made on synecology of plankton based on their correlations with abiotic and biotic factors and on community similarity, species diversity, dominance and evenness of phyto- and zooplankton. In addition, comments are made on trophic status of the studied biotope.

MATERIALS AND METHODS

The observations were undertaken (May 1995 - June 1996) in Nongmahir reservoir (latitude: 25° 8 ' N; longitude: 91° 50 ' E; area: 70 ha; maximum depth: 25 m) located in Ri - Bhoi district of Meghalaya.

Water and qualitative and quantitative plankton samples were collected at regular monthly intervals; the latter were obtained by a nylobolt plankton net (No. 25), preserved in 5% formalin and analyzed for identification and quantitative analysis of different taxa. Water samples were examined for various abiotic parameters (Sharma 1999). Community similarities (*fide* Sorensen's index), species diversity (*fide* Shannon index), dominance (*fide* Berger-Parker index) and evenness (*fide* E 1 index) were calculated following Ludwig and Reynolds (1988) and Magurran (1988). Ecological relationships were based on computation of correlation coefficients (r) between different abiotic and biotic parameters. Comments on trophic status of the sampled biotope were based on phytoplankton indices (Nygaard 1949) and $Q_{B/T}$ quotient (Sladecek 1983)

RESULTS AND DISCUSSION

Mean annual variations in abiotic factors of Nongmahir reservoir are indicated in Table 1. The subtropical ranges of air and water temperatures correspond with geographical location of the sampled ecosystem while its typical alkaline nature is in distinct contrast to acidic waters reported by Sharma (1995). Distinctly lower dissolved mineral content (specific conductivity) of the reservoir warrants its inclusion in class I category of trophic classification Talling and Talling (1965) and Payne (1986). The stated aspect together with salient characteristic soft-waters and low nutrient

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Table 1. Annual variations in abiotic factors (Sharma 1999)

Parameters	Range	Mean ± SD
Rainfall (mm)	1.2 - 600	175.2 ± 206.8
Air temperature (°C)	16.2 - 27.7	22.4 ± 4.2
Water temperature (°C)	13.5 - 26.3	21.1 ± 4.4
Transparency (m)	0.98 - 2.3	1.9 ± 0.4
pH	7.2 - 9.1	8.1 ± 0.6
Specific conductivity (µS/cm)	27.0 - 50.0	35.5 ± 7.7
Dissolved oxygen (mg/l)	5.6 - 7.6	6.5 ± 0.6
Free CO_2 (mg/l)	1.4 - 4.0	2.8 ± 0.9
Alkalinity (mg/l)	20.0 - 38.0	27.5 ± 5.7
Hardness (mg/l)	12.0 - 32.0	21.5 ± 5.7
Calcium (mg/l)	6.3 - 16.8	10.6 ± 3.3
Magnesium (mg/l)	1.4 - 4.1	2.6 ± 0.9
Chloride (mg/l)	5.3 - 8.7	6.7 ± 1.2
Sulphate (mg/l)	1.9 - 10.1	5.5 ± 2.2
Phosphate (mg/l)	0.03 - 0.20	0.13 ± 0.06
Nitrate (mg/l)	0.09 - 3.4	1.3 ± 1.1
Silicate (mg/l)	1.3 - 3.7	2.8 ± 0.9

concentrations can be attributed to leached and weathered nature of rocks and soils (Rai and Hill 1968, Moss 1988) in the region because of high rainfall and the lowered buffering capacity of the de-mineralized waters (Steinitz-Kannan *et al.* 1983). Besides, this study shows moderate transparency, unsaturated dissolved oxygen and low free carbon-dioxide concentrations. Detailed remarks on temporal variations in water quality and abiotic correlations are made separately by Sharma (1999).

Net plankton of Nongmahir reservoir are apparently less speciose. Phytoplankton richness (17 species) nearly corresponds with that of Malse reservoir (Sharma 1995) but depict lower diversity than the reports by Hickel (1973), Nakanishi *et al.* (1988), Tombi Singh and Shyamananda Singh (1994), Alfred and Thapa (1995) and Das *et al.* (1996). Qualitative predominance of the chlorophyta, however, concurs with the stated works but deviates from the Kashmir Himalayan lacustrine environs (Vass and Zutshi 1983, Vaas *et al.* 1988). Phytoplankton communities register low monthly richness (11± 2 species) and do not follow any definite seasonal pattern (Fig. 1). Further, they record (Table 2) community similarity (56.0 - 92.3%; > 70% similarity noticed in about 64% instances) higher than in Malse (Sharma 1995). Interestingly, poor desmid diversity observed in soft-waters (low electrolyte concentration, low calcium content) of Nongmahir reservoir presents a significant departure than general composition of green-algae under identical abiotic attributes in aquatic environs of this region and elsewhere in the world (Rüttner 1953; Woelkerling and Gough 1976). This salient feature may, however, be attributed to alkaline nature of the sampled biotope.

Zooplankton richness (28 species) is higher than in other subtropical environs of India (Negi and Pant 1983, Patil and Gouder 1985, Sharma 1995, Das *et al.* 1996) and in tropical Gumti reservoir, Tripura (Bhattacharya and Saha 1990) while it is lower than the reports by Vass and Zutshi (1983), Sharma and Pant (1985), Yousuf and Qadri (1985), Tombi Singh and Shyamananda Singh (1994). Besides, zooplankton, register (Table 2) higher community similarity (46.6 - 80.9%; > 50% similarity in 63% instances) than that in Malse reservoir (Sharma 1995) but do not depict any definite annual pattern (Fig. 1). Although the present study exhibits lower richness of rotifera, their qualitative predominance in general is, however, in concurrence with composition of zooplankton communities of many freshwater environs of India (Sharma 1998).

Net plankton abundance (80-312, 164 ± 77 n/l) is higher (Table 2) than that in Malse reservoir (Sharma 1995) but lower than the reports of Bhattacharya and Saha (1990) and Alfred and Thapa (1995). Further, they register (Fig. 2) broadly trimodal annual pattern in contrast to bimodal periodicity recorded in Malse (Sharma 1995). The present observations depict net plankton peak in January with distinctly higher abundance during winter season (December - February) and, hence, concur with the results of Wanganeo and Wanganeo (1991). Such a trend is supported by distinct inverse correlation between net plankton and water temperature ($r = -0.899$). In addition, they record significant positive relationship with pH ($r = 0.779$). In spite of relatively lower densities during monsoon months, net plankton do not exhibit

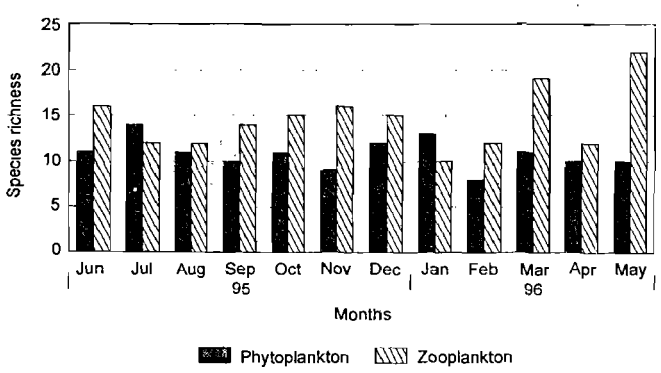

Fig. 1. Species richness of phyto and zooplankton.

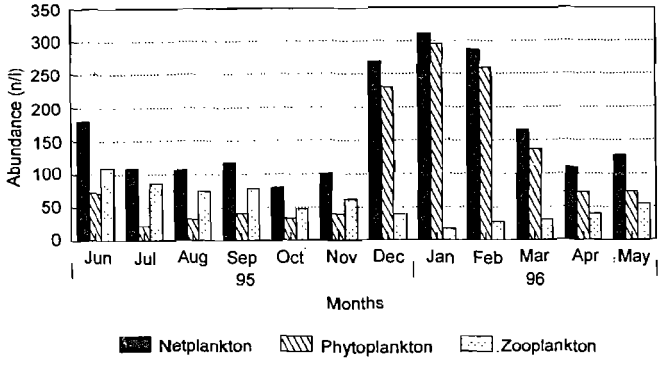

Fig. 2. Abundance of net plankton, phyto and zooplankton.

Table 2. Annual variations in plankton

Parameters	Range	Mean±SE
Net plankton (n/l)	80 - 312	164 ± 77
Phytoplankton	17 species	
Qualitative	Chlorophyta > Bacillariophyta = Cyanophyta > Dinophyta = Chrysophyta	
Species richness	8 - 14	11 ± 2
Community similarity	56.0 - 92.3%	-
Quantitative	Chlorophyta > Cyanophyta > Bacillariophyta > Chrysophyta > Dinophyta	
Abundance (n/l)	22 - 295	109 ± 94
Percentage composition	20.4 - 94.6	56.5 ± 26.3
Species diversity (Shannon's)	0.579 - 2.420	1.596 ± 0.538
Dominance (Berger-Parker's)	0.182 - 0.849	0.488 ± 0.225
Evenness (E 1)	0.232 - 0.917	0.656 ± 0.207
Different groups		
Chlorophyta (n/l)	9 - 272	83 ± 99
(Percentage)	28.2 - 94.4	62.1 ± 23.4
Cyanophyta (n/l)	3 - 20	11 ± 6
(Percentage)	4.3 - 22.5	13.6 ± 6.1
Bacillariophyta (n/l)	1 - 40	7 ± 10
(Percentage)	0.4 - 56.3	11.9 ± 16.1
Chrysophyta (n/l)	1 - 64	6 ± 17
(Percentage)	1.4 - 46.8	5.7 ± 5.6
Dinophyta (n/l)	1 -5	3 ± 1
(Percentage)	0.3 - 8.2	5.6 ± 12.7
Zooplankton		
Qualitative	28 species - Rotifera > Cladocera > Rhizopoda > Copepoda	
Species richness	10 - 22	15 ± 3
Community similarity	46.7 - 80.9%	-
Quantitative	Copepoda > Cladocera > Rotifera > Rhizopoda	
Abundance (n/l)	17 - 109	55 ± 26
Percentage composition	5.4 - 79.6	43.6 ± 26.4
Species diversity (Shannon's)	1.891 - 2.840	2.254 ± 0.289
Dominance (Berger-Parker's)	0.133 - 0.392	0.296 ± 0.098
Evenness (E 1)	0.761 - 0.988	0.838 ± 0.090
Different groups		
Copepoda (n/l)	6 - 73	31 ± 26
(percentage)	22.2 - 72.6	50.5 ± 15.7
Cladocera (n/l)	3 - 34	14 ± 9
(percentage)	11.3 - 43.6	28.2 ± 16.4
Rotifera (n/l)	5 - 13	9 ± 3
(percentage)	6.7 - 40.0	21.1 ± 11.8
Rhizopoda (n/l)	1 - 3	2 ± 1
(percentage)	0.9 - 10.0	3.8 ± 2.7

any adverse general impact of rainfall in Nongmahir reservoir.

Phytoplankton (109 ± 90 n/l) comprise (Table 3) dominant component (56.5 ± 26. 5%) of net plankton and significantly influence annual variations of the latter (r = 0.963). Further, their mean abundance nearly corresponds with that in Malse reservoir (Sharma 1995). The recorded densities are, however, significantly lower than the reports of Nakanishi *et. al.* (1988) and Vass *et. al.* (1989). Like net plankton, phytoplankton of Nongmahir reservoir depict (Fig. 2) distinct winter abundance and peak in January, which is well supported by significant negative correlation with water temperature (r = - 0.899). On the other hand, they indicate notably lower densities during monsoon but do not register significant inverse relationship with rainfall. The positive correlations of phytoplankton with pH (r = 0.828) and dissolved oxygen (r = 0.605) as well as an inverse relationship with free carbon dioxide (r = - 0. 705) exhibit photosynthetic importance of these autotrophs. Besides, phytoplankton indicate direct correlation with transparency (r = 0.588) in the present study.

Chlorophyta (83 ± 94 n/l; 62.1 ± 23. 4%) represent (Table 3) the most dominant group of phytoplankton and significantly influence their abundance (r = 0.972) and that of net plankton (r = 0.939). The qualitative predominance of the green-algae concurs with the results of Bhattacharya and Saha (1990),

Table 3. Phytoplankton abundance (n/l)

Months	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May
Chlorophyta												
Staurastrum rotundum	9	1	-	-	1	-	7	2	6	8	25	20
S. leptocladium	1	1	-	1	1	-	5	3	3	1	6	2
Cosmarium decoratum	3	-	3	-	-	-	-	1	-	-	-	-
C. reniforne	1	1	1	-	2	3	13	6	8	8	10	9
C. leibleinii	-	1	-	-	-	1	1	-	-	-	-	-
Spirogyra sp.	6	3	-	-	-	1	-	-	-	-	-	1
Ulothrix sp.	-	1	4	1	8	18	190	269	220	30	5	25
Protococcus sp.	-	1	10	16	3	3	2	1	-	2	-	-
Gonatogygon kinahani	-	-	1	1	1	-	-	-	-	-	-	-
Cyanophyta												
Microcystis aeruginosa	4	1	4	5	6	6	6	13	15	17	12	5
Anabaena sp.	3	1	1	2	-	-	1	1	-	-	3	-
Spirulina sp.	1	1	1	1	1	1	3	5	5	1	1	1
Bacillariophyta												
Navicula radiosa	40	4	3	7	4	4	1	1	1	-	-	-
N. sp.	-	1	1	-	-	-	1	1	-	1	-	1
Diatoma sp.	-	-	-	1	1	-	-	1	-	1	1	-
Chrysophyta												
Dinobryon sociale	1	1	-	-	-	-	-	-	-	64	6	6
Dinophyta												
Ceratium hirudinella	2	4	2	5	5	1	1	1	2	4	3	3
Chlorophyta	26	9	19	19	16	26	218	272	237	49	46	57
Cyanophyta	8	3	6	8	7	7	10	19	20	18	16	6
Bacillariophyta	40	5	4	8	5	4	2	3	1	2	1	1
Chrysophtya	1	1	-	-	-	-	-	-	-	64	5	6
Dinophyta	2	4	2	5	5	1	1	1	2	4	3	3
Phytoplankton	71	22	31	40	33	38	231	295	260	137	71	73

Alfred and Thapa (1995) and Sharma (1995) but differs from the diatom - blue green algae dominance recorded by Vass and Zutshi (1983), Nakanishi *et al.* (1988) and Vass *et al.* (1988). The present observations indicated winter peak of free-floating filamentous algae and, hence, exhibit distinct departure from the abundance of desmids recorded in the stated reports from N. E. India and several other studies in this region. On the other hand, only 2 desmids i.e., *Stauastrum rotundum* and *Cosmarium decoratum* indicated numerical importance in certain months. The chlorophyta registered significant negative correlations with water temperature (r = - 0.881) and free CO_2 (r = - 0.693) and positive relationships with pH (r = 0.812) and dissolved oxygen (r = 0.558).

The present study indicated poor abundance (Table 3) of cyanophyta > bacillariophyta > chrysophyta > dinophyta which is in contrast to importance of chrysophyta in Malse (Sharma 1995) and that of cyanophyta > dinophyta > bacillariophyta in Gumti reservoir (Bhattacharya and Saha 1990) and also differs distinctly from the results of Vass and Zutshi (1983), Nakanishi *et al.* (1988) and Vass *et al.* (1988, 1989). Blue-green algae in general and *Microcystis aeruginosa* in particular exhibit more densities in winter-spring - early summer and an inverse relationship with water temperature (r = - 0.597). The bacillariophyta (*Navicula radiosa*) and chrysophyta (*Dinobryon sociale*) showed significance only once i. e., during June and March respectively. The dinophyta (*Ceratium hirudinella*) recorded insignificant abundance throughout the study period.

Zooplankton (55 ± 26 n/l; 43.6 ± 26.1%) exhibited (Table 4) higher abundance than that in Malse reservoir (Sharma 1995) but lower than the reports by Zutshi *et al.* (1980), Vass and Zutshi (1983), Nakanishi *et al.* (1988), Vass *et al.* (1988, 1989), Tombi Singh and Shyamananda Singh (1994), Alfred and Thapa (1995) and Das *et al.* (1996). They follow indefinite annual pattern, do not exert any definite influence on net plankton and record higher densities (Fig. 2) during summer and rainy season, the last aspect concurrs with the results of Negi and Pant (1983) and is supported by their significant direct relationships with water temperature (r = 0.899) and rainfall (r = 0.808). Besides, they indicate inverse relationships with dissolved oxygen (r = - 0.682), hardness (r = - 0.600), pH (r = - 0.608), magnesium (r = - 0.600), and direct correlations with free CO_2 (r = 0.799) and phosphate (r = 0.597). An inverse relationship noticed presently between zoo- and phytoplankton (r = -0.678) is in contrast to positive correlations noticed earlier by Zutshi *et al.* (1980), Sharma (1995) and Das *et al.* (1996). Further, mean phyto- / zoo-plankton ratio shows significant fluctuations (0.24 – 17.4)

Table 4. Zooplankton abundance (n/l)

Months	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May
Rotifera												
Anuraeopsis fissa	-	-	-	-	1	1	-	-	-	1	-	1
Brachionus angularis	-	1	-	-	-	-	-	-	-	1	1	1
B. quadridentatus	1	-	-	1	-	-	1	-	-	-	-	-
Keratella cochlearis	-	1	1	1	1	1	1	1	1	1	1	1
Pleosoma hudsoni	1	-	1	-	-	-	-	-	2	-	-	-
Polyarthra vulgaris	1	-	-	1	-	-	-	-	-	1	-	-
Ascomorpha saltans	-	-	-	-	-	1	-	-	-	-	-	1
Lecane bulla	-	-	-	-	1	1	1	1	1	1	1	1
L. luna	1	1	-	-	-	1	1	1	1	1	1	1
Euchlanis dilatata	1	1	1	1	1	-	1	-	-	1	-	1
Trichocerca cylindrica	2	1	-	-	1	1	-	-	1	1	-	1
Conochilus unicornis	-	-	-	1	1	1	1	1	1	3	4	3
Pompholyx sulcata	1	-	1	-	-	-	1	-	-	-	1	1
Asplanchna priodonta	2	2	1	1	1	1	3	2	3	1	2	1
Cladocera												
Chydorus sphaericus	2	-	1	-	-	-	-	-	1	1	1	1
Alonella excisa	-	-	-	-	-	1	-	-	-	1	-	1
Moina micrura	-	-	-	1	1	1	1	-	-	1	1	1
Bosmina longirostris	9	8	5	10	2	1	7	3	9	3	1	4
Bosminopsis deitersi	11	11	5	14	5	4	4	-	-	-	-	1
Diaphanosoma sarsi	2	5	7	9	4	-	-	-	-	-	1	1
Daphnia lumholtzi	1	-	-	-	-	-	-	-	-	-	1	1
Copepoda												
Cyclops sp.	30	15	13	5	3	6	4	3	3	4	5	13
Diaptomus sp.	23	29	29	24	18	31	8	3	1	2	3	5
Nauplius larvae	20	10	8	7	5	8	3	1	2	3	10	11
Rhizopoda												
Difflugia lebes	-	1	-	1	1	1	1	-	-	1	1	1
Arcella vulgaris	-	-	1	-	-	-	-	-	-	-	-	-
Acanthocystis chaetophora	1	-	-	1	-	-	-	1	-	1	1	-
Polymyxa sp.	-	-	-	-	1	-	-	-	1	1	1	1
Other												
Dipteran larva	-	-	-	-	-	-	-	-	-	-	1	-
Copepoda	73	54	50	36	26	45	15	7	6	9	18	29
Cladocera	25	24	18	34	12	7	12	3	10	6	5	10
Rotifera	10	7	5	6	7	9	10	6	10	12	11	13
Rhizopoda	1	1	1	2	2	1	1	1	1	3	3	2
Other	-	-	-	-	-	-	-	-	-	-	1	-
Zooplankton	109	86	74	78	47	62	38	17	27	30	38	54

during the study and their mean annual ratio (3.7 ± 4.9) in Nongmahir reservoir is lower than the reports of Negi and Pant (1983), Vass *et al.* (1989) and Sharma (1995).

Copepoda (31 ± 36 n/l; 50.5 ± 15.7%) comprise the most dominant component of zooplankton and correspond with the results of Negi and Pant (1983), Das *et al.* (1996) and Sharma and Hussain (2001). Such a trend is in contrast to other observations in Meghalaya (Alfred and Thapa 1995, Sharma 1995. This group indicated higher densities during warmer months and monsoon season and primarily influences annual periodicity of zooplankton (r = 0.961); the former 2 aspects are supported by their significant direct relationships with water temperature (r = 0.783) and rainfall (r = 0.887). Besides, the copepods exhibit identical correlations with pH (r = 0.579), free CO_2 (r = 0.813) and phosphate (r = 0.656) and an inverse impact on dissolved oxygen (r = - 0.631) through their respiratory activity. The quantitative dominance of the copepods during the study period reflects the prevalence of stable environmental conditions for these 'k-strategists' as suggested by Allen (1976) and Schmidt-Araya and Zuniga (1992). Further, the calanoids out-number (Table 4) the cyclopoids in Nongmahir reservoir. Interestingly occurrence of nauplii throughout the present observations indicated periods of active reproduction of this group and concurs with the results of Das *et al.* (1996) and Sharma and Hussain (2001).

Table 5. Ecological indices

Months	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May
					Phytoplankton							
Species diversity												
Shannon's index	1.560	2.420	2.081	1.709	2.106	1.656	1.061	0.579	0.686	1.616	1.909	1.772
Dominance												
Berger-Parker's index	0.563	0.182	0.323	0.400	0.242	0.474	0.822	0.849	0.846	0.467	0.352	0.342
Evenness												
(E1)	0.650	0.917	0.668	0.742	0.878	0.754	0.427	0.232	0.330	0.674	0.829	0.770
					Zooplankton							
Species diversity												
Shannon's index	2.058	1.974	1.891	2.078	2.172	1.891	2.463	2.172	2.427	2.840	2.542	2.537
Dominance												
Berger-Parker's index	0.275	0.337	0.392	0.308	0.383	0.500	0.210	0.176	0.333	0.133	0.263	0.241
Evenness												
E1	0.742	0.794	0.761	0.789	0.802	0.682	0.909	0.988	0.904	0.964	0.897	0.821

Cladocera (14 ± 9 n/l; 28.2 ± 16. 4%) depict lower abundance but influence zooplankton fluctuations (r = 0.804). They record (Table 4) higher densities in early part of the study period (June – September) and thus register significant positive relationships with rainfall (r = 0.723) and water temperature (r = 0.610). Besides, they record negative correlations with transparency (r = -0.641), dissolved oxygen (r = - 0.720), hardness (r = - 0.714). The cladoceran periodicity is mainly influenced by the bosminids, *Bosmina longirostris* and *Bosminopsis deitersi*. In general, this group indicates higher abundance than in Malse (Sharma 1995) and lower densities than the reports of Negi and Pant (1983), Patil and Gouder (1985), Yousuf and Qadri (1985), Vass *et. al.* (1989), Alfred and Thapa (1995) and Das *et al.* (1996).

Rotifera (9 ± 3 n/l), practically an insignificant component of zooplankton of Nongmahir reservoir, deviate from their valuable role as noticed by Jyoti and Sehgal (1979), Negi and Pant (1983), Patil and Gouder (1985), Bhattacharya and Saha (1990), Vass *et al.* (1989), Sharma (1995) Das *et al.* (1996). None of the documented rotifer species indicate any seasonal periodicity of occurrence or abundance. Besides, the rhizopods indicate very poor abundance in the present study and other (dipteran larva) is noticed only in April.

The index of species diversity (*fide* Shannon's formula) is influenced by species richness and equitability or relative abundance of species (Sager and Hasler 1969) Phytoplankton exhibit (Table 5) lower species diversity (1.586 ± 0.538) than zooplankton (2.254 ± 0.289) in Nongmahir reservoir because of predominance of fewer species. Further, the former register greater monthly seasonal variations irrespective of their species number, hence, suggest that richness is less important in the index because of narrow range as concluded by Sager and Hasler (1969) and Nakanishi *et al.* (1988). The present report of lowest species diversity of phytoplankton (mainly contributed by filamentous green-algae) concurrent with their peak density stresses emphasis on relative importance of predominant species (Moss 1973). Besides, phytoplankton reflect lower species diversity during winter maxima and peak diversity coincides with their minimum abundance. They exhibit moderate dominance (0.488 ± 0.225) and equitability (0.656 ± 0.207); the former was inversely related to species diversity and evenness. On the other hand, zooplankton record lower dominance (0.296 ± 0.098) and higher equitability (0.838 ± 0.090). They do not depict any definite pattern of species diversity but support an inverse correlation with dominance and a positive relationship with evenness.

Sladecek (1983) suggested *Brachionus*: *Trichocerca* ratio ($Q_{B/T}$) to be useful index for evaluation of trophic status of different aquatic environs. Sharma and Dudani (1992) indicated its usefulness and limitations under Indian conditions. Application of Sladecek's quotient for the present study period resulted in $Q_{B/T} = 2.0$, thereby, indicating mesotrophic status of Nongmahir reservoir. Such a conclusion may provide misleading picture of general tropic status of the sampled water body as species of the mentioned 2 rotifer genera exhibit occassional occurrence, thereby, indicating to general oligotrophic status. This generalization also appears to be valid based on myxophycean index (0.6) and chlorophycean index (0.2) *vide* Nygaard (1948) thus concluding an oligotrophic character of Nongmahir reservoir which, however, indicates a shift towards mesotrophy particularly during winter. This fact is corroborated by water quality parameters (Sharma 1999) and general composition and abundance of planktonic communities as noticed in the present study.

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