

Figure 1. The percentage frequencies of different types of abnormal sperms in a natural population of *P. pictus* (Fabr). Different types of morphologically abnormal sperms: 1. Shrunken; 2. Polyploid; 3. Broken; 4. Zig-zag; 5. Folded, and 6. Coiled.

alterations in the morphology of some spermatozoa in the population, affecting their motility and viability should be considered as their abnormalities since they may not be able to compete with their counterparts in fertilization. The environment, loaded with various types of pollutants, might have played an important role in the production of abnormal sperms in these specimens.

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USE OF UNSUCKLED BOVINE SERUM IN TISSUE CULTURE

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FOETAL calf serum (FCS) has been widely used for the supplementation of synthetic tissue culture media¹. In recent years there has been an acute global shortage of FCS. Experimental studies were therefore planned to test the suitability of local cow and buffalo calf serum and the serum from newborn unsuckled calf for tissue culture. The parameters chosen were cytotoxicity and growth promoting effects on HeLa, Vero and BHK-21 cells. The presence of gamma globulins and antibodies to ten different togaviruses was also studied.

Nineteen sera samples comprising of 3 unsuckled buffalo calf, 3 unsuckled cow calf, 7 buffalo calf and 6 cow calf were studied. FCS (obtained from Microbiological Associates, USA) was used as control. HeLa, Vero and BHK-21 cell lines were maintained in minimum essential medium (MEM) with 10% serum and 10% tryptose phosphate broth respectively. The rate of attachment was studied by observing the cultures before and after fixation at 1/2, 1, 2, 4 and 24 hr. Growth rates were studied by the method of Patterson².

Sera were screened by haemagglutination inhibition (HI) test³ for the presence of antibodies against chikungunya, Sindbis, Japanese encephalitis (JE), West Nile (WN) Dengue type I, II, III and IV, Kyasanur Forest Disease and Batai viruses. The neutralization test (NT)⁴ was carried out to confirm the HI results.

The sera samples were subjected to electrophoresis on cellogel membranes and the patterns of serum proteins were evaluated densitometrically to assess the presence of gamma globulins.

The HeLa, Vero and BHK-21 cells attached to the glass surface within 30 min after incubation, though they appeared rounded. There was wide variation in the period required for attachment of cells firmly to the glass depending on the sera samples and cell types used. It is, therefore, important to use sensitive cell lines while testing sera. Vero cells required the least time for attachment and exhibited less variation

to different samples of sera. BHK-21 cells showed uniformly good response to the sera from the newborn unsuckled calves as against those from the older calves, indicating that the sera from unsuckled calves were least cytotoxic to the cells studied.

It is interesting to note that though the cells attached to the glass with one of the samples (B-18), there

was no evidence of cell proliferation. This indicates that the factors in sera responsible for attachment of cells and the growth of cells are distinct. Sera from the unsuckled calves exhibited higher growth promoting effect than those obtained from suckled calves (figure 1). The higher growth-promoting activity in suckled sera is probably due to their higher α -1-glo-

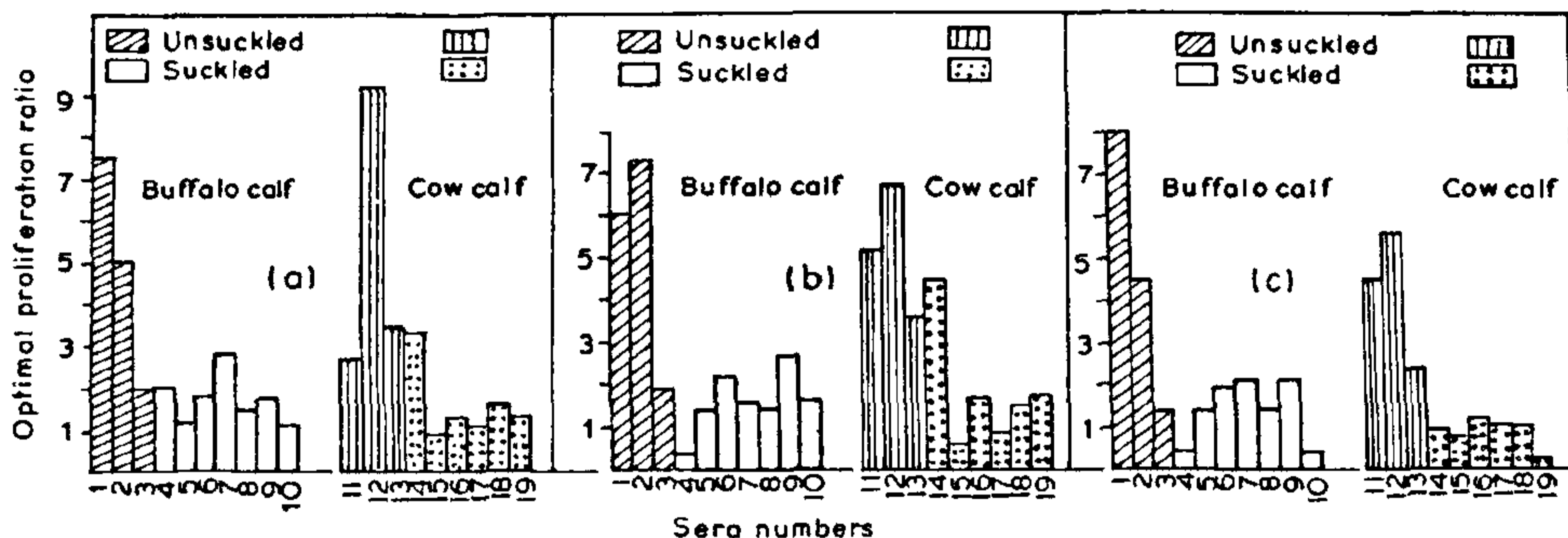


Figure 1a-c. Optical proliferation ratios. a. Vero, b. HeLa, and c. BHK-21 cells in suckled and unsuckled calf sera.

Table 1 Cell growth, γ -globulins and Viral antibodies in different sera

Serum samples	Attachment of cells and growth rate				Antibodies	
	Vero	HeLa	BHK-21	γ -globulin	HI	(NT)
UB-2	III	III	III	-	Batai (-ve)	
UB-4	III	II	III	-	Batai (+ve)	
UB-8	III	II	III	\pm	JE (-ve), WN (-ve)	
UC-9	III	III	II	-	Batai (+ve)	
UC-15	II	III	III	\pm	WN (-ve), Batai (+ve)	
UC-23	II	III	III	-	None (-ve)	
FCS-73	III	III	III	-	None (-ve)	
FCS-74	III	III	III	-	None (-ve)	
B-3	III	II	II	+	None	
B-7	III	II	III	-	None	
B-10	III	II	II	+	JE (-ve), WN (+ve)	
B-11	III	III	II	+	WN	
B-13	III	III	I	+	None	
B-14	III	III	II	+	None	
B-18	II	II	II	+	JE	
B-20	III	II	I	+	Batai (+ve)	
B-25	III	III	III	+	WN, Batai	
C-17	III	II	I	+	JE, WN, Batai	
C-19	III	II	I	+	Batai	
C-22	III	III	III	+	Batai	
C-26	III	II	II	+	None	

B = Buffalo calf, C = Cow calf, U = New born unsuckled calf; HI = Haemagglutination inhibition test; NT = Neutralization test; III = Most of the cells attached and flattened, II = 75% cells attached, many rounded cells; I = 50% cells attached many rounded cells; FCS = Foetal calf serum.

bulin content⁵. The suckled or the older calf sera, though suitable for the growth of cells, showed lower growth-stimulating activity.

The studies revealed no significant difference in the cow and buffalo calf sera. Unsuckled calf sera did not show gamma globulin bands (table 1), which is a special feature of foetal sera. There are reports that the quality of adult sera can be improved by careful removal of gamma-globulins⁶.

Table 1 shows the results of HI and NT tests. Due to the limited quantity of experimental sera, NT could not be performed using all the sera samples. The results indicate that some sera samples contained both haemagglutination inhibiting and neutralizing antibodies while others contain only HI antibodies. Batai is a virus of common occurrence in the cattles of Maharashtra.

Young calves B-10 and B-20 might have received the antibodies for WN and Batai viruses respectively, through the colostrum of the mother⁷. But the presence of antibodies for Batai virus in unsuckled newborn calves suggests that either these might have been derived from transfer of maternal antibodies to the foetus through placenta or due to the immunobiological response of the foetuses⁸. Viruses and mycoplasma have been isolated from the foetal bovine sera⁹. It is desirable to conduct specific tests for the antibodies before using sera for cell cultures employed for virological studies.

Compared to calf sera, unsuckled calf sera had better growth-promoting effect on cells and were least cytotoxic to cells studied. Some sera may contain neutralizing antibodies against viruses. It appears that unsuckled calf sera are a suitable substitutes for the foetal bovine sera.

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AN INTERESTING NEW SPECIES OF *CYLINDROSPERMUM* KÜTZ: *C. ANABAENOIDES* SP NOV—A BLUE GREEN ALGA

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THE present alga was collected from a paddy field at Sirsi in Karnataka State in September 1984. Unialgal cultures were raised by repeated subculturing in soil-water biphasic media and the chief taxonomic features were studied.

Terminal heterocysts followed by large spores and absence of intercalary heterocysts is the characteristic feature of *Cylindrospermum* Kütz and intercalary spores as in the present alga are known in *Anabaena* Bory. Both these genera belong to the family Nostocaceae of Cyanophyta¹. However, since intercalary heterocysts are completely absent both in natural collections and in cultured specimens, the present alga cannot be included in *Anabaena*. The above form resembles *C. marchicum* Lemmermann² and *C. catenatum* Ralfs² in having a series of spores adjacent to terminal heterocyst but differs much from all the known species of *Cylindrospermum* in having intercalary spores. In view of the above distinctive characters, the present alga is identified as a new species and is designated as *C. anabaenoides* sp nov in view of its resemblance to *Anabaena* (figure 1).

Cylindrospermum anabaenoides sp nov

Thallus spreading, mucilagenous, blue green, filaments curved, loosely entangled in colourless