nitro-2-furfuraldehyde diacetate[13] (2.43 g, 0.01 mol) in absolute alchol (20 ml) was treated with concentrated sulphuric acid (0.5 ml) and was heated under reflux for 1–2 hr. On cooling the reaction mixture a yellow solid product separated out, which was crystallized from dioxane to give yellow micro needles of II a, m.p. 182–3°. Other triazoles (I) were similarly condensed with 5-nitro-2-furfuraldehyde diacetate and 5-nitro-2-acetylfuran[14] to give compounds (II) and (III) respectively. The characterization data of these compounds are reported in table 1.

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SPERM ABNORMALITIES IN A NATURAL POPULATION OF *POECILOCERUS PICTUS* (FABR)

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SPERMS are important indicators in reproductive toxicology as they can be used to assess the spermatogenic damage, the fertility effects and the heritable mutations induced either by physical or chemical mutagens or by the surrounding environment itself. The most commonly used system is the mouse sperm test. Chemical induction of sperm abnormalities has been reported in the grasshopper, *Poecilocerus pictus* (Fabr)[1,2]. The physical agent, viz constant high temperature[3], also induced sperm abnormalities in *P. pictus* (Fabr). In the present investigation, a natural population of *P. pictus* (Fabr) collected from Bangalore, was employed for sperm abnormality studies.

Testes were dissected out and fixed in a mixture of acetic acid and alcohol in the ratio 1:3. Heidenhain's iron haematoxylin squash preparations in 45% acetic acid were made. The sperms from uniformly well-spread areas were screened. Different types of morphologically abnormal sperms like shrunken, coiled, folded, zig-zag, broken and polyploid were noted and the percentage frequencies of individual anomalies were recorded (figure 1).

Polyploid spermatozoa have been reported in higher animals[4] like mouse, rabbit, bull and man[5]. In rabbit they form 0.03% of the total sperm, in the bull 0.01% to 0.17% and in man 1.02%. In *P. pictus* (Fabr), 4.21% of polyploid sperms were observed in the present investigation. Sperms were found to coil in bundles in different degrees and this observation resembled the coiling of the sperms of 'Sevin' treated grasshoppers[1]. A sudden bending at frequent intervals in opposite direction, giving a zig-zag appearence to the sperms was common with the extreme conditions resulting in the folded sperms. Many were seen broken either individually or in bundles at a single point or two or more points. Occasionally frequent coiling/coilings were seen in individual/bundles of sperms. The extreme case of folding and coiling followed by their streching might have resulted in the broken sperms. A few of the sperms were reduced in length with irregular bends and splits and were of shrunken nature. Such radical

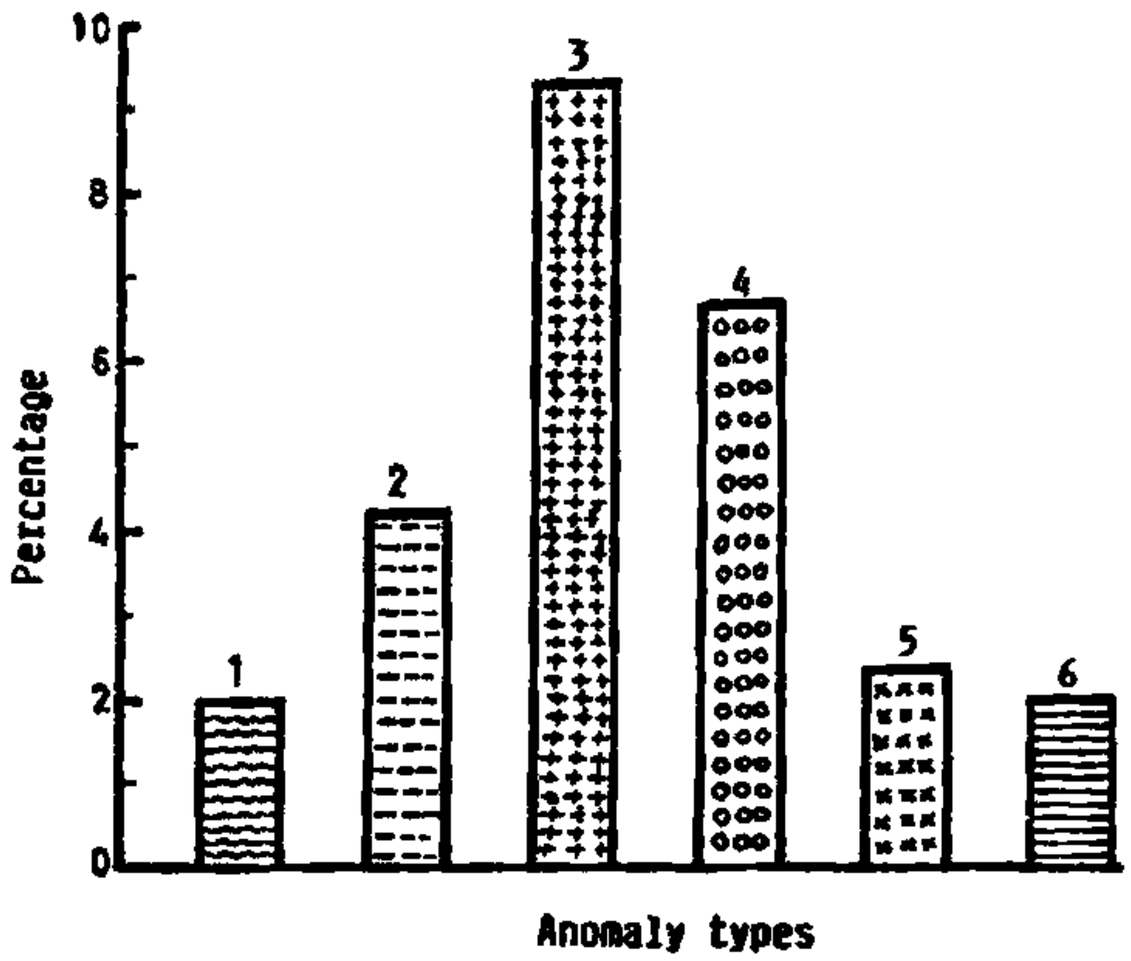

Figure 1. The percentage frequencies of different types of abnormal sperms in a natural population of *P. pictus* (Fabr). Different types of morphologically abnormal sperms: **1.** Shrunken; **2.** Polyploid; **3.** Broken; **4.** Zig-zag; **5.** Folded, and **6.** Coiled.

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 media[1]. 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 Patterson[2].

Sera were screened by haemagglutination inhibition (HI) test[3] 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