CHAPTER - V

THE KINETICS OF SPERMATOGENESIS

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CONTENTS

INTRODUCTION

The investigation on the duration of the meiotic events and spermatogenesis has revealed a number of interesting findings. The cycle of seminiferous epithelium in mammal is a phenomenon of fixed duration (Clermont and Trot, 1969). In rat testis, hypophysial hormones altogether (Clermont and Harvey,1965) and increase in temperature (Chu et. al. 1974;Waites and Ortavant,1976) do not influence the rate of development of the germ cells and the duration of the cycle.

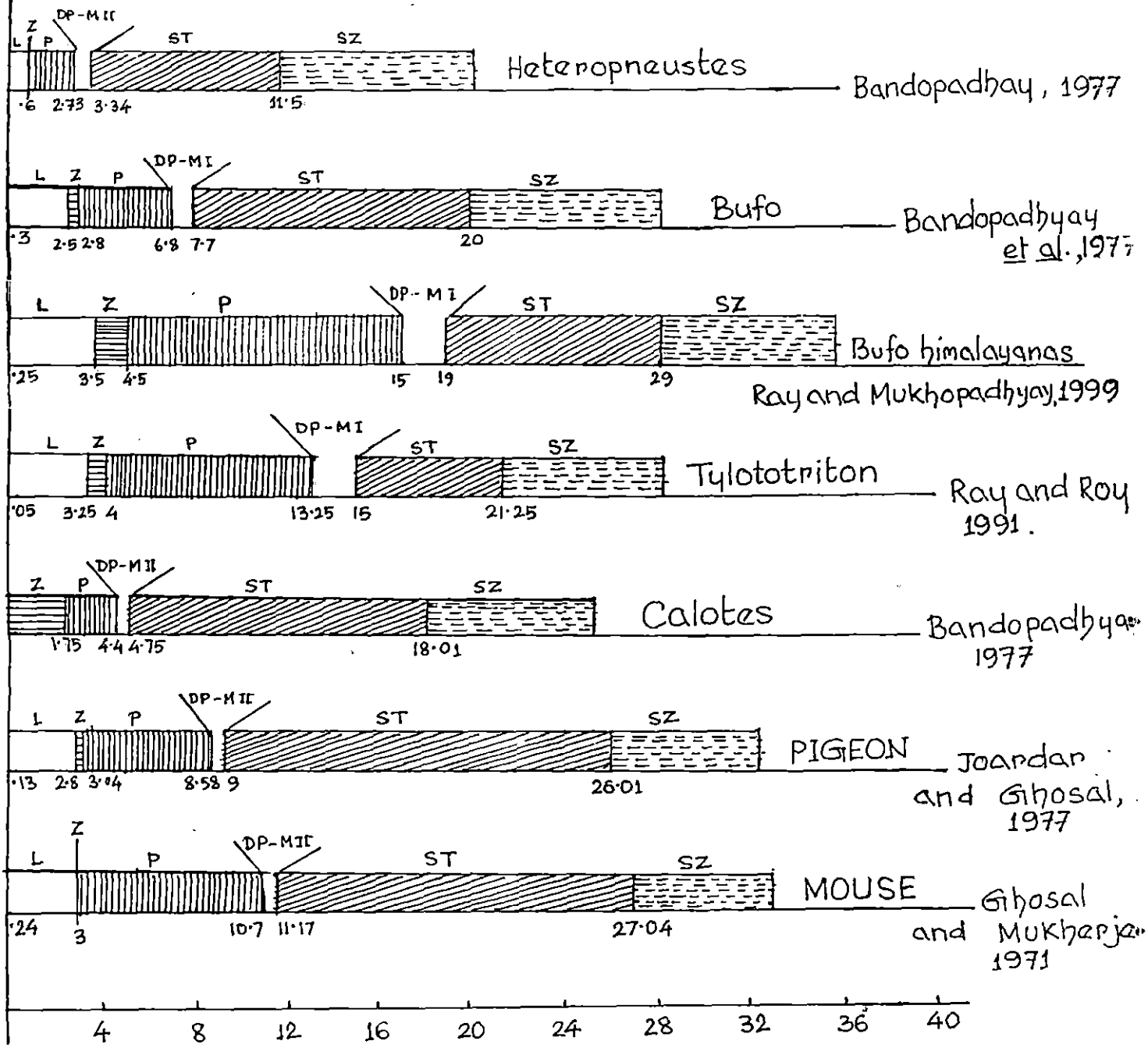
The time cycle of spermatogenesis has been estimated by several investigators using various techniques, such as the colchinine injection (Roosen-Runge,1951) , X-ray exposers (Oakberg,1955), heat-shock (Mcleod and Hotchkiss,1941; Young, 1927) and the injection of toxic substances (Drobeck and Coulston,1962; Muzzanti *et al.* ,1964) to the gonadal tissue. Probably the best of these methods is the one employing a lebelled precursor of DNA. After incorporation of the labels into the DNA of the germ cells, their kinetics till the spermatozoa formation could be traced autoradiographically.

Radioactive tracers such as ^{14}C –adenine, ^{3}H –thymidine and ^{32}P-phosphate has been used widely for determination of the duration of the meiotic stages and spermiogenesis in the mouse(Pele and Howard,1956; Sirlin,1958), rat (Clermont *et al.* 1959), rabbit (Swierstra and Foote,1963,1965), the chinese hamster (Utakoji,1966),Golden hamster (Ghosal and Mukherjee,1971), man (Heller and Clermont,1963), Stallion (Swierstra *et.al.* 1974) and *Mus booduga* (Basu Roy *et al.*,1987). The details of the duration of individual stages of meiosis and spermiogenesis in various vertebrates as estimated by different investigators are shown in Diagram-10.

However information on the details of the amphibian spermatogenic chronology is somewhat sporadic. Gurwitsch (1911), Stieve (1920), Kalt (1976), Bandopadhyay *et al.*(1977), Ray (1978), Ghosal *et al.* (1981), Ray *et al.* (1984), Mallick (1987), Ghosal and Mallick (1988), and Mallick and Ghosal (1988) have recorded the duration or time cycle of spermatogenesis in some salientians and urodel species.

DIAGRAM : 10

A COMPARATIVE DIAGRAM OF THE KINETICS OF MEIOSIS AND SPERMIOGENESIS IN DIFFERENT VERTEBRATES



ABBREVIATIONS

L = Leptotene Z = Zygotene P = Pachytene
DP-MI = Diplotene to Metaphase-I
DP-MII = Diplotene to Metaphase-II
ST = Spermatid SZ = Spermatozoa

AIMS AND OBJECTIVES OF THE PRESENT STUDY

The present study involves the estimation of the total duration of the meiosis and spermiogenesis in Himalayan toad, *Bufo himalayanus* (Gunther) [Anura: Amphibia].

The present study has been engineered to see whether the spermataogenetic cycle of this particular species is continuous or discontinuous in nature and how the duration or time cycle of meiosis differs with other amphibian species.

The present study is also aimed to establish relation ship of the kinetics of meiosis and spermiogenesis with the seasonal breeding habit of this high altitude species.

The present study also will focus on some idea whether extrinsic factors (viz. temperature) has any effect on the kinetics of spermatogenesis. The present study also has an intention to show the phylogenetic significance of the meiotic kinetics and also the phylogenetic relationships between the closely associated classes of animals on the basis of available records.

MATERIALS AND METHODS

Material

Mature and developing testes of the himalayan toad *Bufo himalayanas* were collected from the dissected animals for various methods of preparations for further study and observations.

Methodology

For the study of the kinetics of meiosis and spermiogenesis radio-autographic method suits best. It has also been prooved by various investigators working on this type of experiments.

Radio-autographic method

a) **Selection of tracer :** Different types of labelled precursors like ^{32}P-phosphates (Ortavant,1958) , ^{14}C-adenine (Benette *et al.* 1960), ^{3}H-thymidine (Pollister,1969 ; Wright *et al.*,1970), etc. are used for lebelling DNA when it is synthesized during the premitotic as well as premeiotic S period. The use of ^{32}P- phosphate is not very suitable for our present study as it is incorporated into a variety of compounds besides DNA , while ^{14}C- adenine labels both DNA and RNA. ^{3}H- thymidine is , however, very suitable for its specificity for labelling DNA(Reichard and Estbor, 1951; Mukhopadhyay *et al.* ,1986). The ^{3}H-thymidine has been used in the present investigation, as tritium has additional advantages. It produces very low energy beta rays (a maximum output of energy of 0.018 MeV; Perey, 1964) that are recorded on the overlying nuclear track emulsion or striping film very close to their sites of emission (Messier and Leblond, 1960) resulting in a fine autoradiographic resolution (Taylor *et al.* ,1957). After the injection the tracer diffuses very rapidly throughout the gonad (Utakoji and Hsu, 1965).

b) Administration of tracer : Conventional dosages of 20 μci of tritiated thymidine (Bhabha Atomic Research Center, Trombay) was injected intraperitoneally (sp.act. 10,000 μci/mM) to the animal for investigation at normal breeding season and at the onset of winter.

c) Methods for obtaining the testicular samples: The testis from these specimens were collected after 0-25 days post-injection period. After collection squash preparation was made .

d) Methods for filming with AR-10 stripping film. Slides enshrining squashed testicular material were coated with Kodak-AR –10 autoradiographic stripping film in the dark room conditions (Stevens, 1966) and stored in light proof box containing silica gel (Pfizer) inside the refrigerator at 4 degree centigrade. A At every 5 days interval, used silica gel was replaced by freshly prepared gel. After 4 weeks of exposure, slides were developed for two minutes duration in Kodak D –19 or D- 19b developer (prepared in laboratory) , rinsed in distilled water, fixed in Kodak acid fixer also for two minutes duration, rinsed once again in distilled water and then washed thoroughly for half an hour in running tap water. Throughout the experiment the temperature was maintained at 19 degree Centigrade (Marchant *et al* . , 1965). Then the labelled stages were examined under microscope and selected frames were photographed. In a few cases where identification of the cell or the ellucidation of the archetechture of the chromosome was not possible due to accumulation of grain, the grains were removed and the particular cell then re-examined to identify the stages of the meiosis and spermiogenesis (Plate-36, Figure A-K).

e) Removal of silver grains: There were some case where huge amount of autoradiographic silver grains were accumulated in the slide. These grains were removed by the following procedure (Blanchi *et al.*,1964).

i) Slides were immersed in a slution of 7.5% potassium ferricyanide $[K_3Fe(CN)_6]$ for 15minutes.

ii)Then slides were placed in a 24% solution of sodium thiosulphate for 5 minutes, and

iii) washed in running tap water for 5 minutes and subsequently restained with Azur B Bromide (Gurr) or haematoxyline.

f) Specimen used: Animals were sacrificed at different time intervals (Table-TRK-1). Stages of spermatogenesis were collected by the usual standard technique and radioactivity was recorded by tracing the progression of each stage at various intervals.

OBSERVATIONS

The radioactivity of the gonial cells recorded soon after radioisotope application (i.e. 0-25 days post-injection). The labelled meiotic stages as leptotene, zygotene, pachytene, diplotene and diakinesis were recorded first on 2^{nd}, 4^{th}, 5.5^{th}, 18^{th} and 19^{th} day post- injection respectively.

The spermatids labelled only on 22^{nd} day post-injection and continued to be labelled till 30^{th} day post-injection at when the spermatozoa found labelled for the first time. Duration of leptotene, zygotene , pachytene, diplotene, diakinesis calculated on the basis of the method as stated earlier and the durations are shown in the table (Table-10). The total spermatogenesis process i.e., from onset of meiosis to completion of spermatogenesis is about 29 days (Plate-36, Figure A-K).

In amphibians spermatogenesis exhibits cyclic changes and different cycles were described by different investigators (Bohra and Niazi ,1984; Ghosal *et al.* 1981; Rastogi *et al.* 1985 Ray, *et al.*,1998). According to them the three different cycles were as follows:

(a) *Continuous type*: In *Bufo arenerum, Bufo melanostictus, Rana cancrivora, Rana hexadactyla,* etc., the process of spermatogenesis continues throughout the year.

(b) *Discontinuous type*: In *Rana temporaria* and *Rana pipens* this process is strictly seasonal and never found through the year.

(c) *Continuo - discontinuous type* : In *Rana esculenta, Rana trigrina,* etc., spermatogenesis depends invariably on environmental factors. In winter season spermatogenesis progresses only upto spermatid stage in these species.

Spermatogenetic activity in some anurans with their geographical distribution has been shown in Table-12.

Bufo himalayanas in present study exhibit a discontinuous cycle. Here *Bufo himalayanas* elaborates the production of spermatozoa only in its breeding season which is May- August of the year. This phenomenon also has been reviewed (Laskar,B.1997) (Diagram 8 & 9).

PLATE 36

Legends

Autoradigraphs of developing gonial cell and other stages of meiosis during spermatogenesis through ^{3}H- thymidine treatment

A. Leptotene phase B. Zygotene phase C. Early pachytene phase

D. Late pachytene E. Diplotene F. Diakinesis-Metaphase I

G. Early spermatids H. Early spermatids I. Oval spermatid

J. Late spermatid K. Mature spermatozoa

PLATE 36

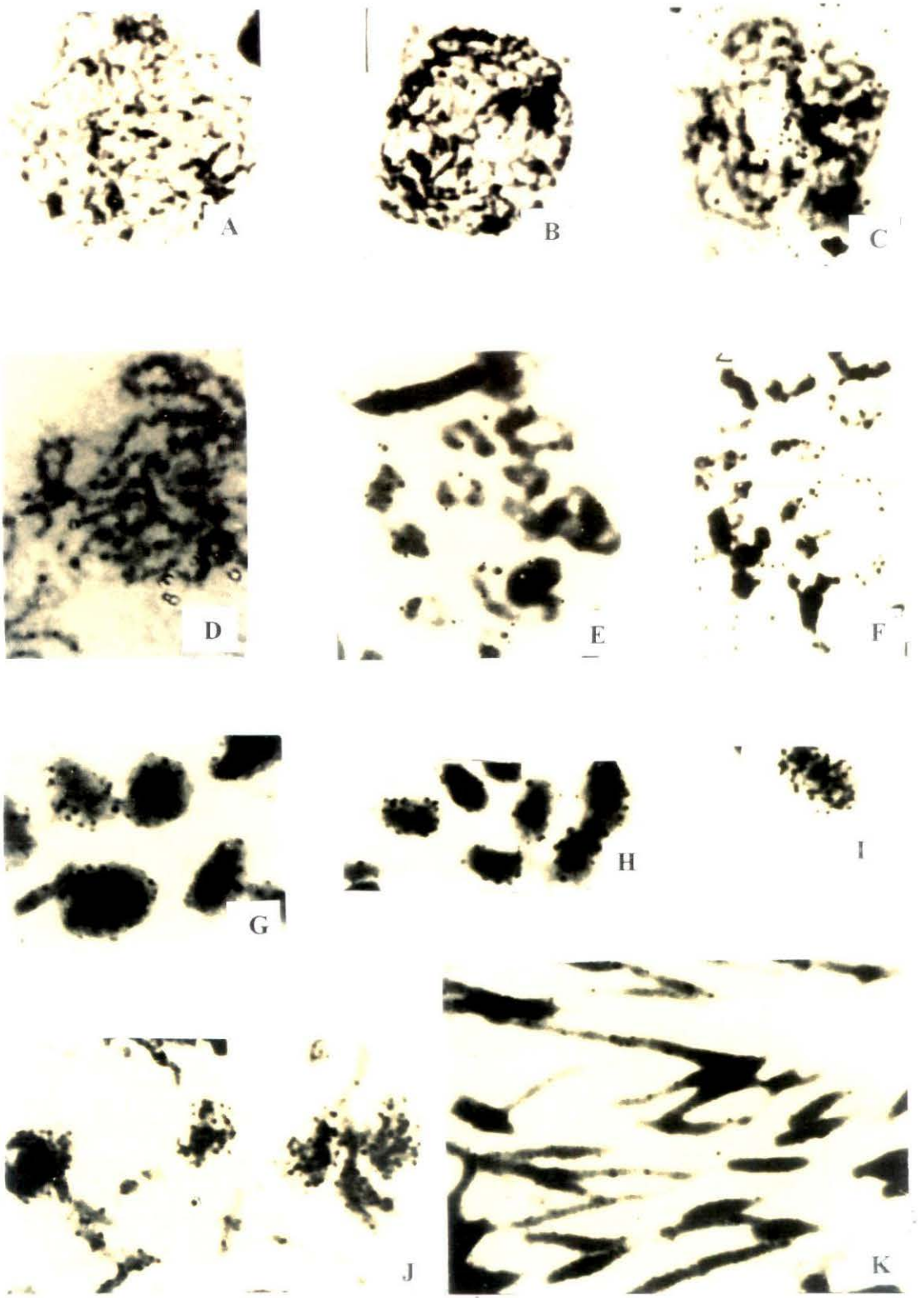


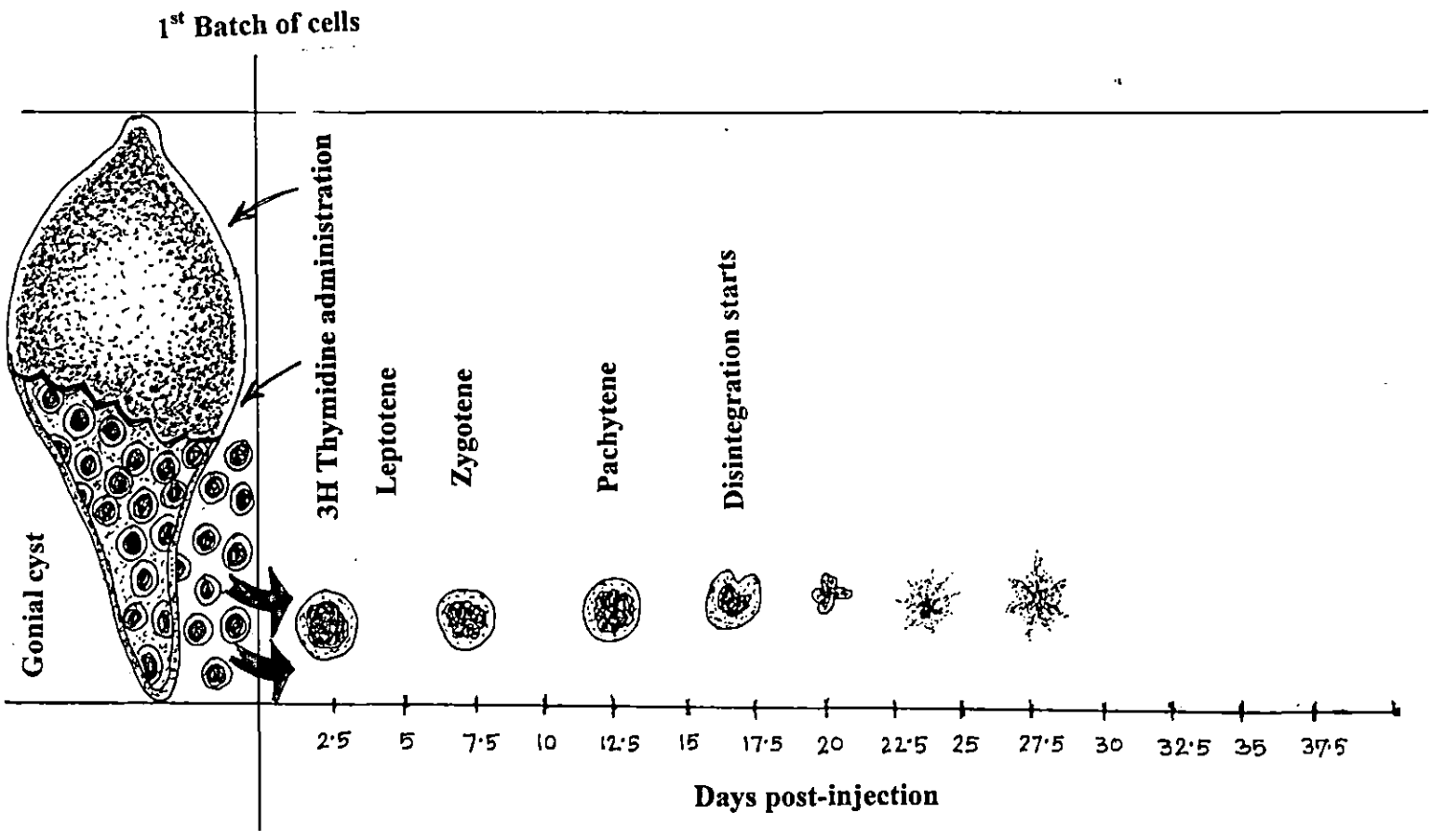
TABLE-12

Spermatogenetic activity in some anurans with their geographical distribution

Species	**Habitat**
Continuous type	
Bufo arenarum	South America
Bufo parachemis	South America
Bufo melanostictus	India, Java
Rana cancrivora *	Java
Rana hexadactyla	India
Pseudis mandidactyla	India
Discontinuous type	
Rana temporaria	Europe
Rana pipens	U.S.A.
Hyla crucifera	U.S.A.
Pleurodema bufonia	South America
Continuo-discontinuous type	
*Rana esculenta**	Europe
Rana gracea	South America
Rana tigrina	India
Rana nigromaculata	Japan

*In this species spermatogenesis goes only upto spermatid formation in winter season.

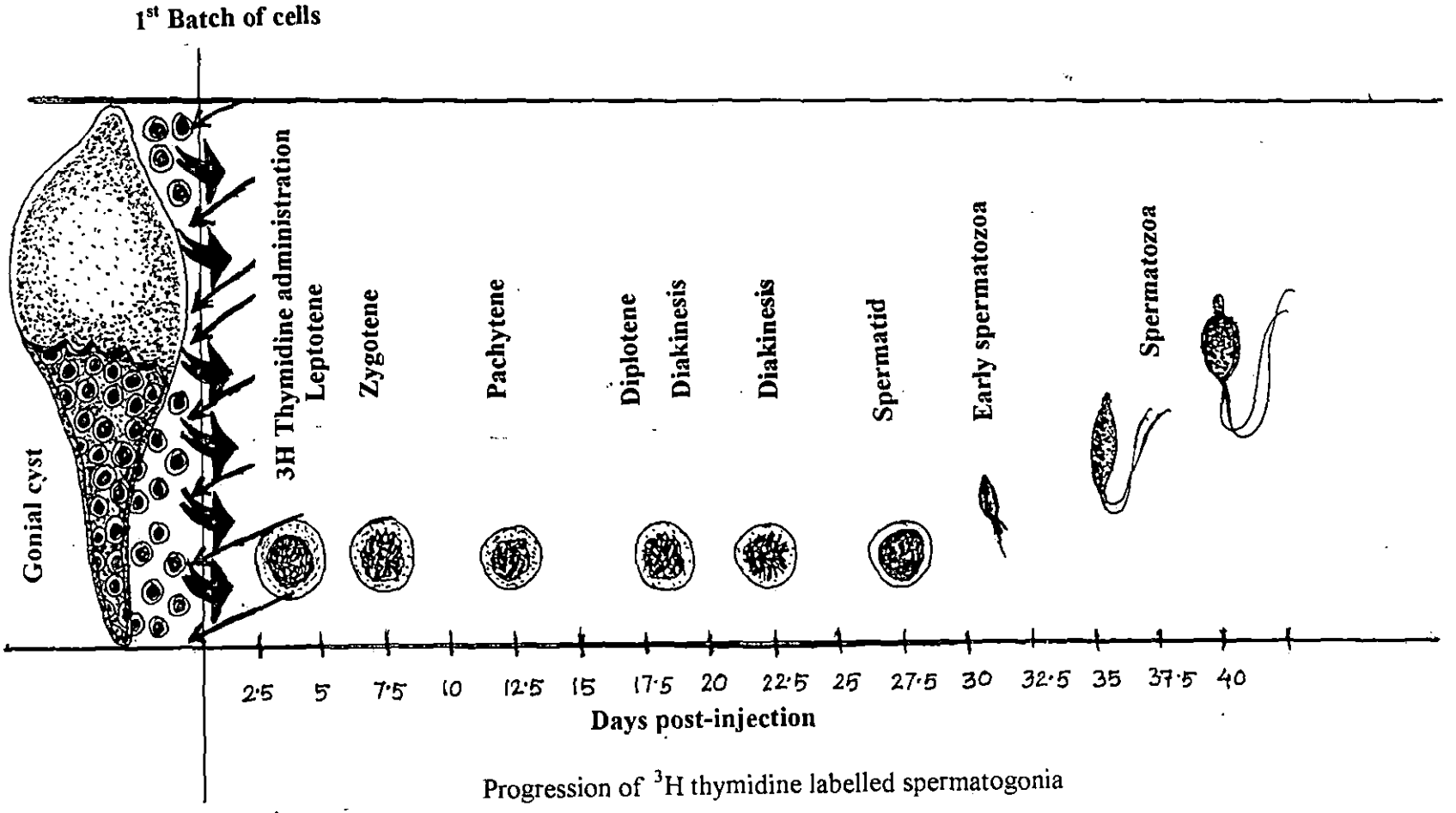
DIAGRAM : 8



Progression of 3H thymidine labelled

spermatogonia in *Bufo himalayanas* during winter season through meiosis

DIAGRAM : 9



Progression of ^{3}H thymidine labelled spermatogonia through various stages of meiosis and spermeiogenesis in *Bufo himalayanas* (breeding season, May-August)

Therefore, kinetics of the spermatoagenesis has been studied only during the breeding season. However the specimens collected from lower altitudes (i.e., below 4000ft. or below) exhibit continuation of spermatogenic activity till September and spermatocytes exhibit anarrest of meiotic transition beyond pachytene stage (Table 11).

The kinetics of meiosis of this species is somewhat is different from other anuran species found in tropical and subtropical regions. The longer duration of each letotene, zygotene, pchytene, diplotene and diakinesis is somewhat similar to *Xenopus laevis* (Kalt, M.R., 1976). In contrast to meiosis , spermatogenesis is remarkably short and is only 9.5 days in duration (Diagram 9). In some species the pachytene last for few months until they degenerate at the onset of winter (Ray and Mukhopdhyay, 1999), (Diagram 8).

Seasonal variation of kinetics of spermatogenesis

In order to study the seasonal variation of the meiosis and spermeiogenesis duration, testicular samples were collected from the specimens which were injected with ^{3}H thymidine both during the normal breeding season when they are actively busy with meiosis and spermeiogenesis (May to August) as well as durig the onset of the winter season when activity of the spermatogenesis seems to be ceased due to temperature drop (September-December).

Animals were sacrificed every month at different time intervals as shown in the Tables 10 and 11. It was found that labelled leptotene stages were

appeared on 2.0 day post-injection in May, June, July and August specimens. Whereas on the 3rd day post-injection, it appeared in September, November and December specimens.

There were some significant changes in duration of other meiotic stages also evident between May-August and September- December specimens. Appearance of first lebelled Zygotene, pachytene, diplotene and diakinesis and spermatids were found respectively on 4.0, 5.5,18.0, 19.0 and 22.0 day post-injection in May –August specimens. But in September-December specimens first labelled stages of pachytene appeared on te 5th day of post-injection. No zygotene phase was recognisable in these winter specimens. It is very significant to note that meiotic process exhibits an arrest of normal meiotic transition beyond pachytene stage (Table 11) in these September-December specimens probably due to fall of environmental temperature at the onset of winter season Ray and Mukhopadhyay, 1999). However the specimens collected from lower altitude (below 5000 ft.), the kinetic pattern remain more or less similar to that of breeding season.

TABLE 10

The progression of lebelled ^{3}H thymidine gonia cells through different stages of meiosis and spermeiogenesis in *Bufo himalayanas* (Gunther). (May-August season)

Days post-injection	Most advanced lebelled stage	Other labelled stage(s)
0.25	Gonia	-----
0.5	Gonia	-------
0.75	Gonia	-------
1	Gonia	------
1.5	Gonia	-----
2	Leptotene	------
2.5	Leptotene	Gonia
3.5	Leptotene	Gonia
4	Zygotne	Gonia,Leptotene
4.5	Zygotene	Gonia,Leptotene
5	Zygotene	Gonia,Leptotene
5.5	Pachytene	Gonia,Leptotene,Zygotene
7.5	Pachytene	Gonia,Leptotene,Zygotene
8.5	Pachytene	Gonia,Leptotene,Zygotene
9.5	Pachytene	Gonia,Leptotene,Zygotene
10.5	Pachytene	Gonia,Leptotene,Zygotene
11.5	Pachytene	Gonia,Leptotene,Zygotene
13.5	Pachytene	Gonia,Leptotene,Zygotene

TABLE 10 (contd.)

The progression of lebelled ^{3}H thymidine gonia cells through different stages of meiosis and spermeiogenesis in *Bufo himalayanas* (Gunther) . (May-August season)

Days post-injection	Most advanced lebelled stage	Other labelled stage(s)
15	Pachytene	Gonia,Leptotene,Zygotene
18	Diplotene	Gonia, Leptotene,Pachytene
19	Diakinesis	Gonia, Leptotene,Pachytene
22	Spermatid	Gonia,Leptotene,Pachytene, Diplotene
23	Spermatid	Gonia, Leptotene,Pachytene, Diplotene
25	Spermatid	Gonia, Leptotene,Pachytene,Diplotene
27	Spermatid	Gonia, Leptotene,Pachytene,Diplotene
29	Spermatid	Gonia, Leptotene,Pachytene,Diplotene
30	Spermatozoa	Gonia, Leptotene,Pachytene,Spermatid
32	Spermatozoa	Gonia, Leptotene,Pachytene,Spermatid
35	Spermatozoa	Gonia, Leptotene,Pachytene,Spermatid

Dose: 20 micro Ci/animal : Sp. Activity-1000Mci (BARC,Trombay)

TABLE-11

The progression of ^{3}H thymidine labelled gonia cells through meiosis in *Bufo himalayanas* (Gunther). September- December season)

Days post-injection	Most advanced labelled stage	Other labelled stage(s)
0.25	Gonia	-----
3.5	Gonia	------
0.75	Gonia	------
1	Gonia	-----
3	Leptotene	Gonia
5	Pachytene	Gonia
7	Pachytene	Gonia
10	Pachytene	Gonia
15	Pachytene	Gonia
20	Pachytene	Gonia
25	Pachytene	Gonia
30	Pachytene	Gonia
35	Pachytene	Gonia

Dose: 20 micro Ci/animal Sp.activity-1000 Mci (BARC,Trombay)

DISCUSSION

Chronology of meiosis and spermeiogenesis

The estimation of the duration of premeiotic 'S' period and the chronology of meiosis in both mammalian and sub-mammalian species have been worked out by various workers by using the technique which involves :

(i) Lebelling of the pre-leptotene spermatocyte's DNA during the premeiotic 'S' period and

(ii) Following the course of migration of these labelled spermatocytes through different stages of meiosis and spermeiogenesis (Crone *et al.* ,1965; Clermont, 1972; Foote *et al.* ,1972; Ghosal *et al.*, 1975; Kalt,1976; Joardar nad Ghosal, 1977; Sinha *et al.*, 1979,1982,1983; Ghosal *et al.*, 1981; Ray *et al.*, 1984; Bandopadhyay and Ghosal ,1985; Ray and Mukhopadhyay,1999).

In mammalian species leptotene duration varies from 3-5 days in different species. For example, leptotene stage lasts for 3 days in rat (Clermont, *et al.*,1959), mouse and golden hamster (Ghosal and Mukherjee 1971) while a little above 5 days in man (Heller and Clermont,1963). In contrast to mammalian species this duration is 2.71 ± 0.09 days in *Calotes* (Bandopadhyay,1977), 2.5 days in *Bufo melanostictus* (Bandooadhyay, 1977), 4 days in *Xenopus laevis* (Kalt,1976), 2.75 ± 0.25 days in Uperodon (Ray *et al.*, 1984), 0.4 day in *Colisa* (Sinha *et al.*, 1979), 0.5 day in *Heteropneustes fossilis* (Bandopadhyay and Ghosal, 1985), 3 days in *Tylototriton verrucossus*

(Ray and Roy , 1989) . In the present investigation we found the leptotene duration is 2.5 days. It first appeared on the second day and lasts upto 3.5 days during breeding season (May-August).

Though the zygotene duration is short as observed in various vertebrates with the exception of *Xenopus* where this stage lasts for as long as 6 days . In amphibian species the duration of zygotene varies from 0.79 to 0.92 day (Mallick,1987). In other sub-mammalian species shorter duration of zygotene has also been recorded (Kalt, 1976; Bandopadhyay *et al.*,1977; Sinha *et al.*, 1979; Ray *et al.* ,1984; Bandopadhyay and Ghosal,1985; Mallick, 1987).

In the present study, we observed longer duration of meiosis in *Bufo himalayanas*. Thus this is somehow similar to *Xenopus laevis* (Kalt, M.R.,1976) . Here zygotene first observed at the 4^{th} day of post-injection and lasts till the 5^{th} day. Thus the total duration of this stage is about two days in the normal breeding season (May- August).

The pachytene stage has got characteristically longer duration. Pachytene lasts for 16.65 ±0.3 days in dog (Ghosal *et al.*,1983), at least 7 days in golden hamster (Ghosal and Mukherjee,1971) and mouse (Ghosal and Mukherjee,1971), 14 days or more in chinese hamster and 10.5 day in bull (Bandopadhyay,1977). Among amphibian species the duration is somewhat variable . It lasts for 12 days in *Xenopus laevis* (kalt.1976), 4 days in *Bufo* (Bandopadhyay et.al.,1977), 5.63 ± 0.38 days in Uperodon (Ray et. al., 1984), 2.92 day in *Rana limnocaris* (Mallick,1987), 6.17 days in *Rana verrucosa* (Mallick,1987).It may be briefly mentioned that among the fishes pachytene duration is very similar with that of amphibian pattern

During the present investigation the pachytene duration appears to be about 10.5 days in *Bufo himalayanas* during its normal breeding season.

The kinetics of transition from diplotene to diakinesis and metaphase- II is extremely rapid in nature and individual stages like diplotene, diakinesis, metaphase –I and metaphase-II lasts for few hours to a day or two in all mammalian and sub-mammalian species studied so far (Bandopadhyay,1977; Bandopadhyay et.al.,1977; Joardar and Ghosal, 1977; Ghosal and Mukherjee,1971).

The present investigation observed longer duration similar with that of *Xenopus laevis* (Kalt,1976). It has been found that the total duration of diplotene to metaphase –I transition is about 2 days in *Bufo himalayanas* in its normal breeding season.

Duration of spermeiogenesis in vertebrates

The total duration of spermeiogenesis is considerably long in mammalian species. It lasts for more than 15 days in golden hamster and mouse (Ghosal and Mukherjee,1971), 21 days in bull (Bandopadhyay,1977), 20 days in dog (Ghosal *et al.*,1983) and about 20 days in lamb (De, 1978). Submamalian species too have longer spermeiogenesis duration , as for example 15 days in quail (Follett,1977),13 days in cock (Joardar,1977), 17.6 days in pigeon (Joardar and Ghosal,1977), 13.26 days in *Calotes* (Bandopadhyay,1977), 8 days in *Heteropneustes* (Bandopadhyay and Ghosal, 1985) and *Colisa* (Sinha *et al.*, 1979).

Amongst amphibia total duration of spermeiogenesis is 12 days in *Xenopus laevis* (Kalt,1976) , 13 days in *Bufo* (Bandyopadhyay et.al.,1977), 9 days in *Uperodon* (Ray *et al.*, 1984), 4.8 days in *Rana*

limnocaris (Mallick,1987), 5.95 days in *Rana verrucosa* (Mallick,1987), 6 days in *Rana cyanophlyctis* (Mallick,1987).

The present investigation recorded the total duration of spermeiogenesis is about 9.5 days *Bufo himalayanas* (Gunther) which shows similarities with *Xenopus* and *Uperodon.*

Kinetics of total spermatogenesis

The total duration of spermatogenesis (meiosis and spermeiogenesis) are characteristics in mammalian species. In man (Helter and Clermont, 1963) the total process is completed in 48 days. The total duration of spermeiogenesis in chinese hamster is 35 days (Utokoji,1966), in mouse it is 25.01 days and in golden hamster it is 27.04 days (Ghosal and Mukherjee,1971). In bull the total spermatogenic duration is about 37 days (Bandopadhyay,1977) and in dog it is 42 days (Ghosal et.al., 1983).

In the present observation the total duration of spermatogenesis is about 9.5 days. The kinetics of the total process of spermatogenesis i.e., from the onset of meiosis to completion of spermatogenesis is about 29 days where kinetics of meiosis only lasts for about 19.50 days which has a close similarity with that of *Xenopus laevis* as described by Kalt (1976). A comparative diagram of the meiosis and spermatogenesis in different vertebrates including *Bufo himalayanas* has been shown in Diagram-10. The total time required for spermatozoa production from any given point i.e., from any specific form of spermatogonia or from pre-leptotene spermatocytes, is of fixed duration in a particular mammalian species (Clermont,1972). Elevation of temperature or hypophysectomy or hypophysectomy-gonadotropin treatment (Clermont and Morgentaler,1955) or hormonal treatment alone (Ray *et al.*, 1981,Ray and Roy,1988) does not alter this total duration. Similarly gonadotrpin

antagonist have no effect on individual duration of meiosis and their transition (Ray and Roy,1988) in the first wave of spermatogenesis following treatment. However prolonged treatment prevents transition of pachytene spermatocytes to diplotene stages (Ray and Roy,1988).

FACTORS CONTROLLING SPERMATOGENETIC ACTIVITY

The seasonal development and activity of the male gonads of amphibians are under the direct control of various endogenous and exogenous factors . The extirpation of the pars-distalis of the hypophysis of the adult male of *Rana nigromaculata* exhibits inhibition of spermatogenesis (Iwasawa and Tojo,1976) at high ambient temperature.However it has been prooved by many investigators that the progress of spermatogenesis and activity of spermatozoa are not influenced by hypophysectomy. Effect of testosterone and gonadotropin on amphibian species has been reviewed by many authors (Basu and Nandi,1965; Iwasawa and Michibata,1972 ; Lofts,1974). Temperature is the major factor controlling spermatogenesis in amphibians. Effect of high temperature on testis and spermatogenetic activity has been investigated by various authors (Kort and Oordt,1965; Oordt and Lofts,1963; Lofts,1974). Rainfall also has similar influence on spermatogenic activty of amphibian species.

The present investigation also suggest that temperature plays an important role in meiotic transition and spermiogenesis. The degeneration of pachytene spermatocytes or arrest of further development during winter season (October-December and onwards) suggest that at low temperature (below10^0C) retards meiotic transition and as a result spermatids and spermatozoa are not formed. Whereas the spermatogenic activity continues at low level in the specimens obtained from lower

altitude where ambient temperature prevails at and around 20^0 C during day time.

In the present study, kinetics of spermatogenesis shows rapid transition and completion of spermatogenesis. It seems to be somehow related with the habit of the seasonal breeding which has an extremely short duration of normal breeding time at this areas of high altitude (4000-8000 ft.). Therefore the Himalayan toad, *Bufo himalayanas* normally has to complete its spermatogenesis within the normal breeding season of this species which ranges between May to August. Seasonal variation as observed is due to low temperature.

Phylogenetically amphibians are related to piscian group on one hand and with reptiles on the other. Their phylogenetic relationship has been established by various methods and techniques. So the kinetics of spermatogenesis can be used as a method to establish such relationship (Laskar,B.,1997;Saidapur,1983; Whittigton and Dixon,1975).

Meiotic duration in piscian group is the shortest among the vertebrates and similar short duration of meiotic stages are found in aquatic amphibian forms such as *Rana, Bufo, Tylototriton* etc. (Oordt and Van,1956; Roy,1990; Saidapur,1983). On the other hand spermatogenesis of terrestrial amphibians is much longer in duration as found in reptiles. Therefore the findings of this current investigation may also have significance in the establishing phylogenetic relationship between such closely related groups of animals.