SURVIVAL OF SPERMATOZOA IN THE FEMALE GENITAL TRACT OF THE INDIAN VESPERTILIONID BAT, *PIPISTRELLUS CEYLONICUS CHRYSOTHRIX* (WROUGHTON)

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ABSTRACT

1. Inseminated females specimens of the tropical vespertilionid bat, *Pipistrellus ceylonicus chrysothrix*, were kept isolated from the males for varying lengths of time during the months of June and July in 1968 and 1969.

2. Every female isolated after the second week of June had undergone copulation. Ovulation and fertilization had not occurred until the 10th of July.

3. Sperms introduced on or before 25th June did survive inside the genital tract of the females and successfully fertilized the ova released during the second week of July.

4. On circumstantial evidence it is concluded that presumably in all the females of this species copulation occurs during the first week of June, and the inseminated sperms are able to survive inside the genital tract of the female and fertilize the ova released some five weeks later in July.

1. INTRODUCTION

THERE seems to be a significant difference between the hibernating bats living in cold and temperate climates and the non-hibernating bats living in tropical and sub-tropical climates with regard to their breeding habits. In the hibernating bats of Europe, North America and Japan there is a long interval of several months between copulation, which occurs in autumn, and ovulation and fertilization, which occur in the following spring (Benecke, 1879; Fries,

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1879; Rollinat and Trouessart, 1895–1897; Grosser, 1903; Nakano, 1928 Rendez, 1929; Guthrie, 1933). During this long interval the inseminated spermatozoa remain dormant within the genital tract of the female and fertilize the ova released in the following spring. This has been demonstrated conclusively by Gates (1936) in *Myotis lucifugus lucifugus*, by Mathews (1937) in two species of British horse-shoe bats, by Folk (1940) in *Eptesicus fuscus fuscus*, by Wimsatt (1942, 1944) in *Myotis lucifugus lucifugus* and *Eptesicus fuscus fuscus*, and by Hiraiwa and Uchida (1956) in *Pipistrellus abramus*. On the other hand in all the tropical bats so far studied copulation is immediately followed by ovulation and fertilization irrespective of whether copulation occurs in spring as in *Miniopterus australis* (Baker and Bird, 1936), *Scotophilus wroughtoni* (Gopalakrishna, 1947, 1948, 1949), *Hipposideros bicolor pallidus* (Gopalakrishna and Moghe, 1960) and *Rhinopoma kinneari* (Anand Kumar, 1965), or it occurs in autumn as in *Megaderma lyra lyra* (Gopalakrishna, 1950; Ramakrishna, 1951; Ramaswamy, 1961) and *Hipposideros fulvus fulvus* (Patil, 1968).

While studying the breeding habits of *Pipistrellus ceylonicus chrysothrix* one of the present authors (Madhavan, 1968) noted that although copulation takes place during the first week of June, ovulation and fertilization do not take place until the second week of July in this species. It became apparent that although this is a tropical species its sex-cycle is unlike any other tropical bat so far studied, and that it resembles, to some extent, the bats living in cold and temperate climates. The present investigation was undertaken with a view to determining if, in this species, the sperms inseminated in the beginning of June survive in the genital tract of the female and fertilize the ova released in the second week of July, that is, after about five weeks.

2. GENERAL NOTES ON *Pipistrellus ceylonicus chrysothrix* (WROUGHTON)

Pipistrellus ceylonicus chrysothrix (Wroughton) is a small bat with an adult body weight of 7 to 8 gm., and a wing span of 22 to 24 cm. These bats usually live in small colonies of a couple of dozens to a couple of hundreds. This species inhabits old houses and dilapidated buildings, and lives inside the crevices in the frames of doors and inside the cracks in the walls. This is a docile animal, and does not get alarmed, nor does it exhibit any agitational movements on approach by human beings. This species remains in partial torpor during the daytime in summer and in the rainy months, and awakes from the partial torpor within a few minutes after being disturbed. However, they fly out of their roosts for feeding within about half an hour after sunset and are very active during the nights.

Pipistrellus ceylonicus chrysothrix breeds in the rainy season (Madhavan, 1968). Although the females come to oestrus, and undergo copulation during the first week of June, pregnancy does not commence until about the second week of July, when all the females within the colony become pregnant. Each of the two cornua of the perfectly bilaterally symmetrical uterus normally bears an embryo, and two young ones are brought forth after a gestation period of 50 to 55 days.

3. Experiments and Observations

Three batches of female specimens of *Pipistrellus ceylonicus chrysothrix* were kept isolated from the males during the period between 12th June 1968 and 26th July 1968. The isolated specimens were kept in small cages with plenty of water in dishes kept inside the cages. The side of the cages were kept covered with wet cloth to give added humidity. Small insects were daily put into the cages. It was noticed, however, that the bats did not survive for long periods under these conditions. The cages were examined several times during the day and the night times, and such of those specimens, which had died, were immediately dissected and their genitalia processed for microscopic examination. Periodically a few specimens were sacrificed and their genitalia were also processed likewise. In each case the o aries, Fallopian tubes, uterus and vagina were examined for the presence of sperms after sectioning and staining. In all the cases the genitalia were fixed in alcoholic Bouin's fluid and preserved in 70% alcohol after 24 hours' fixation. The tissues were dehydrated by passing through graded alcohol and embedded in paraffin after clearing in xylol. The tissues were cut at a thickness of 8 to 10 μ, and the sections were stained with Ehrlich's haematoxylin and counterstained with eosin. The sections were mounted in DPX mountant.

37 females were isolated on 12th June, 23 on 24th June, and 24 on 7th July. From each batch of isolated females the specimens were autopsied at the rate of one or more on different dates following the date of isolation. The last specimen in this series was autopsied on 26th July 1968, and this belonged to the batch isolated on 7th July 1968.

There were swarms of sperms in the uterus and in the Fallopian tubes of all the females isolated on 12th June and examined during the following days up to 24th June, and also in each female isolated on 24th June and examined during the following days up to 8th July. However, ovulation had not occurred in any of the females, but each ovary in each specimen contained a large preovulatory follicle. The 24 specimens isolated on 7th July, and autopsied

on various contained progressively advanced stages of development of the embryo from 15th to 26th July.

Whereas the abovementioned experiments did not conclusively prove that the sperms inseminated during the first week of June fertilize the ova released during the second week of July, they clearly showed that the sperms do remain inside the genital tract of the female for several days. It was unfortunate that no specimen survived the entire period from the time of isolation to the time of ovulation.

In June–July 1969 the experiments were repeated with certain improvements with a view to arriving at some definite results. The isolated females were released into a room 4 meters long, 3 meters wide, and $2\frac{1}{2}$ meters high. Two windows, which were present in the room, were covered with a wire mesh of one cm. square meshes. The window shutters were always kept open to allow a free circulation of air. A wooden box $3\frac{1}{2}$ meters long, 15 cm. wide and 20 cm. deep, and provided with 5 lengthwise partitions, was fitted to the ceiling of the room. The box with its compartments acted as the roosting place for the bats released into the room. The bats were found readily to get adapted to live within these compartments of the box. The floor of the room was slightly sloping towards one of the corners of the room, and water was poured into the room every day. This would collect as a pool at the sloping corner. A few drums and basins filled with water were also kept in the room. A tube-light was fitted near each of the windows to attract insects into the room during the evenings and the nights. The bats were seen to fly about near the lights and catch the insects. On several occasions the bats were seen to swoop down and lap the water in the corner of the room.

The above arrangements gave the bats as near a natural condition as possible, and it was noticed that their survival capacity was considerably higher than in the previous year.

Since there was some overlapping of the successive batches of females isolated and released into the room, different identification marks were made on the bodies of the females to identify the animals belonging to the different batches. These marks were in the nature of shaving off of the fur in different regions of the body in different patterns for different batches. Microscopic examination of these specimens was made as in the specimens studied in June–July 1968.

In the series of experiments conducted in 1969, 51 females were isolated on 14th June, nine on 20th June, four on 23rd June, 11 on 25th June, eight on 27th June, 13 on 29th June and nine on 1st July. These specimens were autop-

sied at the rate of one or more per day during the days following the day of isolation. The last three specimens in this series were autopsied on 14th July, and they belonged to the batch isolated on 1st July. Each of the 51 females isolated on 14th June, and autopsied on different dates up to 29th June, contained swarms of sperms in the uterus and in the Fallopian tubes, Microscopic examination of the ovaries revealed that ovulation had not occurred in any of them. The same situation was noticed in all the females isolated on 20th June and autopsied on different dates up to 1st July, and in the specimens isolated on 23rd June and examined on various dates up to 6th July. Of special interest are the 11 females isolated on 25th June, the eight females isolated on 27th June, the 13 females isolated on 29th June and the nine females isolated on 1st July. In each of these batches the females autopsied on various dates up to 9th July contained sperms in the uterus and in the Fallopian tubes, and a large pre-ovulatory follicle in each of the ovaries. Every female examined on or after 10th July had undergone ovulation and fertilization, and during the following days had embryos at progressively advanced stages of development.

The above experiments show that in *Pipistrellus ceylonicus chrysothrix* the inseminated spermatozoa survive in the genital tract of the female and fertilize the ova released several days later. This is evident from the fact that among the females isolated on 25th June the one sacrificed on 11th July contained a cleaving egg in each of the Fallopian tubes. Although there is no record of the date of copulation of this animal, there is a strong circumstantial evidence to indicate that this female has undergone copulation much earlier than 25th June since every female collected after 14th June had undergone copulation. Madhavan (1968) has shown that in this species the genital tract of every female collected after the first week of June contained swarms of sperms, but that ovulation did not occur until about the second week of July. Evidently, after copulation in June the spermatozoa remain dormant within the genital tract of the female, and fertilize the ova released several weeks later in the second week of July.

4. Discussion

So far only in the cases of the hibernating bats inhabiting cold and temperate climates was it conclusively proved that inseminated sperms survive for long periods inside the genital tract of the female, and successfully fertilize the ova released several months later. In all these species copulation takes place some time in autumn, and the females remain in deep hibernation during the entire winter. Ovulation occurs in the following spring, when the ova are fertilized by the stored sperms (Gates, 1933; Matthews, 1937; Folk,

1940: Wimsatt, 1942, 1944; Hiraiwa and Uchida, 1956). During the period of hibernation the body temperature of the bat is approximately the same as the temperature outside, where it is almost at or below freezing levels. Thus, in these species the sperms are stored at low temperatures although within the body of the female. On the other hand in a tropical bat, such as *Pipistrellus ceylonicus chrysothrix*, and that too during the warm months of June and July, the survival of the spermatozoa inside the genital tract of the female demands special physiological mechanisms so far not known to exist in any tropical animal. Whereas the spermatozoa can be stored for long periods at low temperatures in appropriate artificial media, it has not been possible, so far, to store sperms at higher or even at body temperatures for long periods. Evidently, there is some extraordinary physiological adaptation in *Pipistrellus ceylonicus chrysothrix*, which helps in the survival of the spermazoa at normal body temperature within the genital tract of the female.

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