CHAPTER IV

INDUCED OVULATION FOLLOWED BY EMBRYONIC DEVELOPMENT
IN THE LIZARD, CALOTES VERSICOLOR
INTRODUCTION

The Indian garden lizard, *Calotes versicolor* has been widely used as an example for the study of lizard anatomy in the graduate classes in India and being a hardy lizard, it also forms a good model for research. The embryonic development of the gonads, adrenals and thyroid from oviposition stage (stage 27 according to Muthukkaruppan *et al.*, 1970) has been investigated in our laboratory (Doddamani, 1990). For this study, gravid females collected from nature are brought to the laboratory and the eggs are stripped from the oviduct and incubated till hatching. However, the eggs having embryos prior to stage 27 of development at the time of stripping, normally do not develop. A random natural collection of gravid females does not provide any clue as to what stage of embryo the eggs contain/possess and at times, a large number of gravid lizards are unnecessarily killed. Hence it was hypothesised that if *C. versicolor* possessing preovulatory eggs (during late April or early May) could be induced to ovulate and if the ovulated eggs get fertilized and show normal embryonic development, the study will form a good tool for the study of embryos of known age.

*C. versicolor* possesses the vaginal sperm receptacles and the lizards collected in late April and May invariably have
sperms in the sperm receptacles. In natural cycle, the sperms stored in the sperm receptacles are known to fertilize the eggs in C. versicolor (Shantha Kumari et al., 1990) as in other reptiles which store sperms (Fox, 1963, 1977; Cuellar, 1966a,b; Fawcett et al., 1972; Magnusson, 1979; Conner and Crews, 1980; Bou-Resli et al., 1981; Gist and Jones, 1987, 1989; Uribe et al., 1988). It is known that the mammalian gonadotropin especially FSH can induce ovulation in some lizards (Licht, 1970; Licht and Crews, 1975; Jones et al., 1988). However, there are no studies on the fate of eggs induced to ovulate by exogenous gonadotropins in terms of fertilization and their development into normal embryos. In the present study, serum gonadotropin (Gonadotrophon-FSH) was injected to find out whether it could induce ovulation and if so, whether the ovulated eggs develop into normal embryos or not in C. versicolor.

**MATERIALS AND METHODS**

The garden lizards were collected from the local dealer in the first week of May and were acclimatized to laboratory conditions for three days. They were fed with live cockroaches and were provided water ad libitum. The lizards already having oviducal eggs (presence of oviducal eggs can be made out by abdominal palpation) were discarded. A group of five lizards were
autopsied on the day of commencement of the experiment to serve as the initial control. At autopsy, the weight of the body and that of the ovary was noted. The diameter of the yolky follicles was noted. The remaining lizards were segregated in two groups of eight each. One group of lizards received 25 IU serum gonadotropin (Gonadotraphon-FSH- Paines and Byrne, England, Batch No.775020) in 0.2 ml of 0.7% saline at 9.00 hrs for two days and the second group received two injections of 0.2 ml of 0.7% saline and served as controls. A day after the lizards received the 2nd injection of serum gonadotropin abdominal palpation of the lizards revealed the presence of oviducal eggs and hence the treatment was stopped. Both the serum gonadotropin treated and saline treated lizards were kept in the laboratory without any treatment for 15 days and were then autopsied. At autopsy, the weight of the body and that of the ovary was noted. The ovaries were fixed in Bouin's fluid and processed for histological studies. The eggs from the oviducts of serum gonadotropin treated lizards (no oviducal eggs were found in saline treated lizards) were cut open and the embryos were observed under dissection binocular microscope to study their growth features and to determine their stage of development (Muthukkaruppan et al., 1970).

OBSERVATIONS

The ovaries in the initial control lizards possessed stage VI vitellogenic follicles.
All the lizards treated with saline did not possess any oviducal eggs suggesting absence of ovulation (Fig. 4.1). The ovaries in these lizards were heavier when compared to that in serum gonadotropin treated lizards and possessed stage VI (8.0 mm diameter) preovulatory yolky follicles (Table 4.1). The serum gonadotropin treated lizards possessed oviducal eggs (Fig. 4.2, Table 4.1) and the ovaries possessed stage IV yolky follicles (2 to 3 mm diameter) and a batch of visible corpora lutea (CL). The histological examination of the CL revealed that they were still active.

The examination of the embryos revealed that the embryos were in stage 27 or 28 of development (Fig. 4.3) (criteria for identification of stages of development as described by Muthukkaruppan et al., 1970).

DISCUSSION

The present study revealed that in C. versicolor serum gonadotropin can induce ovulation similar to that described in other lizards after mammalian FSH treatment (Licht, 1970; Licht and Crews, 1975; Jones et al., 1988). However, the present study is the first report which reveals that the eggs induced to ovulate by exogenous hormone treatment can successfully develop into normal embryos in a lizard.
The present study also throws light on another important aspect, regarding the duration/days of oviducal eggs retention before oviposition. Based on the field studies, Muthukkaruppan et al. (1970) reported that the eggs are oviposited in C. versicolor at stage 27 of embryonic development. However, their studies do not report as to how many days the eggs are retained in the oviduct before oviposition in C. versicolor, as it is not possible to know the exact day of ovulation in lizards collected from nature. In the present study, the lizards were autopsied 15 days after the ovulation and the embryos were at stage 27 and 28 of development. Thus it appears that in nature, C. versicolor might be retaining the eggs for 15 days in the oviduct. As the present study was not extended beyond 15 days following ovulation, there was no scope to observe whether the lizards would have oviposited eggs in the laboratory within a day or two beyond the date of autopsy. However, it was observed that the gravid females collected from nature and retained in the laboratory seldom oviposited and the gravid females collected from nature often die when retained in the laboratory for longer periods (15-20 days). The examination of the embryos from such females revealed that the embryos were beyond stage 27 of development.

It is evident from the present study that the lizard C. versicolor can be induced to ovulate by administering serum gonadotropin, an extra pituitary gonadotropin that is mainly a FSH
like gonadotropin. This observation provides support to the earlier reports that it is possible to induce ovulation in some lizards by FSH administration (Licht, 1970; Licht and Crews, 1975; Jones et al., 1988). As this was the first attempt to induce ovulation in the lizard C. versicolor it was limited to the serum gonadotrophon-FSH administration. Further work is necessary to test whether LH/HCG administration will result in ovulation or not.

Since ovulation can be induced in the lizard, C. versicolor, the present study is very useful in obtaining the corpora lutea (post-ovulatory follicles) and the embryos of known age. It will help in investigating the chronological changes that take place in the corpora lutea (such as changes in the luteinizing cells, synthesis of steroids like progesterone and estrogen), and also will provide embryos of known age for the study of chronological embryonic development in this lizard.
Table 4.1: Effect of serum gonadotropin on the ovary of C. versicolor

<table>
<thead>
<tr>
<th>Group</th>
<th>Ovary weight (g)/100 g body weight</th>
<th>Largest follicular stage</th>
<th>Corpora lutea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial control</td>
<td>3.06 ± 0.17</td>
<td>VI</td>
<td>Absent</td>
</tr>
<tr>
<td>0.7% saline treated control</td>
<td>3.51 ± 0.41</td>
<td>VI (preovulatory)</td>
<td>Absent</td>
</tr>
<tr>
<td>Serum gonadotropin treated</td>
<td>1.49 ± 0.10</td>
<td>IV (early vitellogenic)</td>
<td>Present</td>
</tr>
<tr>
<td>(50 IU/lizard/2 days)</td>
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</tbody>
</table>
SUMMARY

1) *Calotes versicolor* during the early breeding phase were injected with serum gonadotrophin-FSH to find out whether it could induce ovulation and if so, whether the ovulated eggs get fertilized and develop into normal embryos.

2) 25 IU serum gonadotrophin-FSH per injection for two days induced ovulation in *C. versicolor*. The ovulation was confirmed by the presence of oviducal eggs which were detected by the abdominal palpation.

3) The lizards induced to ovulate were autopsied fifteen days following ovulation.

4) The eggs stripped from the oviducts of these lizards revealed normal embryos and they belonged to stage 27 to 28 of development. It is interesting to note here that in nature, the freshly oviposited egg in *C. versicolor* shows development up to stage 27 (Muthukkstuppan *et al.*, 1970).

5) It is suggested that *C. versicolor* retains the egg in their oviduct for about 15 days following ovulation. The study provides a tool for studying the embryos of known age and also for investigating the changes that take place in the corpus luteum in a chronological order.