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TECHNIQUES OF ARTIFICIAL INSEMINATION IN *Crotalus durissus terrificus* (*VIPERIDAE-CROTALINAE*)

TÉCNICA DE INSEMINAÇÃO ARTIFICIAL EM Crotalus durissus terrificus (VIPERIDAE-CROTALINAE)

Francisco Garcia de LANGLADA[1]; Selma SANTOS[2]; Iara Lucia Laporta FERREIRA[3]

SUMMARY

Males and females of ***Crotalus durissus terrificus*** which were born in captivity were used to test artificial insemination techniques. The diluted sperm was introduced into the vagina and after 120 days the first 3 snakes were dissected while in other group of snakes, pregnancy was allowed to run its natural course. All the snakes of the latter group gave birth to live and normal young and no atresic eggs were observed.

UNITERMS: ***Crotalus***; Artificial Insemination

INTRODUCTION

The maintenance of snakes in captivity has proved to be a great challenge to the understanding of an important period of the life cycle: the mating and reproductive process.

The quantity of venom which can be produced is dependent upon the amount of snakes which can be maintained in captivity. This is in turn dependent upon the funds available.

Snakes maintained in the laboratory require special carefully controlled conditions; such as temperature, humidity, photoperiod, food supply, etc., that must be carefully regulated. The presence of these special conditions are favorable for adaptation to life in captivity; however, long periods of time are necessary to achieve successful matings and the birth of the offspring (MENGDEN et al.[6], 1980; MONTGOMERY; SCHUET [7], 1982). Due to both limits of space and finances it is necessary for the scientist to explore different alternatives with the limited resources which are available to him. By introducing artificial temperature fluctuations to mimic hibernation in the natural habitat, increases in the success of captive reproduction programmes have been achieved. An alternative for increasing the success of reproduction in captivity is to use methods of Artificial Insemination (A.I.) (MENGDEN et al. [6],1980).

This work reported six cases of reproduction in captivity being induced through use of the A.I. in ***Crotalus durissus terrificus*** (rattlesnake).

MATERIAL AND METHOD

During the course of this study (April and May) six females and six males of ***Crotalus durissus terrificus*** (approximately 30-40 months old) were used. All specimens were born in captivity. They weighed approximately 1kg and their total length (Snout Vent Length + Tail Length) was about 1m.

The snakes were killed and vas deferens were removed and placed on a Petri dish (Fig.1). These were then gently squeased to eliminate the sperm which they contained. The sperm thus obtained was diluted in the proportion of 1:3 in Ringer's solution (HOAR; HICKMAN JUNIOR[1], 1967). The fecundity and motility of the sperm were verified by observations using a light microscope (LANGLADA et al.[5],1991).

The mature females were secured and asseptic conditions were obtained using the technique of LANGLADA; BELLUOMINI[3] (1972). Female snakes were then separated into two experimental groups (A and B). Each group contained three specimens. Group A had semen introduced into the right vagina, and group B had semen introduced into the left vagina. The left uterus was used as a control in group A, and in group B the right uterus.

In order to distinguish between experimental animals in groups A and B, the rattles were differentially colour coded.

A speculum was introduced into the cloaca, exposing the vaginal papilla (Fig.2). After this the area was sterilized with

1 - DM - Butantan Institute, São Paulo, Brazil
2 - Bachelor of Biological Sciences - Butantan Institute, São Paulo, Brazil
3 - Doctor of Physiological Sciences - Butantan Institute, São Paulo, Brazil

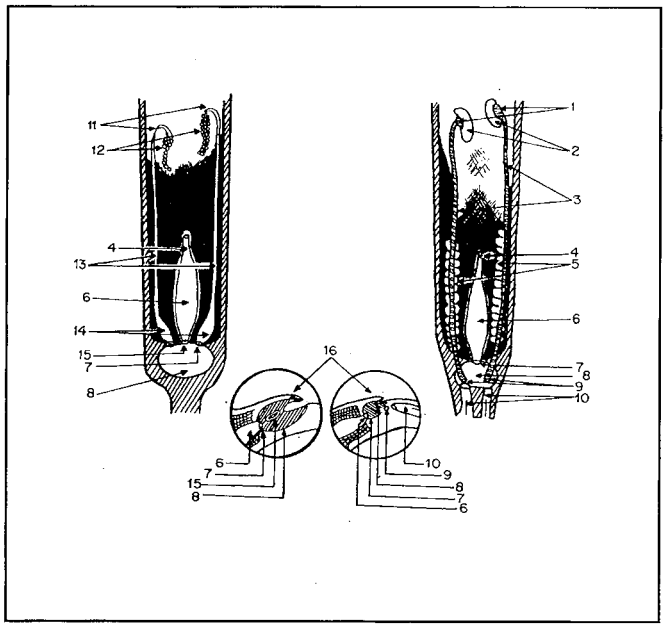

FIGURE 1

A. Male reproduction apparatus

B. Female reproduction apparatus

1-epididymes; 2-testis; 3-vas deferens; 4-intestine; 5-kidney; 6-cloaca; 7-anus; 8-antrum cloacal; 9-seminal orifice; 10-hemipenis; 11-trump; 12-ovary; 13-uterus; 14-vagina; 15-vaginal papilla; 16-cloacal scale.

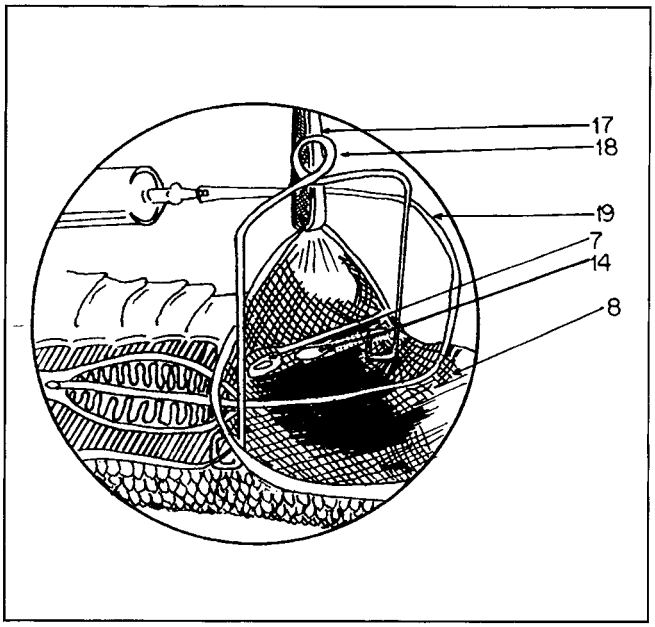

FIGURE 2

Use of speculum (18), polythene tube (19) and syringe in the A.I.

7-anus; 14-vagina; 8-antrum cloacal; 17-tweezers.

gauze soaked in physiological saline solution (0.9 NaCl). A polythene tube (P-90, 4-6cm) was introduced into the vagina following the axial plane. It passed through the entire length of the vagina and entered into the uterus (Fig.2). By using a syringe it was then possible to introduce 1ml of the diluted sperm into each uterus, and this completed the process of A.I. The experimental animals were then housed in individual cages and maintained at room temperature. After A.I., the amount of food offered was constant with that which had been offered previously (20-30g mice/15 days).

In experimental group A snakes were sacrificed 120 days after A.I. In group B, pregnancy was allowed to run its natural course.

RESULTS

The tissue in the vagina and uterus of *Crotalus females* is hard and resistant, this enables the use of this technique without running the risk of damaging the tissues. The vagina appears flattened and is approximately 3-5 times broader than the uterus which then leads into the oviduct (Fig.1).

Physiological signs of pregnancy were only observed in the right uterus in group A which had been inseminated.

When viewed with the naked eye, the fixed placentas resulting from A.I. looked identical to those produced during the "natural" reproductive process in the wild, observed in specimens which arrived in a gravid condition.

The uterus which had not been inseminated, appeared long, smooth, thin and flexible. The wall of the uterus appeared separated into numerous separated compartments, each designed to house one fertilized egg. This change in structure is stimulated by the entry of a fertilized egg and also its consequent embedding. Thus in this experiment a marked contrast in the structure of the inseminated and control uterus was observed.

In experimental group A, no differences were observed between the left and right vaginas. In group B the abdominal diameter was observed to increase after the fifth month of pregnancy. Seven to eight months of pregnancy after A.I. the offspring were born. At the time of birth, the external opening of the vagina was observed to increase about 20% in size. The entire volume of the vagina also increased by 20% to allow for the smooth passage of the juveniles from the uterus to the

external environment.

In control group B the number of youngs which were born varied from 8 to 10 ($\bar{x} = 9$) for each snake. Experimental group B produced 27 viable youngs. Every snake which was born had developed normally. No atresic eggs were found.

DISCUSSION

MENGDEN et al.[6] (1980) while studying ***Bitis gabonica*** (Viperidae) e ***Python anchietae*** (Boidae) reported an unique experiment for A.I. in ***Bitis*** which produced 52 babies, however it was not ascertained whether this was the result of A.I. or resulted from the utilization of stored sperm. LANGLADA et al.[4] (1973) found viable spermatozoa in the oviduct 60 days after natural copulation. Motility was also the criteria used to assess viability of spermatozoa (TSUI; LICHT[10],1974; MENDGEN et al.[6],1980; LANGLADA et al.[5],1991). The viability of these sperm was then proved by using them to fertilize a previously infertilized egg, subsequently a ***Python anchietae*** was artificially inseminated weekly for a period of a month. Vaginal swabs were taken twice to assess the viability of the sperm. In both cases the sperm were found to be viable, but no juveniles were obtained, because the female had not produced eggs (MENGDEN et al.[6],1980). This could be for two possible reasons: either ***Python anchietae*** will not accept A.I. sperm, or due to the timing of insemination and consequently, the season of the year was not a suitable time for reproduction. Although there were large systematic differences between ***Python anchietae*** and ***Crotalus durissus terrificus***, the results of this paper make the second of these hypothesis more likely.

NILSON[8] (1980) while studying the reproductive cycle of ***Vipera berus*** (Viperidae) showed that the zenith of spermatozoa production in the vas deferens was timed to exactly coincide with the time of copulation. In ***Crotalus durissus terrificus*** (LANGLADA et al.[5],1991) found that the number of spermatozoa is constant throughout all of the year, this ensures that no matter when the sperm is collected it will contain a high number of viable spermatozoa. In ***Crotalus durissus terrificus***, copulation is thought to occur in April and May because all reported combat rituals have been observed at this time of year. (LANGLADA[2], 1975; SANTOS et al. [9],1990). Thus sperm can be collected at any time of the year, stored and frozen (MENDGEN et al.[6],1980). It is however very important that A.I. is scheduled for April/May for ***Crotalus durissus terrificus***.

In order to understand the importance of the annual reproductive cycle, it is important to consider whether the vitellogenic process is pre or post nuptial. Only when this information has been gained it will be possible to be certain of the best time of the year to inseminate animals in captivity.

This could have significant uses when trying to preserve endangered species or increasing the success rate of captive breeding programmes in problematical species.

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RESUMO

Machos e fêmeas (n=12) de ***Crotalus durissus terrificus***, nascidos em cativeiro foram usados para testar a técnica de Inseminação Artificial. O esperma diluído foi introduzido na vagina e após 120 dias, 3 fêmeas foram dissecadas para exame. Outras 3 tiveram a gestação levada a termo e produziram filhotes normais. Não foram observados ovos atrésicos.

UNITERMOS: *Crotalus*; Inseminação Artificial

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