Introduction
A series of four one-day capsule veterinary workshops were held at four zoos in India, during the month of October, 1993. These were held on alternate days, immediately following the Asiatic Lion PHVA at Baroda. A team of six US scientists from Smithsonian Institution and NOAA centre (New Opportunities in Animal Health Sciences) of National Zoological Park conducted these workshops. The group included one reproductive biologist, two veterinarians, one geneticist, one reproductive physiologist and a research scholar working on the techniques of animal reproduction. Their names and designations are Dr. David Wild, Reproductive Biologist; Dr. Mitchel Bush, Assistant director, Animal Health, Dr. Terri Roth, Reproductive physiologist; Dr. Budhan Pukazhenthil, Research scholar, -- all from the National Zoological Park, Washington, D.C. and Dr. Lindsay Phillips, Professor, University of California, Davis, California as well as Ms. Janice Martenson, Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland.

These workshops were initiated by Zoo Outreach Organization and hosted by the respective zoos. These zoos were invited to host these workshops on the basis of their proximity to a veterinary college and their relationship and record of cooperation between the same. They were Municipal Corporation Zoo, Baroda, Kamala Nehru Zoo, Ahmedabad, Veermata Jeejabai Bhosle Udyan, Bombay and Sanjay Gandhi Biological Park, Patna. The workshops were held at these centres on 22nd, 24th, 26th and 28th respectively.

One of the major objectives of the team's visit to India was to establish the purity of the Asiatic lion subspecies maintained in these Indian zoos. The group was also interested to study the seminal characteristics of exotic felids that have the history of inbreeding in zoos and to demonstrate these techniques to Indian veterinarians, reproductive physiologists and geneticists working in zoos and universities. While doing so, the team also demonstrated the use of transponders for animal identification, technique of collecting skin biopsies and the procedure of performing vasectomy as a method to control breeding in zoos. This report, accordingly, deals with the following topics....

1. Anaesthetic procedures
2. Electroejaculation & Cryopreservation
3. Genetic tests for recognizing hybrid lions
4. Reproductive control methods-Vasectomy
5. Transponder telemetry
6. Diseases of potential threat to lions

The number of participants at each centre varied from 15 to 30; almost all of them were veterinarians. Most of them were from local universities and veterinary colleges. All participants were given a briefing book and a workshop training manual.

For conducting studies on genetics and reproductive biology, it is obvious that two types of biological materials are essential: blood samples for genetic studies and semen samples for reproductive assessment. For conducting these procedures, anaesthesia is the only choice of restraining wild felids, particularly for electroejaculation.
A. Anaesthetic procedures

Since almost all the participants were veterinarians, a considerable amount of interest was evident during drug-immobilization operations. In fact the success of the whole workshop was dependent on the successful handling of the anaesthetized animals. Therefore, all workshops started with briefing on darts techniques with emphasis on drugs and delivery systems used.

As many as 25 individuals of 6 species of cats were subjected to drug immobilization. These were, lions (Panthera leo), leopards (P. pardus), tigers (P. tigris), clouded leopards (Neofelis nebulosa), jungle cats (Felis chaus) and leopard cats (Felis bengalensis). Multiple samples of blood and semen were collected from most of these animals for performing various laboratory procedures. Skin biopsy samples were also collected from jungle cats, leopard cats and leopards for taxonomic studies.

Drug delivery systems used: Either Blow pipe or Pole Syringe (Jab-stick) were used, depending on the species and the type of enclosures they were in. For all big cats in large enclosures, darts were delivered using blow pipes. Occasionally a pole syringe was used for individuals in confined areas (e.g. squeeze cage). Most of the jungle cats were drugged using a pole syringe and almost all the leopard cats were net-caught and later subjected to drug immobilization. Mini-ject syringes with plastic stabilizers were used in blow-pipes made of aluminium alloy. The advantage of using plastic stabilizers over the conventional wooden stabilizers is that the plastic ones are reportedly more accurate in striking the target. The blow pipe was made of 3 pipes of about 1 metre length.

Drugs used: Telazol was the drug used for most individuals for anaesthesia. Telazol is a 1:1 combination of Tiletamine (a dissociative anaesthetic like Ketamine) and Zolazepam (a sedative like Diazepam). Other drugs used were Ketamine and Xylazine hydrochlorides.

The advantage of Telazol over others is that it is available in a powder form and can, therefore, be made into a high concentration preparation. In fact, the whole 500 mg vial (Tiletamine and Zolazepam: 250 mg each) can be diluted in 1.2 ml of distilled water. This is considerably advantageous because 1-1.2 ml of this preparation would be sufficient to bring down a captive adult lion. Conversely Ketamine, which is the other drug of choice, comes as 100 mg/ml preparation and thus demands a higher volume of drug (say more than 4 ml for an adult lion) even if administered along with 50-100 mg of Xylazine. This means using 2 darts of 3 ml volume; and when animals are to be tranquilized with two darts, there is no guarantee that the interval between these two successive injections would be minimal. During the course of the workshop, some of the other advantages of Telazol also became apparent. Whenever Telazol was used, induction was rapid, anaesthesia was profound, and the total duration of anaesthesia was considerably longer. Like Ketamine, Tiletamine also has a wide safety margin and other properties of Ketamine like maintaining swallowing reflex. However, in spite of all these advantages, the long period taken for recovery should be considered a drawback. Animals immobilized with Telazol often hardly showed any sign of recovery from anaesthesia even after 2 hours.
Telazol was not used for tigers for its alleged side effects in this particular species [readers can refer to page 2 in the 1990 January issue of ZOOS’ PRINT]. Tigers immobilized with Telazol have been reported to develop symptoms of CNS disease including limb ataxia, disorientation, hyperactivity, muscle tremors and petittal ataxia after 3-4 days of recovery from anaesthesia. Because of these reasons, a combination of Ketamine and Xylazine was used for anaesthetizing tigers. Whenever xylazine was used, its effects were reversed by administering Yohimbine hydrochloride. However, Dr. Mitch Bush, the National Zoo Veterinarian, was of the opinion that only 7-10% of the tigers immobilized with Telazol exhibit such side effects, particularly those tigers anaesthetized after repeated drug administration.

Only Ketamine was used for anaesthetizing small cats (including Neofelis) on 2 occasions when Telazol was used in jungle cats. Since Ketamine has a tendency to cause convulsive seizures, diazepam was given intravenously. However, as most of this Ketamine induced seizures are self limiting, animals were monitored for 1-2 seizures to complete before deciding administering this anticonvulsive drug.

Ketamine alone was used for anaesthetizing small cats and clouded leopards at Patna Zoo.

Precautions before drug-immobilization: From animal’s point of view, several precautions were taken before darting. In almost all cases, food was withheld before 24 hours and no water was provided for 6-12 hours before darting. Very old animals were avoided as they were considered weak to withstand the drug effect. It was advised against anaesthetizing animals enclosures with water pools so as to avoid drowning.

Darting procedure: Whenever blowpipe was used, darting distance varied from less than 1 meter to as far as 7 metres. Darting procedures were always systematic. The importance of keeping accurate records of drug-immobilization was stressed every time. The data sheet used can vary in its format from place to place, but should include all the information during anaesthetization including the mistakes made during every operation. Such records are useful at later dates to refer back to the mistakes.

After complete anaesthesia, vital physiological parameters were monitored. Here Dr. Bush is examining a jungle cat at Baroda Zoo.

Collecting biological materials: Once completely anaesthetized, blood samples were collected in Venoject plain vacuum tubes as well as in vacuum tubes with additives (EDTA and/or Sodium Heparin). The site of blood collection was jugular vein in small cats & clouded leopards, and caudal or femoral veins in big cats. Simultaneously, semen samples were also collected using electroejaculation. This has been dealt in the next section on electroejaculation and cryopreservation.

Recovery from anaesthesia: As 3-6 animals were immobilized per day, it was not possible to record the total duration of anaesthesia and the recovery time (the time from induction to getting up) for most cases, particularly those animals anaesthetized with Telazol. Often, big cats administered with Telazol remained in lateral recumbency for more than 3-4 hours after induction. As said earlier, this should be considered as an operational disadvantage while using Telazol. On the contrary, animals anaesthetized with Ketamine (or Ketamine-Xylazine combination) showed signs of recovery after 40-50 minutes of induction.
B. Electroejaculation and cryopreservation

Why should one try artificial insemination (AI) techniques in wild animals? There are many reasons. The ultimate measure of an animal population condition is its reproductive success. The success of any conservation effort, be it captive breeding or habitat protection, lies on the species' ability to reproduce and propagate its numbers successfully. In this respect, reproductive biologists play an important role in conservation biology. Their role becomes more important in the management of captive populations which are more vulnerable to inbreeding and subsequently loss of genetic diversity.

According to Dr David E. Wildt, Reproductive biotechnology using frozen gametes and embryos, has made great strides in recent years, and artificial breeding will find its own management application at least in some wildlife species in the next decade. For example, there are many species like the cheetah, which do not readily breed in captivity. It has been proven that ejaculates from this species contain mostly dead and deformed sperms. In such problem animals, in-vitro fertilization is the only option. Another reason for developing AI techniques in wild animals is that zoos cannot afford to maintain lot of animals to maintain genetic diversity. By AI, the diversity can be maintained in separate populations of a species scattered across several zoos. AI methods are also excellent tools for the propagation of critically endangered species which may not readily breed in captivity.

Almost all the 25 anaesthetized males were subjected to rectal probe electroejaculation. Depending on the species, probes of varying length and diameter were used. For lions, probes with 2 inch diameter, for leopard cats probes with 1 cm diameter and for clouded leopards & leopards, probes with 2 cm diameter were used. The probes contain 3 longitudinal electrodes at the terminal end.

Once anaesthetized, the morphometry (length and breadth) of each testicle was taken using vernier calipers. This procedure has been done to study the relationship between testicular size and semen quality. In animal husbandry practice, it is an established fact that testicular size in bull is positively correlated to seminal quality/volume.

On most occasions of electroejaculation, the electrical stimulation consisted of 3-5 series with 4-6 minutes of rest in between. Each series consisted 10 electrical stimuli or pulses. The voltage used ranged from 1 to 5 volts depending on the species.

Ejaculation procedure: Prior to every ejaculation operation, the prepuce sheath and the penis are cleaned to avoid contamination of the ejaculate. Lubricated rectal probe is inserted into the rectum by gentle pressure. The electrodes are positioned ventrally, i.e. over the underlying accessory sex organs. Penis is projected out of the sheath and a sterile collection vial is kept over the penis for collection. During electrical stimulation (which is done using an electroejaculator which converts the 240 volts electricity to only 1-7 volts; this can be read on the voltmeter), constant pressure is applied over the accessory sex organs by gently pressing the electrodes in the probe. The electroejaculator is switched off after completion of every ejaculation.

Electroejaculation in a leopard

The total volume of the ejaculate is measured and maintained at room temperature. One or two drops of the sample is mounted for microscopic evaluation of its quality. This quality evaluation involves assessing percent sperm motility (marked as 0 to 100%) and rating forward progressive motility (in a scale ranging from 0 to 5.... 5 indicating the best).

All the semen samples were evaluated for quality

Three to five microlitres of the ejaculate is fixed in 100 microlitre of 0.3% glutaraldehyde for analysing morphology. From samples thus fixed, sperm concentration and sperm motility are determined using the conventional methods followed in the animal husbandry departments for evaluating bull semen.

During the four workshops, ejaculates from as many as 25 individuals of 6 species were examined for motility (see table 1 for number of individuals in each species). Broadly speaking, most of the lesser cat samples were of good quality. The sperm concentration and motility in big cats varied according to species. The variability in lions was very noticeable, with some semen samples being poor. In a study conducted by Dr Steve O'Brien (USA), it has been established that the spermatozoa in Asiatic lions show extreme degree of morphological abnormalities. About 79% of the spermatozoa in each ejaculate was
found to be morphologically abnormal. This has been attributed to the result of genetic bottle-neck that the Gir lions underwent during the early part of this century when their numbers were reportedly reduced to less than 20. The quality of semen in tigers and leopards were generally good. All the three clouded leopards of Patna zoo failed to show any live sperms in their ejaculates. One interesting observation could be made on the quality of ejaculates from leopard cats of Patna zoo, apparently bred from a single breeding pair. Ejaculates from 4 males were examined and 2 of them were good with high concentration of progressively moving sperms. Two of them either failed to ejaculate or their scanty ejaculate contained only dead sperms.

Freezing method: Bull semen, as we all know, is frozen in straws that enable easy handling and administration using an appropriate inseminating gun. Apart from this well known method of straw-freezing, there is an old Japanese technique of pellet-freezing. Professional dog breeders in US had apparently failed to get good results in trying to freeze dog sperm in straws until they rediscovered the old forgotten Japanese method of pellet freezing. Cat sperms like dog sperms, for reasons unknown, freeze well while pelleting. The process of cryopreserving feld sperms was demonstrated to the participants during the workshops.

As the sperm concentration is generally low in felids, the ejaculate is centrifuged to get a high concentration of sperms. The supernatant is removed and the sediment is suspended in the cryodiluent (PPV-62) with the cryoprotectant added to it. The sperm sample is then maintained at 4°C in the refrigerator for 20-30 minutes. It is also advised to refrigerate the sterile pipettes, that will be used for pipetting while pelleting, for the same period. The sperm sample is first pelleted on dry ice before transferring into liquid nitrogen. Multiple pits or impressions of 3 mm deep are made on a flat block of dry ice, using a nail-board.

Drops of the diluted sample are pipetted into these depressions using the precooled pipettes. After freezing these multiple pellets in these depressions, the dry ice block is inverted over a bucket of liquid nitrogen. The pellets automatically fall into the liquid nitrogen and sink. Later the pellets are scooped into cryovials using a spoon or by any other suitable equipment. The vial is always kept under the liquid nitrogen during the time of transferring the pellets. The vial is then transferred from the bucket to the liquid nitrogen storage container. One can make up to 2-4 pellets from a good quality semen of a lesser cat.

Thawing and Intra-uterine (IU) insemination: The procedures of thawing and insemination were also dealt during the workshops, though not demonstrated practically. For thawing, 1-4 pellets are transferred into a tube containing Hams F10 (containing 5% fetal calf serum) maintained at 37°C. The sperm motility is evaluated immediately. Unlike in cattle, where the whole semen sample in the straw is inseminated along with the cryodiluent, the sample of felds is centrifuged to separate the medium. This is done by discarding the supernatant by aspiration. This has to
be done because the uterus of felids are apparently more prone to get metritis. The pellets are resuspended in 100-200 microlitre of fresh Ham's F10 (containing foetal calf serum).

In the case of pellets made from a good quality semen sample, 1 or 2 pellets are reportedly sufficient for Intra-Uterine insemination. In bovids, the frozen semen is deposited in the body of cervix from where the sperms progress to the point of fertilization. Cats, for that matter all carnivores, apparently do not conceive when inseminated cervically. Therefore the commonest method practiced is intra-uterine (IU) insemination. Cats are induced ovulators like many species of mustelids like ferrets. However, ovulation can be induced by the administration of gonadotropins. This stimulates follicular development and ovulation. A fibre-optic laparoscope is used for assisting insemination. The site of incision is near the umbilicus. So far successful offsprings have been produced by IU Al using frozen-thawed sperm in 19 species of wild mammals which include bovids, cervids, canids, felids, mustelids, primates and giant panda.

C. Genetic test for detecting hybrid lions

Being an endangered subspecies, maintaining pure Asiatic lions has conservation value because of prospects of reintro-duction. Hybrid lions on the other hand, occupy valuable zoo space and cut into zoo budgets. According to Dr. Wildt, hybrid lions do have some value, particularly in future. They can act as foster mothers for embryo transfer programmes and also as experimental animals for various surgical and diagnostic procedures.

The Asiatic lion differs from the African lion in few morphologi-cal features. The prominent belly fold of skin and the less fuller mane in the Asiatic subspecies are considered as diagnostic. Apart from this, the difference in the infratemporal foramen in the skull is also taken into consideration. These differences are marked as long as the 2 populations are separate and not interbred. The problem has been to differentiate and separate the Asiatic and African lion hybrids based on these morpho-logical features alone.

One of the major objectives of conducting these workshops was to genetically test some of the suspected hybrid lions for purity. The procedure was demonstrated to the participants at Baroda workshop using a newly developed portable laboratory kit. It has been established by this method that the Asiatic lions are genetically monomorphic when compared to African lions which are polymorphic. The presence of any polymorphic isozyme allele denotes that the individual is a hybrid.

Heparinized blood samples are collected either after chemical restraint (using Telazol) of physical restraint (using squeeze cage). Whole blood is centrifuged to separate plasma, erythrocytes and leucocytes. Isozyme extracts are prepared and then subjected to gel electrophoresis, followed by histochemical isozyme development.

What is a hybrid? One of the participants in the Bombay Zoo workshop raised an important question about the definition of HYBRID. He was of the opinion that the word HYBRID has been confusingly used to describe offspring born to two different species/subspecies of wild animals. Being a veterinarian, his contention was that hybridization is an accepted event in livestock breeding programmes where cross breeding is done between two breeds of cattle or birds and, therefore, is proper to term them hybrid. Even though there was some disagreement with the various explanation given to this question, it logical to say that HYBRIDIZATION can occur between any group of animals whenever it is bio-logically feasible. Consequently, a hybrid can be between two species (eg. mule), between two subspecies (eg. Afri-can and Asiatic lion hybrid), between two breeds of livestock (eg. Jersey and zebu hybrid) and so on.

Besides the use of genetic studies in determining hybridization, they can also be used in defining the phylogeny of closely related species. For this, skin biopsies were taken from leopard cats, leopards and jungle cats for cryopreservation. Such preserved tissue samples can serve as a source of DNA and protein products for such studies in taxonomy/phylogeny.

All the biopsy samples were collected from the inner thigh region. A 5x5 cm area of the skin was prepared by shaving off the hairs and cleaning with betadine solution and later with alcohol to remove excess betadine. A piece of skin of approxi-mate 1 cm² was then removed using a sterile scalpel/scissors. The skin was lifted using a forceps to facilitate easy cutting. The area was then left unsutured after applying local antiseptics. All biopsy samples were stored in a tube of biopsy transport medium. Samples can be preserved for 1-2 days in this medium. For cryopreserving biopsy samples, the sample was removed from the transport tube into a petridish and cut into minute pieces. A cryotube containing freeze medium was used for cryopreservation.

D. Reproductive control methods- Vasectomy

While special efforts like electroejaculation, cryorreservation, artificial insemination and embryo-transfer are required to make some of the endangered species breed, others have been breeding prolifically necessitating a special effort to control reproduction. Overabundance is a problem not only in the wild but also in captivity. Many zoos in India have hybrid lions...
and they now realize the need to maintaining pure Asiatic lions. Euthanasia of wild animals is not an accepted practice in India. Controlling the reproduction of such unwanted animals is often the only solution available.

Reproductive control methods can be broadly classified into reversible and non-reversible methods. Use of contraceptive hormonal implants in females belong to the former category. Though animals return to normal oestrus cycle once the implants are removed, there is always a risk of developing cancer in the urogenital organs. Surgical operations like castration and vasectomy in males and ovariohysterectomy & oviductomy in females belong to the category of non-reversible methods. Methods like castration totally eliminates gonadal sex hormones which are essential for the growth and maintenance of secondary sexual characteristics like mane in lions. One acceptable alternative is vasectomy.

Vasectomy of male lions has been recognized as an acceptable practice of reproductive control. ZOOS' PRINT on two different occasions has published articles on vasectomy. One was on a leopard in India (May 1990 issue) and the other one recently on lions in Sri Lanka (June 1993 issue). Most veterinarians probably have some idea about this simple surgical procedure. But the method followed during the workshop was slightly different from those reported by Dr Babwre and Dr Jayanthi in 1986. Such variations in this surgical procedure is due to the fact that the Vas Deferens, the subject of surgical intervention in this procedure, can be severed at any place during its course before it disappears into the abdominal cavity through the inguinal canal.

Anaesthesia: During this workshop, vasectomy was demonstrated on a lion in Ahmedabad zoo. Anaesthetic used was Telazol (450 mg for the adult lion), administered using a blow pipe. During vasectomy operations published earlier in ZOOS’ PRINT (May 1990 & June 1993 issues), the leopard was anaesthetized with Xylazine and Ketamine, 150 mg each. In the second case in Sri Lanka, Ketamine alone was used at the rate of 150 mg/kg BW for the lion. This amounts to almost 2,000 mg of Ketamine (20 ml of a 100 mg/ml solution) for an adult male lion weighing 130 kg.

Site of incision: After induction of anaesthesia, the animal was positioned laterally on the right side. The left leg was lifted by tying to a rope, to expose the perineal region. The choice of incision site and the number of incisions seems to be dependent on the operator's choice. Scrotal neck (base of the scrotum on the anterior side) was the site chosen for the incision in the reports published in ZOOS' PRINT. While a single mid-line incision was done in the case of the leopard, two parallel incisions were done in the case of the lion in Sri Lanka. In this workshop, the incision site was different. After preparing the penile and scrotal area aseptically, local anaesthetic was administered subcutaneously. An incision long incision was made first on the left of the anterior part of the penis, all along the chord line.

Ligating the Vas Deferens: After incising the skin, the spermatic chord was exposed by blunt dissection of the subcutaneous tissue. In animals with considerable amount of subcutaneous fat, there can be some difficulty in finding the chord, as happened during the demonstration at Ahmedabad. One can feel the movement of the chord in this area by pulling the testicles posteriorly. After locating the chord, it was pulled out and held firmly over a forceps. The Vas Deferens was uncovered by cutting through the tunica vaginalis using a forceps and scissors. The Vas can be easily differentiated from others by its white colour and firm consistency. The Vas was then ligated in 2 places 2 cm apart using a 3.0 chromic catgut. The mid portion was excised and preserved in 10% formalin for a later histological confirmation. The spermatic chord was then released into its original position. Later the subcutaneous tissue and skin were sutured using a 2.0 chromic catgut, with continuous and simple interrupted sutures respectively. The same procedure was done on the right side also. An antibiotic injection of long acting penicillin was administered subcutaneously after dressing the wound.

E. Transponder telemetry

Transponder telemetry is the latest technique now available for identifying individual animals. The transponder system consists of 3 components:
(i) A 4 mm long microchip which is injected into the animal
(ii) An applicator for injecting the microchip and
(iii) A scanner/reader for reading the identification number of the injected microchip

Microchip and the reader

Advantages & disadvantages: Using transponders for animal identification has its own advantages. It is permanent and remains life long inside the animal, demanding no further application unlike ear tags and tattooing. Being a non-radioactive material, it does not cause any ill effects. This marking system is very useful for animal identification during transport as it is not subjected to damage during transport unlike the conventional type of ear tags and tattoos.

Transponders have their own disadvantages also. As they cannot be seen externally, and have a short reading distance, animals have to be restrained every time for reading the identification number. Group living animals like primates & ungulates with transponders, therefore, cannot be subjected to behavioral studies like focal animal sampling. Moreover, transponders being more sophisticated, are also costlier than the conventional ones.

Implantation procedure: The recommended site of implantation of the microchip is the dorsal aspect of the tail base. This has
So far the Asiatic lions in Sakkara Boazar zoo and Gir forests have proved to be negative to this virus presence. In fact FIV has not been detected from any of the feline species including domestic cats in India. However, one must admit that only few limited samples have been subjected to the diagnostic procedure. A simple diagnostic kit for diagnosing FIV and FeLV (Feline Leukaemia Virus) was demonstrated to the author by Ms Janice Martenson at Patna Zoo. This so called CITE Combo FeLV Ag/FIV Ab Test, has been designed to diagnose infection by one or both of these viruses in a single test procedure. It is an enzyme linked immuno-absorbent assay (ELISA) for detecting FIV antigen and antibody to FIV. As both these viruses cause immunosuppression, it is difficult to differentiate them based on clinical symptoms alone. Using this kit, the test can be performed with whole blood, serum or plasma. Even hemolyzed samples can be used.

None of the blood samples collected during the workshops were positive for FIV and FeLV. The Coimbatore Zoological Park & Conservation Centre has plans to conduct a prevalence survey of FIV and FeLV in domestic cats after obtaining the diagnostic kit from US.

### Table 1. Details of anaesthesia (all are adult animals).

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<th>SPECIES</th>
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<td>Lion</td>
<td>Ahmedabad</td>
<td>NR</td>
<td>Telazol</td>
<td>425</td>
<td>Not required</td>
<td>6</td>
<td>NR</td>
</tr>
<tr>
<td>13</td>
<td>Lion</td>
<td>Ahmedabad</td>
<td>NR</td>
<td>Telazol</td>
<td>450</td>
<td>50 mg had to be</td>
<td>6.5</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>given twice as it</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>started growing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Lion</td>
<td>Bombay</td>
<td>NR</td>
<td>Telazol</td>
<td>450</td>
<td>Not required</td>
<td>7.5</td>
<td>NR</td>
</tr>
<tr>
<td>15</td>
<td>Leopard</td>
<td>Bombay</td>
<td>NR</td>
<td>Telazol</td>
<td>250</td>
<td>Not required</td>
<td>8</td>
<td>NR</td>
</tr>
<tr>
<td>16</td>
<td>Asiatic lion</td>
<td>Bombay</td>
<td>NR</td>
<td>Telazol</td>
<td>400</td>
<td>Not required</td>
<td>7.5</td>
<td>NR</td>
</tr>
<tr>
<td>17</td>
<td>Leopard</td>
<td>Bombay</td>
<td>NR</td>
<td>Telazol</td>
<td>180</td>
<td>100 mg Ketamine</td>
<td>15</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>after 17 hrs</td>
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<tr>
<td>18</td>
<td>Clouded leop.</td>
<td>Patna</td>
<td>NR</td>
<td>Ketamine</td>
<td>250</td>
<td>Not required</td>
<td>7.5</td>
<td>53</td>
</tr>
<tr>
<td>19</td>
<td>Clouded leop.</td>
<td>Patna</td>
<td>NR</td>
<td>Ketamine</td>
<td>250</td>
<td>Not required</td>
<td>8</td>
<td>42</td>
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<tr>
<td>20</td>
<td>Clouded leop.</td>
<td>Patna</td>
<td>30</td>
<td>Ketamine</td>
<td>275</td>
<td>50 mg more after</td>
<td>16</td>
<td>36</td>
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<td>11 mts</td>
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<td></td>
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<tr>
<td>21</td>
<td>Leopard cat*</td>
<td>Patna</td>
<td>50</td>
<td>Ketamine</td>
<td>50</td>
<td>40 mg after 10</td>
<td>7</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>minute</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 mg after 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>minute</td>
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<td></td>
</tr>
<tr>
<td>22</td>
<td>Leopard cat*</td>
<td>Patna</td>
<td>100</td>
<td>Ketamine</td>
<td>100</td>
<td>Not required</td>
<td>8</td>
<td>NR</td>
</tr>
<tr>
<td>23</td>
<td>Leopard</td>
<td>Patna</td>
<td>250</td>
<td>Ketamine</td>
<td>100</td>
<td>Not required</td>
<td>7</td>
<td>NR</td>
</tr>
<tr>
<td>24</td>
<td>Leopard cat*</td>
<td>Patna</td>
<td>100</td>
<td>Ketamine</td>
<td>100</td>
<td>Not required</td>
<td>4.5</td>
<td>NR</td>
</tr>
<tr>
<td>25</td>
<td>Leopard cat*</td>
<td>Patna</td>
<td>100</td>
<td>Ketamine</td>
<td>100</td>
<td>Not required</td>
<td>5</td>
<td>NR</td>
</tr>
</tbody>
</table>

*Estimated body weight before darting
*Total dose in mg.
*Induction time in minutes
*Revival time in minutes (from induction to getting up)
*Not recorded
*In these two cases, apnoea developed after 10 minutes. Endo tracheal tubes were inserted using a laryngoscope and artificial respiration provided using a manual resuscitator.
*As the animal got over-excited prior to drug administration, subsequent administrations were required. Hyperthermia developed after 15 minutes (up to 108°C). Libral spraying of water brought down body temperature to 105°C in 20-30 minutes.
been chosen because it is safer to place the reader at the rear side of wild animals, particularly carnivores. Locations like shoulder, neck etc. are closer to an animal’s head and thus invariably require drug-immobilization for implantation as well as reading. Whenever tail base is chosen, the implantation can be done simply by restraining carnivores in squeeze cages.

The selected area is clean shaven and prepared surgically using antiseptic lotions like Betadine solution and alcohol. The 4 mm long microchip that comes within a 16 gauge disposable needle is attached to the special syringe that functions almost like the customary Artificial Insemination gun (meant for frozen semen). The identification number of the microchip can be seen on its pack. The microchip is implanted subcutaneously like giving a subcutaneous injection. Before releasing the animal, the number is read on the reader for confirmation.

F. Diseases of potential threat to lions
The disease working group in the PHVA on Asiatic lion, held at Baroda during Oct 18-21 1993, inferred that two incidents of disease in Asiatic lions need special attention. One was the frequent reports of maggot wounds and the other the incidence of viral papilloma. Most of the zoo veterinarians during the veterinary capsule workshops agreed that the Asiatic lions and their hybrids are more prone to get their wounds infested with maggots. In fact two of the seven lions anaesthetized during these workshops had maggot wounds. Both animals were treated with subcutaneous injections of Ivermectin at the rate of 200 microgram/kg body weight.

During the 1992 collaboration with US scientists and the forest department of Gujarat, lions from the Sakkaraubg Zoo were examined for disease prevalence. Papillomatous lesions were detected on the anterior part of the ventral surface of tongue. When subjected to histopathological and virological diagnostic tests, a papilloma virus was found to be the cause. Interestingly, it is the same viral papilloma that has been found to affect bob cats, Florida panther and snow leopards. This condition has not been found in African lions so far. During these workshops, two of the seven lions examined had viral papillomas on their tongue. Samples of the lesions were collected & frozen and some preserved in formalin. Determining the prevalence of this suspected pathological condition in Asiatic lion was one of the objectives of the US team’s visit to India. Even though, only a limited number of Asiatic lions have been subjected to any such systematic investigation, Dr. Bush & Dr Philip were of the view that about 25% of all the pure Asiatic lions have viral papilloma. It is not known whether this virus can cause any disease to these wild felids.

Another virus that has disease causing potential is the Feline Immunodeficiency Virus (FIV). FIV is a recently identified lentivirus that can cause depletion of T-lymphocytes in domestic cats. FIV is closely related to HIV (the Human Immunodeficiency Virus), the cause of AIDS in humans. Exposure to FIV has been detected in African lions, bobcats, leopards, snow leopards and jaguars. Like the Papilloma virus, there has been so far no evidence of FIV causing any disease in these species, however.
BARODA ZOO WORKSHOP -- 20 October 93

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Dr. K. N. Vyas, Principal, Veterinary College, Anand

Dr. J. M. Anjarla, Professor and Head, Department of Zoology, Banaras Hindu University

Dr. S. C. Patel, Asst. Director, Veterinary Investigation Centre, Vadodara

Dr. W. K. Soni, Asst. Director, Animal Husbandry, Veterinary Services, Vadodara

Dr. K. C. Patro, V.A.S. Nandankanan, Orissa

D. R. K. Sahu, Dy. Director, Animal Husbandry, Orissa

AHMEDABAD ZOO WORKSHOP, 21 October 1993

Dr. N. M. Shah, Assoc. Prof., Department of Veterinary Pathology, Gujarat Veterinary College, Ahmadabad

Dr. G. R. Patel, M.V.Sc., Dy. Director, Veterinary Hospital, Andhra Pradesh Veterinary College, Anand

Dr. R. C. Goswami, Vety. Officer, Animal Husbandry Branch, Directorate of Animal Husbandry, Madhya Pradesh, India

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BOMBAY ZOO WORKSHOPS -- 26 October 1993

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Mr. V. Sharma, Dy. Director, Veterinary Hospital, Chandigarh

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ZOO'S PRINT First DRAFT Report of P.H.V.A. for Asiatic Lion (Panthera leo persica) JAN/FEB 1994
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National Cancer Institute
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Knoxville Zoo, Tenn.

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