The assessment of captive breeding in the Caucasian viper (Gloydius halys caucasicus) in Iran

Sedigheh Shakoori, Fatemeh Todehdehghan, Abdolhossein Shiravi, Vida Hojati

Abstract
This study assesses captive breeding in Caucasian Viper, Gloydius halys caucasicus. Twenty three Caucasian Viper male and female snakes were collected from Taleghan and Damavand Counties to study the reproductive potential and assessment of captive breeding. The results indicated that the average weight of a pregnant female Caucasian viper was 68.92 ± 10.41g and the mean body length from snout-vent length (SVL) was 48.39 ± 2.69 cm. It is good to mention that the Caucasian Viper female has an annual reproductive cycle. Mating occurs in late spring (May-June) or early summer (July). Then, growth and maturation of follicles continues during the active season and stops during hibernation and will be resumed in the spring of next year. Due to giving birth in September (end of summer), the duration of pregnancy is estimated to be 2.5 months. The average litter size was 3–5. The average baby weight was 2.81 ± 0.39 g and the mean length (SVL) was 14.82 ± 0.84 cm as well. The Caucasian viper lives in mountainous areas and hibernates when the temperature drops during the winter. Hibernation lasts for three months.

Keywords: Caucasian Viper, hibernation, captive breeding, litter size.

Introduction
The Caucasian Pit Viper, Gloydius halys caucasicus, is a viviparous and venomous species. It inhabits in the mountain areas including southwest and southeast of the Caspian Sea, Azerbaijan southeast, south Turkmenistan, former Soviet Union, northern Iran, northwest of the Afghanistan. Its habitat in Iran includes Tehran, Gilan, Mazandaran, Golestan and Semnan Provinces [1, 2]. Vipers are in danger of extinction due to factors like indiscriminate hunting by hunters, global warming, climate change, pandemics, land use changes, conversion of natural habitats to agricultural and residential lands, habitat destructions and etc. One way to prevent the extinction of vipers is captive breeding where they are kept in special circumstances and hence, the toxins produced by them could be used in pharmaceutical industry and serum research institutes regarding to the treatment cardiovascular, hypertension and strokes, and bites [3]. Captive breeding is defined as the reproduction of rare and endangered species in certain societies to accomplish controlled environmental factors that are limited by certain constraints [4]. Also, captive breeding meets economic and conservation aspects. The economic outlook can be extracted from the venom in production of anti-snake bite serums in serum institutions along with its commercial purposes for the skin and their oil plus its application in pharmaceutical industry as drugs used for cardiovascular disorders [3, 5]. From the conservation point of view, it can be stated that snakes are helpful for the environment. Snakes are the best hunters of mice and therefore, could act as rodents population controllers. Snakes are also a food source for the wildlife. Hence, it would be a better idea to study their behavior in the laboratory to prevent nature manipulation and snakes population. So far, no research has been conducted in Iran in this area. Researchers have classified the reproduction pattern of female snakes into two categories: high-frequency and low-frequency reproduction [6]. However, the reproduction pattern of male snakes is divided into four categories: 1. After mating (summer). 2. Complex. 3. Prior to mating. 4. Continuous or sequential [7]. The length of pregnancy in snakes has been reported between two to eight months [8]. Also, severity and duration of winter are believed to be the hibernation determinant while hibernation period is known as one of the essential factors for the next year reproduction [7]. The aim of this study was the assessment of captive breeding in the Caucasian Viper in Iran.
Materials and Methods
Twenty three Caucasian viper snakes (18 females and 5 males) were collected from two mountainous regions: Damavand (52°4' N, 35°43' E) and Taleghan (36°12' N, 50°47' E) counties during 2013-2014. After removing the parasites and the quarantine period, they were transferred to the department of venomous animals, Razi Vaccine and Serum Research Institute, Karaj, Iran (temperature of 22 ± 2 °C). Snakes were divided into two groups: the first group was composed of 14 pregnant females that were removed by abdominal palpation (Figure 1). After weighing the specimens by point scale and measuring their length by meter (calibration) during the end of July (summer), they were sent for delivery into the vivarium (40 × 36 × 70 cm) in a room with an average temperature of 24.4 ± 0.97 °C and an average humidity of 41.9 ± 5.74 %. The room was lit with a 12 light / 12 dark (220 V) set (Figure 2).

At the end of pregnancy, the parturition and litter size was registered. The second group of nine Caucasian vipers (4 females and 5 males) was moved for hibernation preparation into the snake vivarium in the room in (19°C) October (fall). At first, the snakes were fed for 10 days with one-day-old mice (Figure 3-A). After that, they remained without food for five weeks until their intestinal contents were empty (Figure 3-B). Then, after preparations, the snakes were transferred to a special room for hibernation. The initial temperature of the winter home was 15°C and the initial moisture was 47%. Each week the temperature was reduced to one degree. In the end, the temperature, humidity, and hibernation durations were recorded.

Results and Discussion
After an year of investigation, it was concluded that the female Caucasian viper reproductive cycle was annual. Mating occurred in late spring (May) or early summer (July). It seems that ovulation occurs during mating. The vitellogenic cycle began in the spring or early summer. Growth and maturation of follicles continued during the active season, interrupted during hibernation and resumed in the spring of next year. Moreover, early embryos were seen in mid-July and embryos were formed in late August. In September, fetus reached its full growth. Due to parturition in late summer and early fall, the duration of gestation period is estimated from mid-July to late summer (2.5 months). Pit Viper can reproduce in vitro and the litter size is 3 to 5 (Figure 4). Three young were able to survive for three months in vitro (Figure 5). Pit Viper lives in the mountainous areas; therefore, it hibernates in the winter due to temperature drop. It seems that hibernation is essential to stimulate the reproduction in the future and further investigation is required. In this study, by applying natural climatic conditions in the laboratory, and essential preparations before hibernation, pit vipers were able to hibernate from December to February and spent three months in the dead of winter and consequently, used their body fat to feed on for three months. Table 1 shows that female pit vipers were smaller than others and due to their smaller size; they have less litter size.

<table>
<thead>
<tr>
<th>Litter Size</th>
<th>Female SVL (cm)</th>
<th>Young SVL (cm)</th>
<th>Litter Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–5</td>
<td>48.39 ± 2.69</td>
<td>14.82 ± 0.84</td>
<td>3–5</td>
</tr>
<tr>
<td>5–10</td>
<td>79</td>
<td>17.47 ± 0.7</td>
<td>5–10</td>
</tr>
<tr>
<td>3–8</td>
<td>68.7 ± 3.2</td>
<td>16.27 ± 0.7</td>
<td>3–8</td>
</tr>
<tr>
<td>10</td>
<td>75</td>
<td>17.5 ± 1.41</td>
<td>10</td>
</tr>
<tr>
<td>Bothrops newiedi pubscens</td>
<td>76.2 ± 9.10</td>
<td>21 ± 2.1</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 1: The female and Young snout-vent lengths (SVL) and their litter size in Gloydius halys caucasicus and some other species for comparison.

Fig 1: A pregnant female Gloydius halys caucasicus

Fig 2: Vivarium for keeping pregnant female Gloydius halys caucasicus

Fig 3: A: feeding of Gloydius halys caucasicus before hibernation. B: defecation of Gloydius halys caucasicus before hibernation.

Fig 4: Young of Gloydius halys caucasicus born in vivarium (2 days old).

Fig 5: Young ones of Gloydius halys caucasicus were born in vivarium (3-months-old).
Based on measurements, it was observed that the female Caucasian viper had an average length of 48.39 ± 2.69 cm. Moreover, the vitellogenesis cycle began in spring or early summer. Growth and maturation of follicles continued during the active season and stopped during hibernation and resumed in the spring of next year. In September, the fetus reached its full growth and due to giving birth in late summer and early fall, the gestation period was estimated from mid-July to late summer (2.5 months). It hibernated in the winter and used its fat reserves for energy. Vitellogenesis of female snakes *Crotalus durissus* started in the fall and continued during winter, until ovulation and fertilization occurred in the spring. Parturition occurred at the end of spring and during summer. Primary follicle yolk of female *C. durissus* was available all year round; nevertheless, the yolk secondary follicle was available from late autumn to late spring (July to November). *C. durissus* female parturition snakes in captivity of northeastern Brazil occurred in the summer (February/March) and early autumn (April) [10]. The pregnancy period of female snakes (*C. durissus*) was from spring (October and November) to summer (February/March) and early autumn (April). Pregnancy lasted for 3 to 4 months. Seasonal or non-seasonal cycle of reproduction is dependent on latitude [10]. In Colubrid brown snakes (*Storeria dekayi*) from Louisiana area, like in most females, the yolk deposition began in early spring and most female ovulate in April [11]. Pregnancy lasted 2.5 months and most births occurred in the second half of June and July [11]. But vitellogenesis cycle of female *Micrurus corallines* started in the beginning of rainy season (September to December), mating occurred in October hatching in rainy season (December to January) [12]. Fertilization of *Crotalus durissus terificus* occurs in spring [13]. In the present study, because of the mountainous natural habitat of the snake pit viper mountain, an artificial hibernation condition was provided and stones, boxes, cabers and algae were used as resting place for snakes [14]. During the hibernation, the snakes refused to eat any food. Other researchers on the condition snakes from mountainous areas such as *Triceratolepidophis sieversorum*, *Vipera latifi*, *Protobothrops cornutus*, *Montivipera albicacornuta*, *Vipera ammodytes*, *Vipera nikolskii* and *Vipera urssini* have shown that they need to hibernate for reproduction ability in the next year [4, 5, 15, 16, 17, 18, 19].

The egg-laying snakes produce egg clutches that are, on average, 20 percent larger than the litters of ovoviviparous snakes. However, live-born snakes tend to be slightly smaller than those hatching from eggs, resulting in litters that typically contain more individuals than clutches of eggs do; litters average 11 young while the average clutch contains seven eggs [20].

The presence or absence of predators is an important factor influencing the size of offspring and litters. In general, when comparing closely related snakes, the one with fewer natural predators often produces litters of fewer, larger young ones than those from predator-rich environments [20].

According to this study, it is recommended that female Caucasian vipers should be reproduced in vitro conditions and healthy babies must bear alive. Also, babies could survive in vitro as well. Finally, in the winter, as the temperature drops, the Caucasian viper uses its fat storage as the food source.

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**References**
11. Kofron CP. Reproductive system of female *Storeria dekayi* from Louisiana was examined. Copeia 1979; 9:463-466.