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Establishment of live birth following intravaginal artificial insemination with chilled epididymal dog semen collected post mortem: case report
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The collection and cryopreservation of epididymal semen after the sudden death of a valuable dog is often requested by the owner. However, it has been shown that canine epididymal semen has a lower tolerance to freezing compared to ejaculated semen. It may therefore be preferable to chill the semen and look for a suitable female in heat during the days after the death. In this case, we were confronted with the sudden death of male Chihuahua, and the owners demanded to produce offspring from their beloved dog. Since the Chihuahua is a popular breed, we advised the owner to chill the epididymal semen, while they had to find a fertile bitch during the next few days.

The male dog died 4 hours before arrival upon the clinic. During transportation, the owners tried to keep the dog at 37°C by using a heat mat. Upon arrival, epididymal semen was collected as earlier described for cat epididymal semen: briefly, the testes and epididymes were collected, the cauda epididymis and ductus deferens were separated from the testes and incised repeatedly using a scalpel to allow the spermatozoa to swim out during 10 minutes in Hepes TALP. In total, 850 million spermatozoa could be collected, with a total motility (TM) of 70% and progressive motility (PM) of 42% (evaluated by CASA). By means of eosin-nigrosin staining, we evaluated the live/death ratio to be 92/8, unfortunately only 14% of the spermatozoa had a normal morphology, 12% had an abnormal head, 12% an abnormal tail and 62% had a proximal protoplasmic droplet.

Semen was centrifuged to obtain a pellet of 0.5 ml and was diluted in Tris-citric acid- egg yolk-glucose based extender in a ratio of 1:3. Subsequently, the diluted semen sample was placed in a water beaker (at 37°C) and slowly cooled in the fridge to 4°C. At day 2 after epididymal semen collection, a Chihuahua bitch of 3 years old, was presented at the clinic. She was in heat: vaginal cytology revealed 85% anuclear cells and progesterone concentration was 17.02 ng/ml. Half of the epididymal semen was inseminated intravaginally at day 2 and the other half at day 3 of the procedure. After both inseminations, the hindquarters of the bitch were elevated for 10 minutes and vaginal and uterine contractions were stimulated. Afterwards, the bitch was encouraged to walk in order that she was not allowed to urinate or sit down for 10 minutes. At day 3 of chilling, the epididymal semen had still a TM of 70% and a PM of 35%. Live/death ratio was evaluated to be 90/10, and 17% had a normal morphology, 17% of spermatozoa had an abnormal head, 10% an abnormal tail and 55% a proximal cytoplasmic droplet. At 28 days after the first insemination, ultrasound examination revealed the presence of one foetus, which was confirmed by RX at day 58. One healthy female puppy was born naturally at 61 days after the first insemination.

In this case report, we describe for the first time the birth of a healthy puppy after intravaginal insemination with chilled epididymal semen collected post-mortem. The semen quality after 3 days of chilling, was comparable to the quality of the semen at the moment of collection. Further research is needed to investigate the possibility of storing epididymal semen at 4°C, which can be a helpful tool to store semen of valuable dogs or wild carnivores after death or castration for a short time and circumvent the lower freeze-tolerance ability of epididymal semen.