after death from epididyma and deferent ductus from an adult Komodo dragon (13 years old) by slicing the tissue. The sperm cells were collected and diluted using a commercial extender for semen without permeable cryoprotectants (Gent A; Minitub Iberica, Spain), and maintained at 4C for 0, 24, 48, 72, 96 and 120 hours. Sperm viability was assessed by counting viable sperm cells after eosin-nigrosin staining. Initial viability was 96% (0h) and remained above 80% for 96h of refrigeration, whereupon 60% viability was recorded at 120h. Motility features were assessed using a computer-assisted sperm analysis system (CASA system; Proiser SL, Valencia, Spain). Average motility after collection was 87.1% with 75% of progressiveness. Motility decreased over cooling time ($R2=0.93$; $p<0.01$) and remained above 55% for 96h, whereupon 32.9% was recorded at 120h of chilling. Progressiveness decreased over cooling time ($R2=0.98$; $p<0.01$) and remained above 55% for 48h, whereupon a first drop to 34.7% (72h) was recorded and later critically dropped to 7.1% (120h). Our data suggest that epididymal spermatozoa from Komodo dragon can be stored at low temperatures without cryoprotectants maintaining sufficient levels of sperm motility for several days. This should allow transport of semen between institutions and theoretically be sufficient to achieve artificial insemination in Komodo dragons.

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**Conflict of interest:** None to disclose.

## P36
## AMPHIBIAN ART OVER THE GENERATIONS: FROZEN SPERM OFFSPRING PRODUCE VIABLE F2 GENERATION

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Sperm cryopreservation is a key element in the Assisted Reproductive Technologies (ART) toolbox for stabilizing genetic diversity of captive and wild amphibian populations threatened with extinction. When expressed as spermic-urine, mature amphibian spermatozoa have characteristically long needle-shaped heads with a high proportion of surface membrane to cytosolic volume, making the cells extremely fragile and susceptible to osmotic stress. Despite these challenges to sperm cryopreservation, we have produced F1 generation offspring from cryopreserved spermic-urine in both caudates (Tiger salamander, Ambystoma tigrinum) and anurans (Mississippi gopher frog, Lithobates sevosa; Boreal toad, Anaxyrus boreas). A key question now presents itself- what is the reproductive capacity of this frozen sperm produced F1 generation? In 2015, male and female A. tigrinum were treated with human chorionic gonadotropin and gonadotropin releasing hormone for gamete development and release. Frozen sperm (5% Me2SO+0.05% BSA) thawed for IVF, yielded F1 offspring ($n=21$). In 2018, a mature F1 female and two males were analyzed using ultrasound for oocyte and testicular development and hormonally treated to obtain gametes. The F1 female produced 550 viable eggs; a subset($n=45$) underwent IVF. Fertilization, cleavage and early embryonic development of the F2 generation to blastula stage was achieved($n=8/45$;18%). The same sperm sample yielded blastula stage embryos($n=74/174$; 42%) from an F0 female, control. Similarly, in 2013 spermic urine from hormonally induced L. sevosa that was frozen(10% dimethylformamide+10% trehelose) and thawed for IVF resulted in F1 offspring (26.3% frozen; 45.6% control). In 2018, spermic urine from adult F1 males and a control F0 male were used to fertilize eggs from an F0 female. The development and survivability of the F2 generation to advanced stage tadpoles/metamorphs was similar ($p>0.05$) between F1 frozen males (9%, 7%) and F0 control male (8%) for the same egg mass. Thus, a frozen sperm F1 generation can produce viable gametes and offspring.

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## P37
## OPTIMAL CRYOPRESERVATION YIELDS INTACT ALGINATE MICROSPHERES AND HIGH CELL VIABILITY AFTER THAWING

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The possibility of long-term storage of clinically relevant cells combined with daily availability of genetically stable material gives cryopreservation a high potential in modern cell therapeutics. Although cryopreservation of alginate-encapsulated cells is very promising for further clinical application, there is still no optimal cryopreservation protocol for freezing and thawing of cells within intact and mechanically stable alginate microspheres. Alginate-based semipermeable capsules allow for permanent protection of cells against physical damage caused by the cryopreservation process. In this work, a range of parameters such as alginate cross-linking time, concentration of dimethyl sulfoxide, its loading time and cooling rate were analysed for freezing of 300, 800, 1000 and 1500 μm alginate microspheres. The micro-spheres were generated using electro-spraying (300 μm) and air flow (800, 1000 and 1500 μm) encapsulation methods. The structural integrity of cell-free alginate micro-spheres was evaluated upon freeze-thaw cycles using an Axio Imager M1m microscope with Linkam cryostage to identify an optimal combination. Verification of optimal cryopreservation protocol yielding intact capsules has been performed using multipotent stromal cells derived from the common marmoset (Callithrix jacchus). Freezing of alginate-encapsulated cells has been conducted using a controlled-rate freezer Planer Kryo 560-16 and Askion C-Line workbench. Analysis of cell viability after thawing has been performed using Calcein-AM / EthD-1 cell viability assay following image analysis using μVision software. Among the optimal conditions, the following ones yielded the highest cell viability (83%) and recultivation efficiency (70%) 24 h after thawing: cross-linking time 15 min, 10% (v/v) Me2SO, loading time at 4°C of 45 min and a cooling rate of 2.5 K/min. The results of this study demonstrate a high potential for clinical application of mechanically stable alginate capsules for efficient treatment of rare diseases.

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## P38
## EFFECT OF VITRIFIED-WARMED BOVINE OOCYTES ON THE BLASTOCYST HATCHABILITY

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This study evaluates the effect of vitrification of blastocyst which derived from IVF following vitrified-warmed matured bovine oocytes. During experiment, IVM oocytes or blastocysts were exposed to medium containing 5% dimethylsulfoxide (DMSO) and 5% ethylene glycol (EG) for 30 sec, followed by exposed to medium containing 12% DMSO, 12% EG and 0.25M sucrose for 30 sec, finally exposed to vitrification solution containing 20% DMSO, 20%EG and 0.5M sucrose for 30 sec. Oocytes or