Probl Cryobiol Cryomed 2025; 35(4):245
https://doi.org/10.15407/cryo35.04.245

Evaluation of the impact of hypoxic conditions on the viability of spermatozoa after capacitation and cryopreservation

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For the successful penetration of spermatozoa through the oocyte envelopes and the initiation of fertilization, the process of capacitation is essential (Balbach M, 2023). *In vivo*, it occurs in the female reproductive tract (Gallo A, 2021). *In vitro*, capacitation is modeled by culturing spermatozoa in specialized media. Oxygen concentration is one of the key in these media, however regulators of this process are insufficiently studied. Furthermore, it remains unclear under which conditions cryopreservation of spermatozoa is more effective: before or after capacitation.

The objective of this study was to assesses the survival rates of non-capacitated and capacitated spermatozoa under different oxygen concentrations (1, 5, 21% O_2).

Ejaculates from healthy donors (n = 30) were used. A basic semen analysis was performed according to the WHO guidelines (2010). Spermatozoa were isolated by two-step gradient centrifugation (80%/40% SpermGrade), and the pellet was resuspended in Global Total for Fertilization medium (CooperSurgical, USA). Capacitation was carried out in a CO_2 incubator at 37 °C for 60 minutes under 1, 5, and 21% O_2 conditions. Cell motility was evaluated using CASA, and the acrosome reaction was assessed using FITC-PSA (Sigma-Aldrich, USA) following the manufacturer's protocol. Statistical analysis was performed using one-way ANOVA.

After 60 minutes of incubation, the best motility, viability, and percentage of cells with induced acrosome reaction were observed in the group incubated under 5% O_2. In the groups incubated under 1 and 21% O_2, these parameters were significantly lower. After cryopreservation, the highest survival rate was recorded in the non-capacitated spermatozoa group (84.5 ± ± 12%) and in the group incubated under 1% O_2 (73.9 ± 3.3%).

Conducted research showed that oxygen levels during sperm capacitation affected their viability, motility, and acrosome reaction. Cryopreservation of non-capacitated spermatozoa increased their survival rate.

Hypoxia (1—5% O_2) during incubation reduces oxidative stress and protects sperm membrane integrity, ensuring higher viability after cryopreservation. Normoxia enhances lipid peroxidation and protein degradation, leading to reduced cell survival following the cryoprocedure. The use of hypoxic incubation conditions is a promising approach to improve the efficiency of male reproductive material cryopreservation.

Probl Cryobiol Cryomed 2025; 35(4):245
https://doi.org/10.15407/cryo35.04.245a

Fish reproductive cells collection of the Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine: history, significance and perspectives

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Biodiversity conservation in the current era of climate change remains an important global challenge (Brooks *et al.*, 2006). Although the most desirable way to save rare species is to conserve them within a minimally transformed environment (Marini *et al.*, 2019). In the context of current Ukrainian conditions, this method of biodiversity restoration is complicated by the impact of military activity (Vasyliuk *et al.*, 2025). One of the methods widely used to secure rare species outside of the wild environment is cryopreservation of reproductive cells and embryos (Kopeika *et al.*, 2019), and the storage of fish sperm by cryopreservation is widely used (Cherepanov & Kopeika, 2007; Martínez-Páramo *et al.*, 2009; Puhovkin *et al.*, 2016).

To conserve genetic diversity among rare and especially valuable fish species as well as breeds, the researchers of the Institute for Problems of Cryobiology and Cryomedicine organized more than 54 expeditions between 1986 and 2000. The expeditions covered the territories of Ukraine and neighbouring countries. During the field expeditions, more than 300 specimens of 16 different species were collected. These species represent three families: Cyprinidae, Acipenseridae, and Salmonidae.

Cyprinus carpio samples are the most represented in the collection by five different breeds and one wild population. The collection also includes specimens of *C. rubrofuscus*. Some specimens from this species originate from the wild Amur population, the others relate to the breeding form of Koi. In addition, the collection includes specimens of two species of the genus *Hypophthalmichthys*, *H.°molitrix* — silver carp, and *H. nobilis* — bighead carp.

Salmonids are represented in the collection by specimens of four species of the genus *Oncorhynchus*, namely *O. mykiss*, *O. gorbuscha*, *O. keta*, *O.°kisutch*, and two species of the genus *Salmo* — *Salmo salar* from the Baltic population and *S.°labrax* from the Crimean population. Both of the *Salmo* listed populations are endangered, however, while the Baltic population of *Salmo salar* has an EN status in The IUCN Red List of Threatened Species, very little is known about the condition of the Crimean population of *S. labrax*. In the occupied Crimea a significant anthropogenic transformation of small rivers, which is a habitat of the Crimean population of *S. labrax*, is happening.

The sturgeons in the collection are also represented by two genera, the genus *Huso* with species *H. huso* and *H. dauricus* on the one hand, and the genus *Acipenser* with the species *A. ruthenus*, *A. gueldenstaedtii*, *A. stellatus* and *A. nudiventris* on the other. Last of these species of the *Acipenser* genus, *A. nudiventris*, is the rarest of the above, as it has disappeared from the wild in Ukraine and is rarely kept in aquaculture.

Overall, the collection of the Institute for Problems of Cryobiology and Cryomedicine includes various forms of Cyprinidae, Salmonidae and Acipenseridae which can be used in the future for the restoration of aquatic bioresources of Ukraine.