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Зниження токсичності кріопротекторів без шкоди для кріозахисту яєць морського їжака (частина 1)

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Reduction of the Toxicity of CPAs is Without Compromising the Cryoprotection in Sea Urchin Eggs (Part 1)

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Cryoprotective agents (CPAs) are fundamental chemical compounds to apply to cryopreservation and vitrification. The aim of this work is to study the effect of combinations of CPAs on sea urchin eggs to try to find a promising CPA cocktail in the future development of cryopreservation protocols.

Two protocols were used with sea urchin unfertilized eggs, the first consisted of the addition of CPA in a single step to a fixed volume, with an exposure time of 15 min. The second protocol consisted of the addition of CPA in 15 equimolar steps of one minute, up to a final volume of 2 mL, followed by dilution of the CPA with filtered seawater in 12 steps of 1 min. After exposure to the cryoprotectant at room temperature (18–20ºC) and its subsequent dilution and filtration, the sea urchin eggs were fertilized and incubated at 18ºC for 48 h, until reaching the 4-armed pluteus stage.

The 15-step protocol allowed greater growth and survival of sea urchin larvae than the addition of 1 step at a fixed volume, coinciding with previous findings on the toxicity of CPAs for sea urchin larvae *Paracentrotus lividus* carried out by E. Paredes and J. Bellas (2009) and E. Paredes (2014). In one step morphological damage occurs and we can observe tissue damage in the larvae arms.

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Зниження токсичності кріопротекторів без шкоди для кріозахисту яєць морського їжака (частина 2)

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Reduction of the Toxicity of CPAs is Without Compromising the Cryoprotection in Sea Urchin Eggs (Part 2)

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DMSO is one of the most widely used cryoprotectants and has been shown to be an effective CPA with several marine invertebrates, including sea urchins (Barros *et al.* 1996; Barros *et al.* 1997; Tervit *et al*. 2005; Adams *et al.* 2009; Paredes *et al.* 2009). In this work, eggs from the sea urchin *Paracentrotus lividus* were used. Toxicity tests were carried out that consisted of a controlled exposure to CPAs for a certain time and at a constant temperature (Paredes and Bellas 2009; Heres 2021) in a concentration range from 0.5 M to 3 M, using two different addition methods looking for a CPA combination with low toxicity. DMSO was used as the base of all solutions and was combined with well-known CPAs like propylene glycol, ethylene glycol, methanol, sucrose and fructose. The methanol and DMSO cocktail was the one with the highest percentage of normality, opening a possible line of work to design a CPA solution with these two chemicals as base components, although a decrease in toxicity was not observed when combining DMSO with other cryoprotectants.