THE SURVIVAL OF RAT TESTICULAR INTERSTITIAL CELLS IN HYDROXYETHYL STARCH AND DEXTRAN BASED SERUM-FREE MEDIA

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Introduction

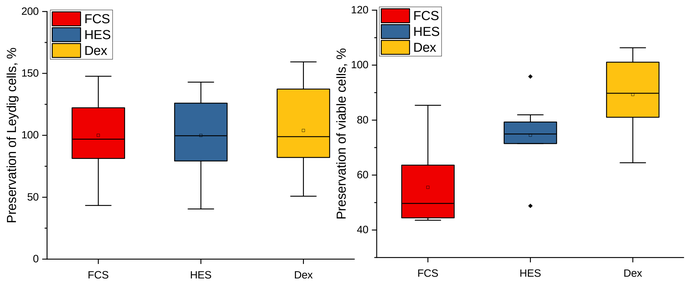
The material of cryopreserved testicular cells can be used for study of testicular defects, preservation of endangered animals, or in reproductive technologies.

Cryopreservation media for the interstitial cells (ICs), in addition to Me_2SO, may contain blood serum or polymers that improve the outcome of cryopreservation. However, the use of serum may impose some problems

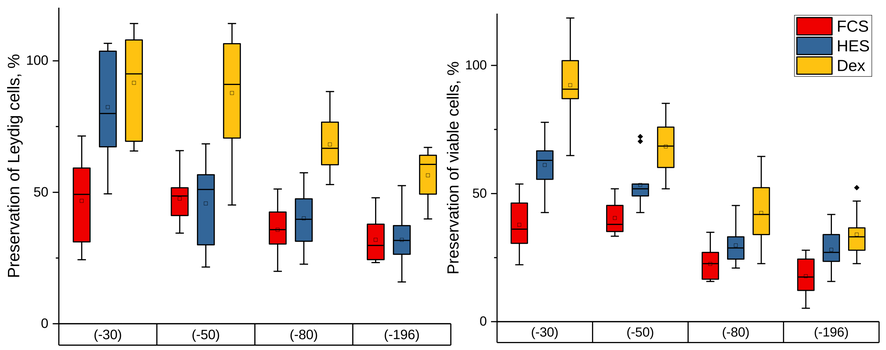
The objective is to investigate cryoprotective properties of serum-free media containing hydroxyethyl (HES) or dextran (Dex) for preservation of ICs.

Results:

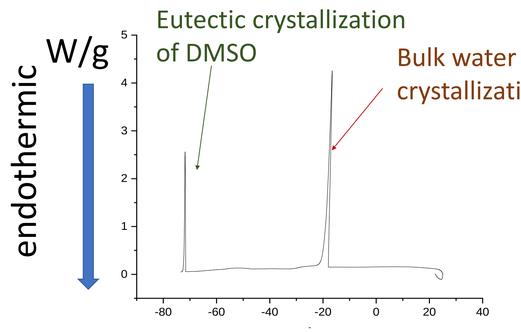
Incubation at +4°C



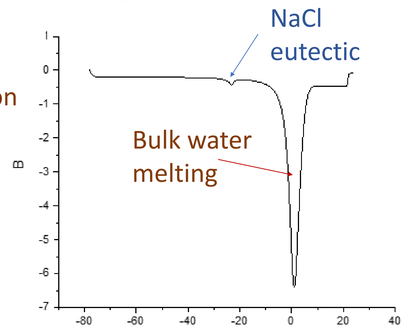
Preservation of cells in different temperature intervals



Cooling of FCS containing medium:



Heating of medium with cells:



- NaCl and DMSO eutectics were not seen when media were supplemented with Dex were used

Materials and Methods

- The viability of ICs was measured using trypan blue dye; Leydig cell count was assessed by histochemical staining on 3β-hydroxysteroid dehydrogenase; The metabolic activity of ICs was measured by MTT-test.
- ICs were incubated at +4°C for toxicity assessment;
- The cells were cooled at a rate of 1°C/ min to the final temperature of -30, -50, -80°C followed by warming or to -80°C followed by their immersion into liquid nitrogen;
- Ham's F12 media containing 100 mg/ml HES starch (Mm. 200 kDa) or Dex (Mm. 40 kDa) in combination with 0.7 M Me_2SO were used for cryopreservation; The medium supplemented with 10% FCS and 1.4 M Me_2SO shown in some researches was used as a control.

Conclusions

- Cryoprotective properties of FCS are not directly linked with the polymeric properties of its proteins. Thus, the mode of cryoprotective action of FCS is distinct from that of developed by HES and Dex based solution.
- HES and Dex based media have lower toxic effect on interstitial cells than the media with FCS.
- The intervals that resulted in the marked cell loss in the FCS based media are (+4 to -30C) and (-50 to -80C) where the water, salt and Me_2SO crystallization may take place.
- The best results were obtained with the media supplemented with 100 mg/ml Dex and 0.7 M Me_2SO