EVALUATION OF DECONDENSATION EFFECT BY HEPARIN AND DITHIOTHRETIOL (DTT) IN FREEZING-THAWING BOAR SPERMATOZOON.

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The spermatic nucleus of the mammals is highly resistant to a variety of chemical agents that solubilize other cellular structures. (1) This resistance is provided partially by the Perinuclear Theca (PT), cytoskeletal structural element that is surrounding the nucleus and blocks the entrance of external factors. The PT increases its stability during sperm epididimal transit, by disulfide bonds formation (2,3) and a substructure (sPT), the last is located in the apical zone of postacrosomal-PT layer(2). After fertilization, loosening and dispersion of the postacrosomal PT sheath precede sperm chromatin decondensation. The acrosomal reaction causes a morphological change on PT, and it is associated with the disappearance of the sPT. If the TP persists, decondensation is carried out in an irregular way. On the other hand the freezing-thawing causes a defect in decondensation, reported as an overcondensation producing a delay in the male pronucleus formation (4,6). For in vitro study of spermatic nuclear decondensation several chemical has been used such as sulfhydryl reducing agents, alone or in combination with neutral detergents, anionic detergents, glicosaminoglicans (GAG's), high salt concentration and proteases. (5,7). The high number of disulfide bonds formed among the protamine molecules of the spermatic nucleus of the mammals, transform DTT into one of the most popularly used decondensating agent. Heparin favors decondensation by competing for charges with protamine (7). It has been reported that in humans the freezing-thawing process damages the spermatic cell in such a way that the DTT and the Heparin favored their decondensating action (7). The present research looked for establishing the effect that different Heparin and DTT concentrations have on the decondensation process of boar fresh semen, and the possible differences that could be seen on the thawed sperms due to the action of the same decondensating agents and if this variations were present because of the damage done to the PT morphology. PT fresh and frozen-thawed sperms were suspended in NaCl (154mM) managing a concentration of $35x10_6$ sperms/ml and treated with Brij 36T (1.2%) during 10 min to expose their PT. There after, sperms were incubated with DTT 3.75mM. or heparin (5,10 and 15 USP). Aliquots samples were fixed at pre-established times. Fixed samples were processed for their observation, by negative stain, to Transmission Electronic Microscope, where the integrity of the sPT was used as morphological marker to evaluate the damage on PT after the process of freezing-thawing. The fresh sperms showed an undamaged sPT; on the other hand post thawed spermatozoa have different on grades of damage or a total absence of sPT. Starting from the 15 seconds of DTT treatment, produces an alteration of the sPT in fresh sperms (Fig. A); this alteration was progressive passing first through an expansion of the same one, losing continuity and ending like a diffuse line getting lost completely at the 3rd min, the above-mentioned coincided with the decondensation of the head, which presented small areas with loose of material, and at min 5 it was visible a true decondensation of the subacrosomal sheath (Fig. B). Due to the fact that the subacrosomal sheath was first decondensated, could be attributed to the greater amount of proteins stabilized by disulfide bonds. In the case of the post thawed sperms, decondensation was less homogeneous in the population, since at min 3, heads were found with a true decondensation, although most of the population presented an unchanged head even after min 10 of exposure. These results suggest that the freezing-thawing alters the decondensation pattern and that a relationship exists between the grade of integrity of the sPT and this process, because the thawed cells presented a difference in the damage under want by the sPT and they also presented a greater heterogeneity response to

DTT decondensation . On the other hand, heparin was not able to cause a decondensation at the used concentrations, which coincides with reported cases in other researches that mention the human sperm as the only specie where heparin has a decondensing action. On the other hand it is important to remark that heparin was able; in fresh semen, to extract the sPT at min 3 of exposure to a 10 USP concentration (fig.C), whereas thawed semen exposed to a lower concentration of 5USP was able to extract the sPT in only 15 seconds (fig. D), this suggests that the freezing-thawing process causes a damage in the stability of the sPT that makes it more sensitive to the action of heparin. Treatment with heparin (5USP) produced inclusive an PT alteration, since the progressive lost of material was remarkable in fresh spermatozoa as well as in thawed ones, furthermore in the last, the lost of material was greater, since in fresh sperm up to 1 hrs of treatment, PT residues could be distinguished (fig E), while in thawed sperm PT was almost lost at 5 min treatment (fig.F), confirming their greater susceptibility

References

[1] W. E. Gall. Experimental Cell Research (1976) 102: 349-358

[2] A. Mujica, et al., Microscopy Research and Technique (2003) 61:76-87

[3] P. Sutovsky ,et al., Microscopy Research and Technique (2003) 61:362-378

[4] S. Hamamah, et al., Reprod Nutr Dev (1990) 30: 59-64

[5] S. Jager, et al., The Journal of Experimental Zoology (1990) 256:315-322

[6] A. Cordova, et al., Theriogenology (2002) 57:2119-2128

[7] B.R.Zirkin, et al., Gamete Research (1985) 11:349-365

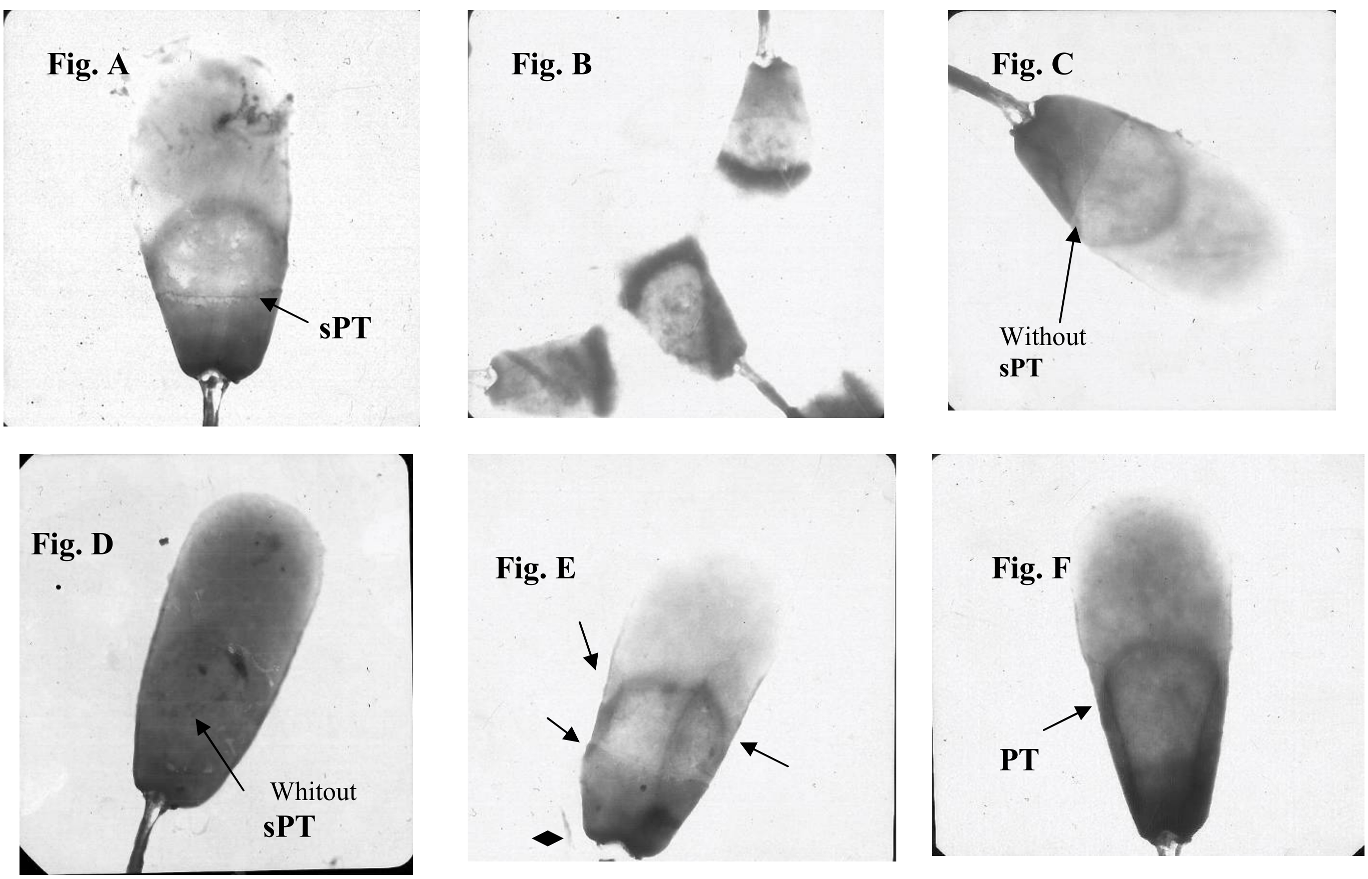

Fig A. Complete sPT structure.

Fig.B Decondensated spermatozoa, it is only apreciated the calix region (postacrosomal)

Fig.C sPT its not visible and where it should be found it is noticed a electrolucid zone.

Fig.D sTP has completely disappeared.

Fig E. Arrows show the places where electrodenses zones are still apreciated, corresponding to PT, the rhombus signals the lost of material .

Fig.F The portion that shows PT is lower and thiner .

(**notices:** Pictures are not in sequence for a single treatment, but for more information refer to text).