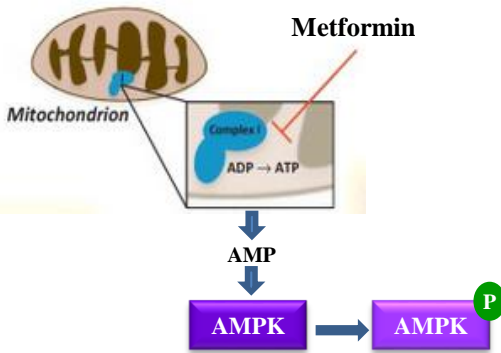


EFFECTS OF METFORMIN ON THE MOTILITY AND ACROSOME REACTION OF FRESH AND FROZEN-THAWED CHICKEN SPERMATOZOA

Thi Mong Diep Nguyen, François Seigneurin, Elisabeth Blesbois

Introduction



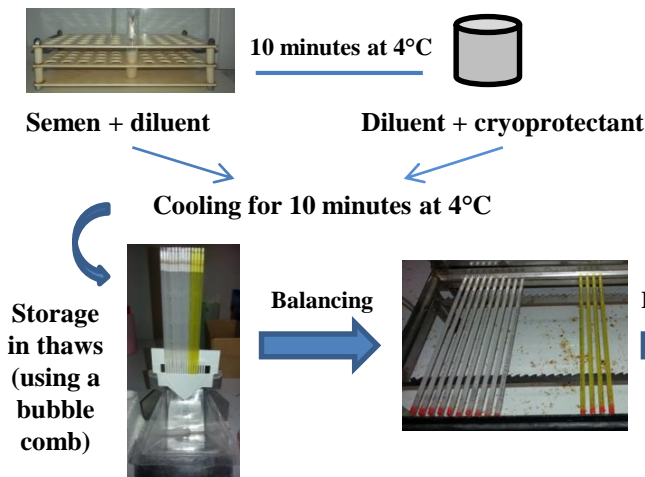
AMP-activated protein kinase (AMPK) is a key regulator of energy metabolism. Metformin is an activator of AMPK.

Recently, we have characterized AMPK in spermatozoa and found it to be involved in fundamental functions: motility and the ability to carry out the acrosome reaction. However, the presence and role of AMPK in frozen-thawed spermatozoa is still unknown. Spermatozoa are highly specialized cells that require a great deal of energy to ensure their motility and fertilization capacity.

The aims of this study were to investigate the effects and molecular mechanism of metformin in frozen-thawed spermatozoa.

Materials & methods

1. Freezing process



2. Semen quality assessment

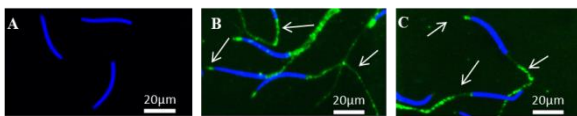
Viability is measured by Sybr 14 and PI (propidium iodide). Motility parameters are assessed by HTM-IVOS. Acrosome reaction (AR) is detected by FITC-PNA in the presence of Ca²⁺ and perivitelline membrane (PVL).

3. Immunocytochemistry

Spermatozoa were incubated with the primary anti-phospho antibody AMPK α (diluted 1:100).

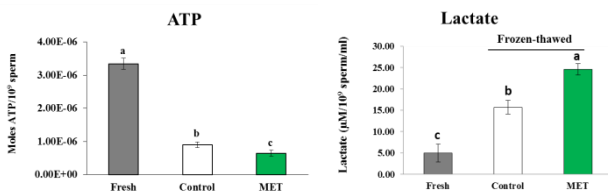
Results

Localization of AMPK in spermatozoa.

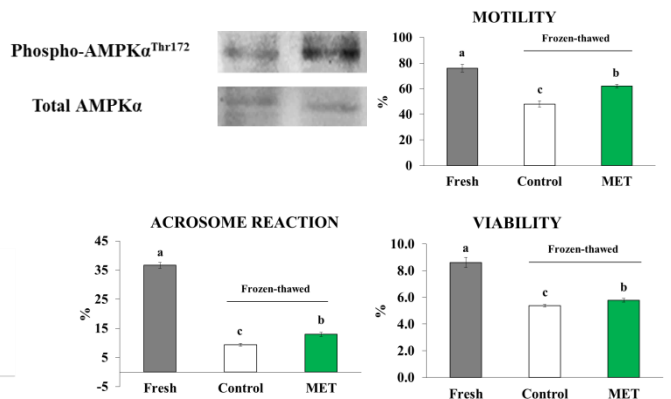


AMPK is found in the acrosome region, in the midpiece and throughout the flagellum.

Metformin stimulates ATP and lactate production.



Effects of metformin on AMPK phosphorylation and spermatozoa viability, motility and AR.



Conclusion

Our results showed that chicken spermatozoa cryopreserved with AMPK activator had improved membrane integrity, motility, and acrosome response. This is the first assessment of the effects of metformin on frozen-thawed chicken spermatozoa through its influence on AMPK activity to reduce damage to avian semen caused by cryopreservation. These data will be useful for developing and improving avian semen handling and storage techniques. In this context, an *in vivo* study using physiological doses of metformin will be carried out to assess the subsequent fertility of breeding stock.