Evaluation of arachidonic acid supplementation in the diluent on acrosomal membrane integrity in post-freezing goat sêmen

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INTRODUCTION

The cryopreservation process can generate an increase in oxidative reactions generating an excessive production of reactive oxygen species - ROS (AITKEN; MARSHALL, 2002). This excessive production can generate oxidative stress, resulting in metabolic and functional disorders in male germ cells (DOBRAKOWSKI et al., 2017), such as damage to sperm motility and viability (VARNER et. al., 2015), damage to the plasma membrane (FOLCHINI et al., 2012), the acrosome and cellular DNA (GUTHRIE; WELCH, 2012).Damage to the integrity of sperm membranes, caused by oxidative stress, can impair their biological functions. Damage to the acrosome can cause damage to the acrosomal reaction, resulting in fertilization failure. Therefore, it is necessary to add substances that can improve the antioxidant defenses of biological systems, combating the high production of ROS.

OBJECTIVE

To evaluate the effect of arachidonic acid supplementation in TRIS-gem diluent on acrosomal integrity during cryopreservation of goat spermatozoa.

RESULTS

Table 1 Acrosomal membrane integrity (AC) of post-thaw goat spermatozoa after supplementation of different concentrations (0.5 µM, 5 µM and 50 µM) of arachidonic acid in the diluent

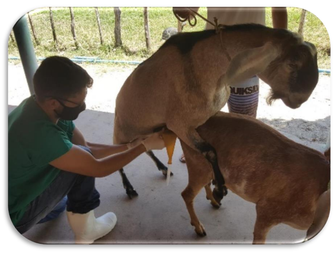
Treatment	AC (%)
Control	60.00 ± 7.84 [a]
0,5 µM of arachidonic acid	53.20 ± 9.85 [a]
5 µM of arachidonic acid	51.60 ± 7.50 [a]
50 µM of arachidonic acid	32.00 ± 17.17 [b]

Values are expressed as mean ± standard deviation. Mean values with different superscript letters in the same column indicate significant differences (P < 0.05) by DUNCAN test

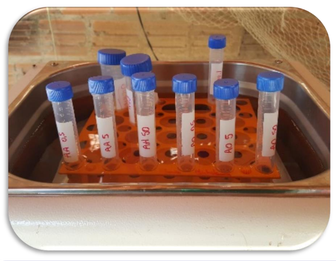
CONCLUSION

It is concluded that the addition of 0.5 µM and 5 µM of arachidonic acid to the diluent maintained the acrosomal membrane integrity in post-cryopreservation goat semen, being beneficial for sperm capacitation process and fertilization.

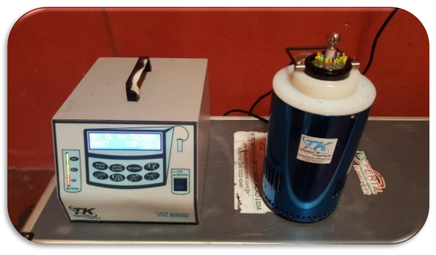
MATERIAL AND METHODS



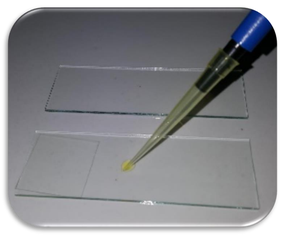
Semen collection from four AngloNubian



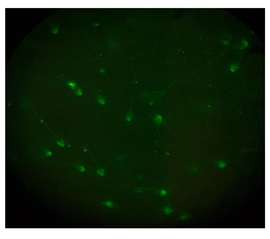
- Pool formation
- *dilution*
- Treatments (Tris-Gema; Tris-Gema + 0.5 µM arachidonic acid; Tris-Gema + 5 µM arachidonic acid; Tris-Gema + 50 µM arachidonic acid)



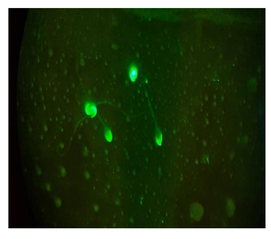
Cryopreservation of semen in Tk-3000 machine



Sample are thawed. Made the smear with 20µL of FITC-PNA . Incubated 20 minutes in a humid chamber at 4°C in the absence of light



Acrosome intact - Acrosomal region stained



Reacted Acrosome - Green fluorescent band in the equatorial region of the sperm head