EXPRESSION OF HETEROLOGOUS BOVINE SPEMADHESIN-1 AND FUNCTIONAL ASSAYS IN POST-THAWED SPERM CELLS

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INTRODUCTION

Seminal plasma proteins can protect sperm cells against thermal and oxidative stress. In this sense, some proteins have a relevant role in the biological protection system, and their positive effect has been described in cryopreservation. Spermadhesin-1, also known as acid protein of seminal fluid (aSFP), has been considered one of the main ones to mitigate the deleterious effects of oxidative stress in the cryopreservation process. The expression and extraction of aSFP in a bacterium, and the performance of bioassays in sperm cells, might relevant for the development of additives in cryopreservation and post-thaw processes.

OBJECTIVE :To produce and to purify the aSFP in Escherichia coli bacteria and addition in post-thaw sperm cells in cryopreservation processes.

MATERIALS AND METHODS

The expression of BL21DE3 was evaluated with IPTG concentrations and post-induction temperature at 37 ° C. The raSFPH6 was released by ultrasonic disruption and confirmed by polyacrylamide electrophoresis. The raSFPH6 was added in different concentrations and the post-thaw semen quality parameters were evaluated.

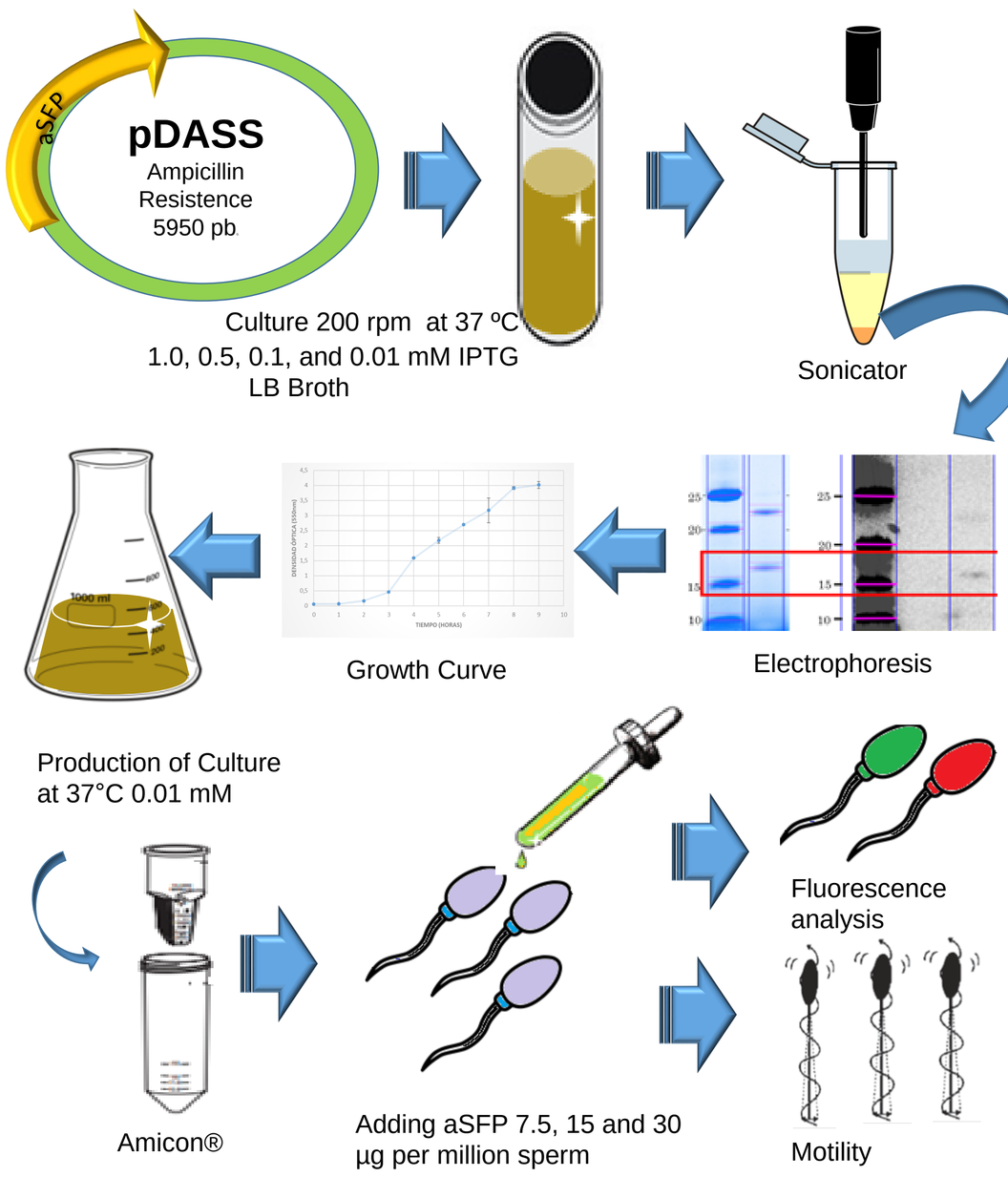

Figure 1. Expression of recombinant aSFP using *E. coli* and bioassay.

MATERIALS AND METHODOLOGY

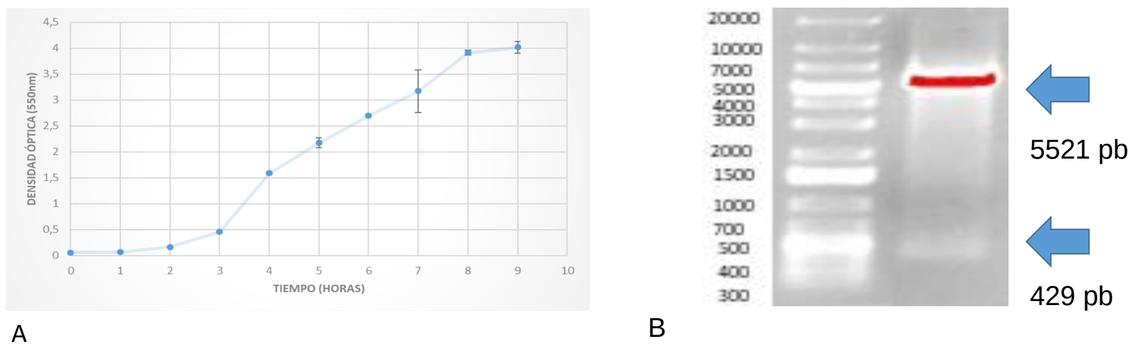

Figure 2 . A) Growth curve of *E. coli* BL21 DE3 B) BamHI - HindIII Digestion Enzymes pDass 5950 pb .

The transformation was confirmed with the digestion enzymes and the induction with IPTG at 2 hours and a half.

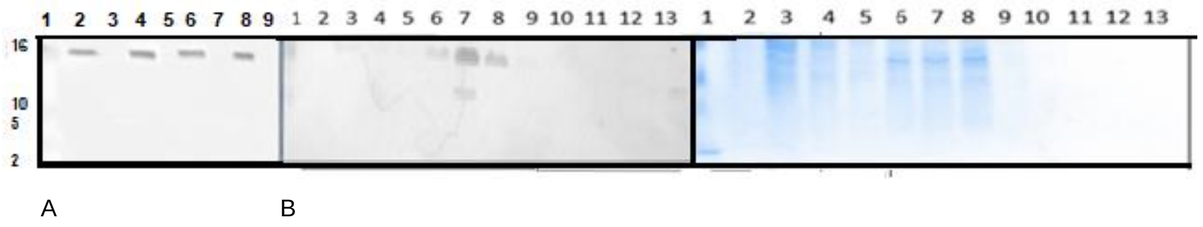

Figure 3: Western blot developed with anti-his of: A) soluble raSFP (Lines 2,4,6,8) and insoluble (no bands detected) in BL21 DE3. The band with 0.01 mM IPTG is highlighted; and B) Purification with 200 mM Imidazole of raSFP (Line 6,7,8).

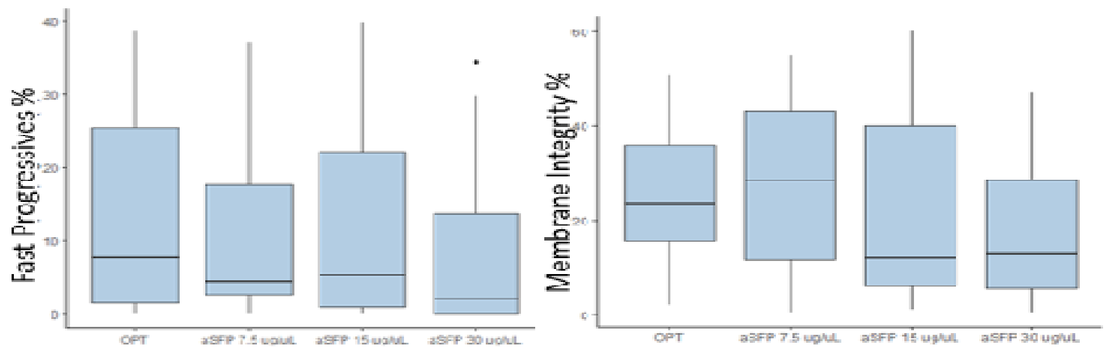

Figure 5. Statistic analysis.Shapiro's normality test and the Kruskal-Wallis nonparametric test were performed. There are no significant differences between the treatments.Shapiro's normality test and the Kruskal-Wallis nonparametric test were performed.

CONCLUSIONS

The bovine aSFP protein was produced recombinantly and due to the translocation signal peptide, disulfide bonds are formed, improving folding and reducing inclusion bodies, obtaining raSFPH6 of 15 kDa. Quality tests did not show changes in post-thaw sperm cell motility and viability. However, there was a trend towards a higher protein concentration and decreased motility, possibly related to a mechanism that prevents the energy consumption of the sperm before fertilizing the oocyte. Protein amounts less than 7.5 µg per million sperm should be considered in further experiments.

ACKNOWLEDGMENTS

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