

## INTRODUCTION

Artificial insemination with frozen semen in jennies is associated to few pregnancy rates. The main cause can be the high immune response in the endometrium. Jennies are more likely to get acute endometritis postinsemination than mares when the used semen is frozen. This is due to anatomic, histologic and physiologic differences of the reproductive tracts of both species. These endometritis are a cause of infertility because they generate a bad uterine environment. It is a reaction to specific species and varies depending on the type of insemination, semen quality and where it is deposited during AI.

## OBJECTIVES

The aim of this study is to analyse the factors involved in the endometrial inflammatory response in jennies and the available treatments, to optimize the artificial insemination protocols with frozen semen and to improve the results of this reproductive technology in jennies.

### Reproductive characteristics jennies vs mares

Jennies	Mares
Heterotypical behaviour	uncommon
Oestrus cycle is not affected by the season	Seasonally polyestrus
Oestrus cycle 23-25 dies	Oestrus cycle 21-22 dies
CLs homogeneous echogenic texture	CLs non-echogenic central area
Oestrus behaviour: predictive of ovulation	Oestrus behaviour: not predictive



Jenny's cervix



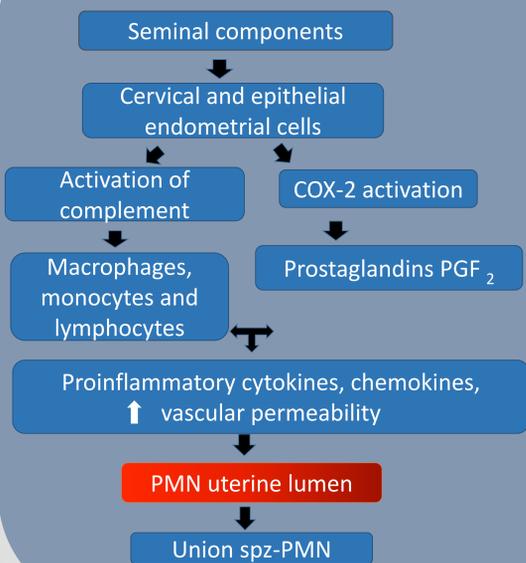
Mare's cervix

### Artificial insemination with frozen semen

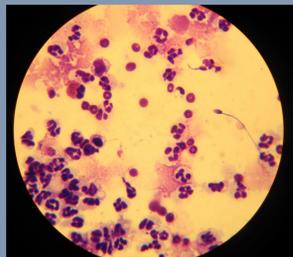
For the cryopreservation of semen it is necessary to remove seminal plasma and add extensors and cryoprotectors. Freezing induces cryogenic damage on spermatozoids and produces physical and chemical changes in the sperm membrane.

Female species	Fresh semen diluted with milk and cold (2 males)	Frozen semen with 2.2% glycerol (6 males)
Jennies	9/20 (45%)	4/38 (11%)
Mares	33/73 (45%)	18/50 (36%)

### Induced endometritis by frozen semen

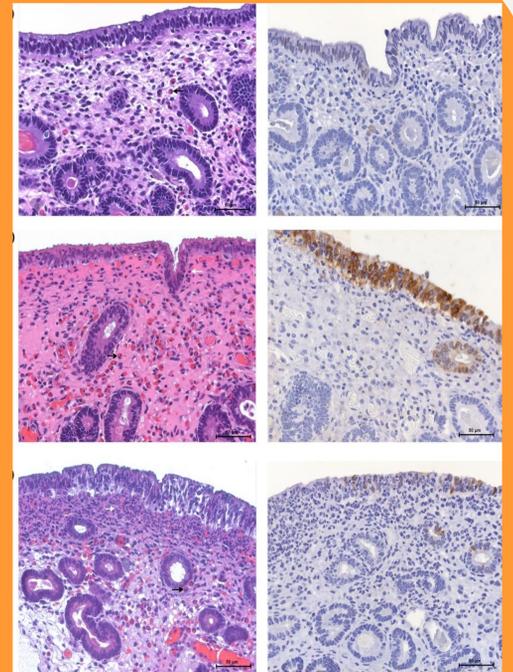


When frozen semen is used it produces an inflammatory response, not only because of the presence of spermatozoids, but also because of the elimination of immunomodulator proteins that exist in the seminal plasma and the addition of extenders.



### Role of seminal plasma

Seminal plasma is not necessary for fertilization to occur, but it facilitates interactions between the most competent male gametes and the uterus. Seminal plasma has favourable effects on sperm motility and endometrial inflammatory response, as it reduces the union of spermatozoids-PMN and inhibits COX-2 gene expression at the luminal epithelium and stratum compactum.



### Treatment of endometritis

Ketoprofen is a strong inhibitor of the COX-2 expression in the stratum compactum, but donkeys have a low tissue distribution and a rapid elimination. An improvement in the fertility of Jennies when frozen semen is diluted in seminal plasma was observed, with an increase of 61.5% in jennies fertility. A high concentration of spermatozoids causes PMN saturation and free spermatozoids are able to fertilize. When a deep-horn intrauterine insemination is used, spermatozoids are deposited along the oviduct helping to avoid contact with PMN.

### In vitro model



Neutrophil activated by SP



Degranulated neutrophils activated by FMLP

## CONCLUSIONS

The cleaning mechanisms of the jennies uterus are different than mares. The presence of eosinophils in the stratum compactum seems to be a characteristic of the endometrium in healthy oestrus jennies not observed in mares. The role of seminal plasma on the control of endometrial inflammation post AI with frozen semen seems to be important. Ketoprofen IV is able to reduce COX-2 expression post AI, but not to control PMN amount and activity. The combination of high frozen sperm concentration, Deep-horn intrauterine AI, and the addition of seminal plasma, with or without Ketoprofen administration as an AI protocol, might improve pregnancy rates in jennies when semen is frozen.