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## USE OF AMOXICILLIN IN EXTENDERS FOR STALLION SPERMATOZOA

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Antibiotics in extenders are necessary to control the normal flora present in the stallion semen [1, 2]. Penicillin (Pen) and Gentamicin (Gent) are recommended and has been largely used in stallion semen extenders in France. Pen is now reserved for human hospitals in France. So the aim of these studies was to validate the replacement of Pen by another betalactam. Amoxicillin (Amox) presents a wider antimicrobial spectrum and is largely marketed in veterinary and human medicine.

Different experiments/trials were performed to evaluate Amoxicillin :

- 1) its safety for stallion spermatozoa after storage by measuring their motility by a computer-assisted sperm analyzer (2 experiments with fresh semen and 2 experiments with frozen semen).
- 2) its antimicrobial activity on stallion semen flora by counting the number of aerobic and facultative aero-anaerobic bacteria (number of colony-forming unit (CFU)) (technique OIE, 1988).
- 3) its global effect on semen stored < 12 h by estimating the fertility per cycle of stallions.

Stallion spermatozoa were diluted either in UHT milk ( $20 \cdot 10^6$  spermatozoa /ml) and stored at 4°C or in freezing extender (INRA82 + 2% egg yolk + 2.5% glycerol), after centrifugation, and frozen. In these extenders, Gent (Gentalline 80 or 160, Schering-Plough ND) was used at 50 mg/L, Pen ( $10^6$  IU, Diamant or Panpharma or Sigma ND) was used at 50 000 IU/L if non specified. Amox used were Clamoxyl 500 mg (ND, Beecham) in experiment 1a, and Clamoxyl 1g IM (ND, Glaxo-Smith-Kline) diluted in apyrogenic water in all other experiments and trials.

### Results

1) Within experiment, the motility was similar :

a - after storage for 48 h in milk containing no antibiotics, Pen+Gent, Pen (40, 400 or 5100 IU/mL) or Amox (20, 200 or 1000 mg/L) (3 stallions x 2 ejaculates)

b - after storage for 72 h in milk containing no antibiotics, Pen+Gent, Pen (50 or 500 IU/mL) or Amox (25 or 250 mg/L) (5 stallions x 1 ejaculate)

c – after freezing and thawing in freezing extender containing Gent+Pen or Gent+Amox (400 mg/L) (6 stallions x 2 ejaculates)

d – after freezing and thawing in freezing extender containing Gent+Pen or Gent+Amox (100 mg/L) (122 stallions, > 877 ejaculates/group): 49% vs 50%, respectively.

2) The log of CFU was lower for fresh stored semen diluted in extender containing Gent+Amox (80 mg/L) (3,35) than Gent (3,93), Gent+Pen (3,56) being intermediate, all of these were lower than the control with no antibiotics (4,7) (8 stallions (from 4 studs) x 1 ejaculate x 24 h storage)  $P < 0.05$ .

3) Fertility per cycle was similar for 5 draught stallions whose semen was diluted, stored at 4°C for 2-8 h, with Gent+Pen (64%, n= 243 cycles, in 2004) and with Gent+Amox (100 mg/L) (69%, n= 288, in 2005), it was the same for 2 light stallions (59% (n=80) and 54% (n=61)).

These experiments has proven the safety of Amoxicillin for the equine spermatozoa, even at high dosage. Its antimicrobial action has been demonstrated: Amoxicillin is almost as efficient as Penicillin for the control of normal flora of stallion semen. This is consistent with previous data [1]. So this antibiotic could be used instead of Penicillin for this indication. In that case, the recommended dose for Amoxicillin in extender for equine spermatozoa is 100 mg/L.

However, in case of pathogens portage or genital infection of the stallion, use of semen for artificial insemination (AI) must be reconsidered. AI can be used if the bacterial infection could be controlled by antibiotics (the ones tested here or others). In that case, the antibiotics must be used at an appropriate dosage that remain compatible with the safety of the spermatozoa [2].

[1] Clement F, Vidament M, Guerin B (1995). Microbial contamination of stallion semen. In : Equine Reproduction VI. Biology of Reproduction, Monograph Series I. Sharp D.C., Bazer F.W. Eds. Society for the Study of Reproduction Publ., Madison, USA, 779-786.

[2] Varner DD, Scanlan CM, Thompson JA, Brumbaugh GW, Blanchard TL, Carlton CM, Johnson L (1998). Bacteriology of preserved stallion semen and antibiotics in semen extenders. *Theriogenology*, 4, 559-73.