Seminal plasma proteins and semen characteristics in relation with fertility in the stallion

I. Barrier-Battut, Jean-Louis Dacheux, Jean-Luc Gatti, P. Rouvière, Cristina Stanciu, Françoise Dacheux, Marianne Vidament

Animal Reproduction Science (4th International Symposium on Stallion Reproduction. 21-23 October 2005, Hannover, Allemagne) 89 (1-4), Abstract 22 p:255-258.

Seminal plasma proteins and semen characteristics in relation with fertility in the stallion

I. BARRIER-BATTUT[1], J.L. DACHEUX[2], J.L. GATTI[2], P. ROUVIERE[2], C. STANCIU[2], F. DACHEUX[2], M. VIDAMENT[2]

[1] *Ecole Nationale Vétérinaire, Pathologie de la Reproduction, 44307 Nantes, France*
[2] *UMR 6175 INRA- CNRS-Université de Tours-Haras Nationaux, 37380 Nouzilly, France*

Summary

Several parameters were measured on semen from 36 stallions (fertiles and subfertiles). Conventional parameters were significantly related with fertility, as well as results of hypoosmotic swelling test and induction of acrosome reaction. Total protein concentration in seminal plasma and concentration of human epididymal 1/cholesterol transfer protein were not related with fertility, whereas concentrations of lipocalin-type prostaglandin D_2 synthase and angiotensin I-converting enyzme were strongly related with fertility.

1. Introduction

Identifying subfertility in a stallion before a breeding season is extremely important for the equine industry. Conventional parameters including concentration, morphology and motility allow detection of the stallions most likely to be fertile. However, some of them are or become subfertile despite acceptable results of the conventional "breeding soundness examination". Due to this limitation, functional tests including hypoosmotic swelling test (Jeyendran et al., 1984) and acrosome reactivity after induction of acrosome reaction (Cummins et al., 1991) have been developped in other species and adapted for equine (Meyers et al., 1995; Neild et al., 2000; Varner et al., 2002).

The influence of seminal plasma on stallion fertility is still unclear. Several proteins have been identified in stallion seminal plasma (Calvete et al., 1994; Fouchécourt et al., 1999; Gatti et al., 1999; Fouchécourt et al., 2000). Among them, lipocalin-type prostaglandin D_2 synthase (PGDS), angiotensin I-converting enyzme (ACE) and human epididymal 1/cholesterol transfer protein (HE1), were shown to play a role in fertility in other species.

The aims of the present study were : 1) to quantify the amounts of PGDS, ACE and HE1 in stallion seminal plasma and describe individual variations in stallions of known fertility (fertile or subfertile), 2) to evaluate the predictive value of seminal plasma proteins on stallion fertility, compared to conventional parameters and some functional tests (hypoosmotic swelling test (HOS), acrosome integrity before and after induction of acrosome reaction).

2. Material and methods

Thirty six stallions belonging to the french National Studs were used. Per cycle fertility ranged from 25% to 80% (17 to 170 cycles per stallion and per breeding season, mean 66 cycles). Three groups of stallions were considered : "fertile" : per cycle fertility $\geq$ 55% (mean

63%, n=22), “intermediate” : 45% ≤ fertility <55 % (mean 48%, n=8), and “subfertile” : fertility < 45% (mean 37%, n=6).

For semen analysis, 2 ejaculates were collected per stallion. Conventional parameters were measured : semen volume, sperm concentration, total and progressive motility immediately after collection (0h) and after 24 h storage at 4°C in UHT skim-milk, percentage of alive spermatozoa (exclusion of eosine), percentage of morphologically normal spermatozoa. Integrity of the flagella membrane was evaluated using HOS test. Acrosome integrity and the ability of spermatozoa to undergo acrosome reaction after exposition to calcium ionophore A23187 were evaluated after 2h of incubation using staining with fluoresceinated Pisum Sativum agglutinin.

Total protein concentration was measured using the Bradford assay kit, with bovine serum albumin as a standard. PGDS, ACE and HE1 were quantified by electrophoresis and immunoblotting (Fouchécourt et al., 2002), using antibodies previously produced in our laboratory.

3. Results

Most conventional parameters were significantly correlated with fertility (positively : concentration, motilities, % alive sperm, or negatively : volume, % abnormal sperm). The percentages of swollen spermatozoa after HOS test, of intact acrosome before ionophore test, and of spermatozoa responding to induction of acrosome reaction were also significantly positively correlated with fertility ($p<0.05$). Consequently, all conventional parameters (except total sperm number) and results of functional tests differed significantly between the fertile and subfertile groups (% very swollen spermatozoa (HOS) : 49 vs 37 respectively, difference between % of reacted acrosomes before and after induction : 67 vs 51, respectively).

Concentrations of PGDS, ACE and HE1 were highly repeatable between the 2 ejaculates of the same stallion. Total protein concentration and HE1 concentration were not significantly related to fertility and did not differ significantly between fertile and subfertile stallions. PGDS and ACE concentrations were highly positively correlated with fertility, and differed significantly ($p<0.01$) between fertile and subfertile stallions. However individual patterns showed high intensities only on samples from fertile males, and low intensities on samples from a wide range of fertility (see Fig. 1). Determination of a threshold value to identify fertile and subfertile stallions, with acceptable specificity and sensitivity, seems therefore very difficult.

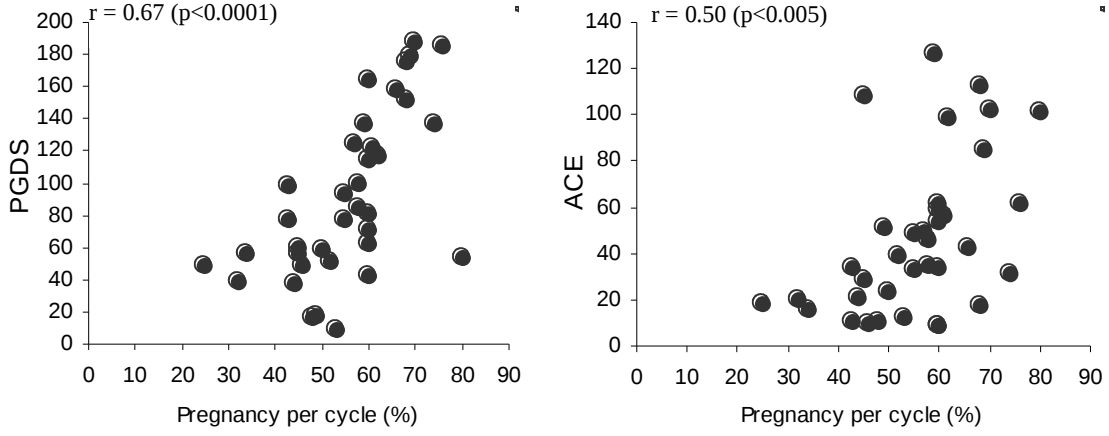

Fig. 1. PGDS and ACE concentrations (arbitrary units) in seminal plasma from 36 stallions of known fertility. Each point corresponds to the mean value of 2 samples for each stallion. Spearman coefficients of correlation with fertility are indicated.

Discussion - Conclusion

Conventional parameters differed significantly between fertile and subfertile stallions. This is in agreement with previous reports (Kenney et al., 1971; Dowsett & Pattie 1982; Jasko et al., 1992; Kenney et al, 1995). Results of HOS test differed significantly between fertile and subfertile stallions, which is consistent with observations in other species. Induction of acrosome reaction also differed significantly between fertile and subfertile stallions, which confirms previous findings in several species including the stallion (Varner et al., 2000), using here a larger number of animals.

Total protein concentration and HE1 concentration were not related with fertility. PGDS and ACE concentrations were strongly related with fertility, as observed in bovine (Gerena et al., 1998) but not in ovine (Fouchécourt et al., 2002; Métayer et al., 2001). This suggests that PGDS and ACE could play a role in fertility in the stallion.

Acknowledgements

The authors thank the french National Studs for financial support, and access to stallions.

References

Calvete et al., Reprod Dom Anim 1994; 29:411-426
Cummins et al., J Androl 1991; 12:98-103.
Dowsett & Pattie, J Reprod Fert 1982; Suppl 32:1-8.
Fouchécourt et al., Biol Reprod 1999; 60:558-566.
Fouchécourt et al., Biol Reprod 2000; 62:1790-1803.
Fouchécourt et al., Biol Reprod 2002; 66:458-467.
Gatti et al., Biol Reprod 1999; 60:937-945.
Gerena et al., Biol Reprod 1998; 58:826-833.
Jasko et al., Am Vet Med Assoc 1992; 200:979-985.
Jeyendran et al., J Reprod Fert 1984; 70:219-228.
Kenney et al., Biol Reprod 1995; Mono 1:647-653.
Kenney et al., Proc 17th Ann Conv A.A.E.P. 1971:53-67.
Métayer et al., Biol Reprod 2001; 65:1332-1339.
Meyers et al., J Androl 1995; 16:47-54.
Neild et al., Andrologia 2000; 32:351-355.
Varner et al., Anim Reprod Sci 2000; 60-61:493-509.
Varner et al., Theriogenology 2002; 58:303-306.