Comparison of prostanoid concentrations in extended boar seminal plasma to pregnancy status of sows after artificial insemination

S. Barnes[1,a], E. Mas[2], T. Rozek[2], C. Oger[3], J-M. Galano[3], T. Durand[3], J. Kelly[1], R. Kostecki,[1] and E. Noschka[1]
[1]University of Adelaide, Faculty of Science; Adelaide, South Australia, Australia
[2]Genetics and Molecular Pathology, SA Pathology; Adelaide South Australia, Australia
[3]Institut des Biomolécules Max Mousseron, Pôle Chimie Balard Recherche, CNRS, Université de Montpellier, ENSCM, France.

Corresponding author: serena.barnes@adelaide.edu.au

Quantification of semen quality in relation to subsequent successful pregnancy remains elusive; therefore, additional indicators are required. Non-enzymatic [isoprostanes (IsoP) and neuroprostanes (NeuroP)] and enzymatic (prostaglandins; PGF) lipid peroxidation products may be viable indicators of male fertility. However, their use as biomarkers in boars is currently inconclusive. This pilot study evaluated prostanoids, including IsoP, NeuroP, PGF, and their metabolites (n=13), in boar seminal plasma (SP) to identify differences in female pregnancy status after insemination. It is hypothesised that specific prostanoid concentrations in semen would influence pregnancy rates. Females (Parity 0-4; n=46) located in South Australia were given fenceline contact with a boar on Days 3 to 7 from weaning (Parity 1-4) or Days 4 to 7 from cessation of altrenogest (Parity 0) in October 2022, December 2022, and May 2023. Upon oestrus detection, females were inseminated with single use pouches containing pooled extended semen (80ml) collected from boars housed in Victoria, Australia. A second insemination occurred approximately 16h later in animals displaying receptive behaviour. Semen was scavenged from discarded pouches after insemination for analysis. Spermatozoa concentration and total live spermatozoa were determined using a nucleocounter (Chemometec, New South Wales, Australia). SP rich supernatant was obtained from a semen aliquot by centrifugation (1000g, 5 min) and stored at -80°C until analysis. Prostanoids were quantified in frozen-thawed samples using liquid-chromatography tandem mass-spectrometry. All non-parametric data were log transformed prior to respective one-sided independent t-test analyses to compare prostanoid data in SP that did (n = 43) or did not (n = 7) result in pregnancy. SP prostanoid data were compared to spermatozoa viability (%; live spermatozoa/concentration) using Spearman's correlation analyses. Alpha was set to 0.05. Data are presented in Table 1. $PGF_{2\alpha}$ affects uterine contractility which may improve sperm transport in the female reproductive tract, as exogenous administration of $PGF_{2\alpha}$ induces myometrial contractions and increases pregnancy rates after artificial insemination in sows. In this study, SP concentrations of $PGF_{2\alpha}$ from non-pregnant sows were similar to published results that were unable to induce uterine contractions *in vitro*, while $PGF_{2\alpha}$ concentrations in SP that resulted in pregnancy were significantly higher. Various NeuroPs and IsoPs have been associated with spermatozoa quality in other species. While no significance differences were detected between pregnant and non-pregnant groups, 20-F_{4t}-NeuroP, 4(*RS*)-4-F_{4t}-NeuroP, and 17-F_{2t}-dihomo-IsoP displayed mean differences in concentrations between pregnancy status groups that were outside the standard error, suggesting the sample size may not be high enough in the non-pregnant group to achieve significance. Prostanoids could be additional biomarkers to characterise boar semen quality; however, larger studies are required to determine if they are accurate predicators of semen fertility regarding pregnancy success after insemination.

Table 1: Prostanoid concentrations (ng/ml) in seminal plasma rich supernatant of semen used to inseminate animals that did or did not become pregnant are presented as mean ± standard error. Correlation results include coefficient and significance values.

Prostanoid	Pregnant	Non-pregnant	T-test Sig	Correlation Viability (R; Sig)
$PGF_{2\alpha}$	0.423±0.085	0.088±0.027	**P<0.001**	P>0.050
15-F_{2t}-IsoP (8-iso- $PGF_{2\alpha}$)	0.243±0.076	0.136±0.039	P=0.236	P>0.050
2,3dinor-15-F_{2t}-IsoP	5.526±0.795	5.426±1.027	P=0.419	P>0.050
5-F_{2t}-IsoP	0.362±0.047	0.323±0.053	P=0.405	P>0.050
5-F_{3t}-IsoP	0.368±0.074	0.389±0.115	P=0.368	P>0.050
8-F_{3t}-IsoP	0.225±0.019	0.244±0.044	P=0.339	**0.655; P=0.014**
18-F_{3t}-IsoP	1.638±0.179	1.234±0.295	P=0.135	P>0.050
ent-7(*RS*)-7-F_{2t}-dihomo IsoP	0.175±0.027	0.199±0.040	P=0.299	P>0.050
17-F_{2t}-dihomo-IsoP	0.255±0.079	0.099±0.031	P=0.139	P>0.050
7(*RS*)-ST-Δ^8-11-dihomo-IsoF	0.551±0.053	0.361±0.025	**P=0.002**	P>0.050
4(*RS*)-4-F_{4t}-NeuroP	6.500±1.681	2.941±0.655	P=0.192	P>0.050
20-F_{4t}-NeuroP	3.245±0.641	2.168±0.431	P=0.277	P>0.050
14-F_{4t}-NeuroP	0.936±0.101	0.919±0.250	P=0.421	P>0.050