

ARI Final Report

- A. Date: 26 August 2002
- B. Reporting Period: 1 July 2000-30 June 2002
- C. Project Number: 46870
- D. Project Title:
Analysis of Platelet Activating Factor in the gravid reproductive tracts of swine
- E. Principal Investigator (s):
Dr. Bill Plummer
Animal Science Department-Cal Poly
(805) 756-2113 office
(805) 756-5069 fax
wplummer@calpoly.edu
- F. Co-Principal Investigator (s):
Dr. John Diehl
Animal and Veterinary Science Department, Clemson University, Clemson, SC
(864) 656-5166 office
jrdiehl@clemson.edu
- G. Cooperator (s):
None
- H. Prepared by:
Dr. Bill Plummer
Animal Science Dept-Cal Poly
San Luis Obispo, CA 93407
(805) 756-2113 office
(805) 756-5069 fax
Principal Investigator
- I. Abbreviated review of initial proposal

Executive Summary. Depressed hog prices in 1998 and 1999 were due to not enough shackle space (kill capacity) and not to a glut of slaughter swine, however many swine producers were put out of business in California (Scotto, 2000). In 2000, market prices rebounded due to increased shackle space and reduced numbers of slaughter swine available as a result of the sellouts of the previous two years. The economic roller coaster ride emphasized the need for maximizing profit while streamlining operations. Increasing numbers of pigs per litter, and thereby reducing the cost per pig produced, is one aspect of streamlining. A significant step in improved litter efficiency would be to utilize platelet activating factor (PAF) in commercial

swine operations. Platelet activating factor is involved in signal transduction within many of the body's physiological systems. Of primary interest in the reproductive system is PAF's enhancement of embryonic development, placental attachment and sperm functioning. The amount of embryo-derived PAF produced is correlated to pregnancy potential, for higher PAF levels lead to greater numbers of embryos developing to the blastocyst stage (Abeydeera *et al.*, 1998). It is produced by the embryos of various mammalian species (Battye *et al.*, 1991; Collier *et al.*, 1988; O'Neill, 1985; Zhu *et al.*, 1991), exerts significant effects upon the preimplantation embryo (Harper, 1989), placental attachment (Ryan *et al.*, 1987), and perhaps maternal recognition of pregnancy and sperm functioning (Roudebush, Fukuda and Minhas, 1990). Presently, only Dr. John Diehl at Clemson University in collaboration with others (Mook *et al.*, 1998) has published data to suggest that PAF is found in either male or female swine reproductive tracts. Most notably, PAF was found in the uterine luminal fluid (ULF), the developing filamentous embryo and the endometrium (Diehl and Roudebush, 1999).

Expected outcomes. It was anticipated that significant levels of PAF would be found in the reproductive tracts of early pregnant gilts and that this knowledge will lead to the ability to increase litter numbers.

If PAF levels were as expected then enhancement of reproduction in swine can be attempted. It is not possible at this time to actively treat the female, so a more appropriate approach would be to pre-treat sperm with PAF prior to artificial insemination or select boars whose sperm produce significant levels of PAF. Pretreatment has resulted in increased numbers of developing embryos (Roudebush, Fukuda and Minhas, 1993). In addition, sperm evaluation for endogenous PAF could be used as part of an overall reproductive soundness exam (Diehl, 1999).

Materials and Methods. Fifty-two terminally crossbred (normally would have been harvested as fat hogs) gilts were maintained and bred at the Cal Poly Swine unit and utilized in a study to evaluate swine reproductive tracts for the presence of (PAF). Of the 52 gilts, 43 were utilized in the study, 6 had abnormal reproductive tracts upon recovery and 3 were not utilized for health reasons. Reproductive tracts were removed at harvest, endometrial tissue and embryonic tissue samples taken and processed, all tissues quick frozen and then sent to Dr. Diehl's lab. Students in Dr. Diehl's lab processed the tissues for the presence of PAF, PAF receptors (PAFr) and for DNA coding for PAF and PAFr.

***Results.* They were successful in finding all of the above, thereby confirming that swine do produce and utilize PAF and that PAF plays a significant role in establishing peri-implantation embryonic development and maternal recognition of pregnancy.**

J. Major Accomplishments:

State the situation of the research and the findings. Describe how the research was beneficial as well as the solutions that were found and by what means. Identify these points by numbers and/or bullets.

- Collaboration occurred between Cal Poly-SLO and Clemson University. Cal Poly has animals that can be used for research but does not have the appropriate labs for classical bench top research. Clemson has the bench top labs but does not have animals available for research.
- Cal Poly undergraduates at and Clemson graduate students were able to perform procedures via a strict protocol applying a scientific approach to data collection and analysis.
- Platelet Activating Factor had been identified in other species, but not confirmed in swine until this study.
- DNA for the production of PAF and PAFr was identified in both swine endometrial and embryonic tissues.
- A significant connection between PAF production and estradiol levels was established and considered as an indication of the mechanism for the maternal recognition of pregnancy in swine.
-

K. Impact Statements:

List the project impacts in priority order using as many numbers as needed.

1-With it established that swine embryos produce PAF and have PAFr, research can now be pursued to add exogenous PAF to extended boar semen in an attempt to increase the numbers of pigs/litter.

1a- PAF is routinely added to embryo cultures (particularly with human and other primate embryos) to enhance the viability of embryos when only a few are cultured together.

1b- Semen contains PAF and decreased levels have been associated with reduced numbers of pigs/litter in swine.

1c- In processed and extended boar semen PAF levels fall to zero by Day 3 post collection.

2- Swine now join a long list of mammals in which it has been proven that platelet activating factor is a necessary component of fertilization, embryogenesis and pregnancy.

L. Dissemination, publications and presentations of research:

1. List all activities in chronological order by category and date (most recent first) including any planned activities.
2. Include all names of those presenting and/or coauthoring material.
3. State name of publications and identify as refereed journal/paper or trade publication.
4. State names and places where presentation(s) were or will be made.
5. Attach copies of all disseminated materials including senior projects and graduate theses.

Refereed publications

W Yang, JR Diehl, M Yerle, JJ Ford, WE Roudebush, **WE Plummer**. Studies on structure and mapping of porcine platelet-activating factor receptor (PAFr) gene and

temporal expression of PAFr and estrogen receptor alpha (ER) genes in pig endometrial and embryonic tissues. Accepted, *Mole Reprod Dev.* 2002.

W Yang, JR Diehl, L Grapes, MF Rothschild, WE Roudebush. Rapid communication: The pig platelet activating factor receptor gene is expressed at the mRNA level in different tissues and is mapped to chromosome 6. *Anim. Reprod. Sci.* In Press. 2002.

W Yang, JR Diehl, WE Roudebush. Comparison of the coding sequence of the platelet-activating factor receptor gene in three species. *DNA Sequence* 12:239-251. 2001.

Abstracts:

W Yang*, JR Diehl, M Yerle, JJ Ford, RK Christenson, WE Roudebush, **WE Plummer**. Organization and chromosomal location of porcine platelet-activating factor receptor (PAFR) gene and temporal expression of PAFR and estrogen receptor (ER) genes in pig reproductive tissues. Soc Study Reprod. (annual meetings, Baltimore, MD, July 2002)

D R Gaddam*, JR Diehl, JJ Ford, TJ Rathje. Effect of photoperiod on platelet-activating factor concentration in boar spermatozoa. Soc Study Reprod. (annual meetings, Baltimore, MD, July 2002)

A B Hulon*, JR Diehl. The effect of storage time of the PAF content of boar semen at refrigeration temperature. 13th Annual Research Colloquium, SC Governors School for Science and Mathematics. 2002.

Platelet-activating factor in bull sperm has a positive and significant relationship with sperm motility. JR. Diehl, JC Spitzer, W Yang, WE. Roudebush. *Biol Reprod* 64:301 abstract 496. 2001.

GenBank submissions:

W Yang, JR Diehl, WE Roudebush. *Sus scrofa* platelet-activating factor receptor (PAFr) gene, intron 2, exon 3, and intron 1B. GenBank Accession No AF460980. 2002.

W Yang, JR Diehl, WE Roudebush. *Sus scrofa* platelet-activating factor receptor (PAFr) gene, promoter, exon 2, and intron 2. GenBank Accession No AF460979. 2002.

W Yang, JR Diehl, WE Roudebush. *Sus scrofa* platelet-activating factor receptor (PAFr) gene, promoter, exon 1, and intron 1A. GenBank Accession No AF460978. 2002.

Wensheng told me he has another GenBank submission to come because he has identified another exon in the PAFr gene. We are in the process of writing an invited review for *Biology of Reproduction* "Platelet-Activating Factor and its Receptor." He will have 4 manuscripts and close to 12 (I haven't counted them all) GenBank submissions.



Jenny Hansel (l) and Lisa Branstad (r) processing endometrial and embryonic tissues.



Apparatus for preparing tissues for freezing.



A normal 16 day pregnant gilt uterus.



One uterine horn extended for collection of embryos.



Jenny as a reference for the length of the extended horn (approximately 4 feet).