Estrogen and Interleukin-1β regulation of trophinin, osteopontin, cyclooxygenase-1, cyclooxygenase-2, and interleukin-1ß system in the porcine uterus

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Embryonic loss during early gestation limits litter size in swine production. Failure of the conceptus to attach properly to the uterine surface may contribute to the high rate of embryonic loss observed in swine. Attachment to the uterine surface is a highly synchronized event that requires precise communication between the expanding conceptus and endometrial tissue. Conceptus attachment to the uterine surface includes upregulation of adhesion molecules at the maternal/fetal interface for attachment as well as a pregnancy specific inflammatory response. Trophinin and osteopontin are cell adhesion molecules that may function in initial attachment between conceptus trophectoderm and uterine luminal epithelium of the pig and human. Leukocytes infiltrate the endometrium during implantation, and the pro-inflammatory cytokines Cyclooxygenase (COX)-1 and COX-2 are expressed in human and pig endometrium during pregnancy, where they are proposed to regulate conceptus implantation and uterine angiogenesis. Interleukin-1β (IL-1β) increases during implantation in the mouse, human and pig and may regulate uterine inflammatory cytokines. Estrogen also controls uterine events necessary for attachment and implantation of the mouse and pig conceptus and may act in synergy with IL-1β to prepare the uterus for the implanting embryo. Furthermore, trophinin expression was induced by IL-1β in human endometrial cells, and uterine osteopontin expression is regulated by estrogen in the pig and mouse. The objective of the current study was to evaluate the hypotheses that estrogen regulates the uterine inflammatory response induced by IL-1β during the establishment of pregnancy.

Cyclic gilts were treated with corn oil or estradiol cypionate (5 mg) on Day 11 of the estrous cycle. On Day 12, gilts were subjected to mid-ventral laparotomy and uterine horns were randomly infused with either saline or porcine IL-1β (15 μg). Uterine horns were removed at 4h and 36h post-infusion (4 gilts/trt/sampling periods) and endometrial mRNA was quantified by quantitative RT-PCR.

Estrogen did not influence ($P > 0.1$) concentrations of endometrial COX-1 and COX-2 mRNA; however, IL-1β increased ($P = 0.01$) endometrial COX-2 mRNA by 3.5 fold and tended ($P = 0.06$) to increase COX-1 mRNA by 2.5 fold 4h post infusion. Cyclooxygenase-1 and COX-2 regulate uterine prostaglandin secretion, which is essential to normal implantation and pregnancy in pigs (Kraeling *et al.* 1985). Cyclooxygenase-2 null mice are infertile and fail to implant; however, implantation is not impeded in the Cox-1 null mouse (Lim *et al.* 1997). Although the conceptus induces uterine COX-2 expression at implantation sites, estrogen did not increase COX-2 mRNA in ovariectomized mice which is true in our pig study (Chakraborty *et al.* 1996). Furthermore, IL-1β regulates ovulation in mice through COX-2 and prostaglandin production (Davis *et al.* 1999). We hypothesize that conceptus IL-1β regulates uterine prostaglandin secretion by increasing endometrial COX-2. Prostaglandins regulate angiogenesis

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and the conceptus may increase uterine vascularity through IL-1β induced COX-2.

The elongating porcine conceptus secretes IL-1β on Day 12 of pregnancy during conceptus attachment and maternal recognition of pregnancy, and this correlates with endometrial Interleukin-1 receptor type 1 and interleukin receptor accessory protein gene expression suggesting conceptus interleukin upregulates its own endometrial receptors (Ross *et al.* 2003). In the current study, IL-1β increased ($P = 0.05$) its own expression by 3.5 fold and tended ($P = .08$) to increase Interleukin-1 receptor type 1 by 2.5 fold; however, estrogen not IL-1β increased ($P < 0.05$) interleukin receptor accessory protein (2.5 fold increase). Similarly, IL-1β increased Interleukin-1 receptor type 1 in human endometrial stromal and glandular cells (Soloff *et al.* 2004). Interleukin-1β and estrogen differentially regulated uterine interleukin receptors; therefore their combined effects may alter the endometrial response to IL-1β during implantation.

Endometrial osteopontin and trophinin were increased ($P < 0.05$) at 36 h but not 4h after estrogen treatment. Estrogen treated gilts had 11.3 fold greater OPN and 3.6 fold greater trophinin endometrial mRNA than gilts treated with corn oil. Trophinin mRNA and protein are located at the maternal/fetal interface during implantation of pigs, mice, and humans (Suzuki *et al.* 2000, Nakano *et al.* 2003, Sugihara *et al.* 2008). Uterine trophinin is increased by estrogen in mice but IL-1β in human endometrial cells (Sugihara *et al.* 2008), suggesting expression is pigs and mice are controlled by similar mechanism which differs from humans. The fact that OPN and trophinin receptors are located on conceptus trophectoderm suggests a role in attachment and communication at the maternal/fetal interface. We hypothesize that IL-1β and estrogen secretion by the pig conceptus differentially modulates uterine expression of COX-1, COX-2, trophinin, osteopontin, and the interleukin-1 β system providing an inflammatory environment that is essential to establish pregnancy.

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