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DEVELOPMENT OF METHODS FOR STUDYING EMBRYO-UTERINE INTERACTIONS

Z. Zhang and D. L. Davis

Summary

The endometrium (lining of the uterus) functions to support and nurture developing embryos. However, 20 to 30% of pig embryos are lost in early pregnancy. Therefore, we developed methods to study the endometrium. Our initial work addresses the production of prostaglandins by the endometrium.

Prostaglandins are known to play important roles in the establishment of pregnancy. In the pig, this process occurs near the end of the second week of pregnancy. Therefore, we determined the prostaglandin production by glandular and stromal cells of pig endometrium collected on d 13 of pregnancy. Glandular cells produced more prostaglandin $F_2\alpha$ ($PGF_2\alpha$) than prostaglandin E (PGE). In contrast, stromal cells secreted more PGE than $PGF_2\alpha$. Progesterone inhibited PGE and $PGF_2\alpha$ production by both glandular and stromal cells. Four-hydroxyestradiol, an estrogen produced by pig blastocysts and endometrium, inhibited PG production of both prostaglandins by glandular cells but stimulated prostaglandin production by stromal cells. Our data indicate that glandular and stromal cells of the pig endometrium possess different characteristics of prostaglandin production, and these differences may be important in conceptus signaling for the establishment and maintenance of pregnancy.

(Key Words: Uterus, Embryo, Pregnancy, Prostaglandin, Estrogen.)

Introduction

In swine, pregnancy is commonly initiated with the presence of 14 to 16 viable embryos. However, on the average, only 9 to 11 pigs are born. The peak of embryo losses occurs near the end of the second week of pregnancy and corresponds to periods of attachment to the uterus and other events collectively referred as the establishment of pregnancy. These processes involve interactions between the lining of the uterus (endometrium) and the embryos. The endometrium consists of stromal and epithelial cells (Figure 1) and is active in prostaglandin production. Therefore, we developed procedures to study the endometrium. Since prostaglandins are powerful regulators of many physiological processes, studies of PG production by the endometrium will lead to a better understanding of the establishment of pregnancy and allow us to test procedures for improving embryo survival.



Figure 1. Uterine endometrium of the pig (S = stroma, G = uterine gland, E = luminal epithelium).

Experimental Procedures

Endometrial tissues were collected surgically from anesthetized gilts and sows on day 13 of pregnancy. Glandular (epithelial) and stromal cells were separated by enzymatic dispersion of the tissue. Cells were cultured in RPMI 1640 medium with 10% bovine fetal serum for two days. Then the medium was discarded and new medium, containing various hormones, was added. After an additional 24 h of culture, we collected the medium for assay of prostaglandins and other products. Cells were assayed for protein or were subjected to immunocytochemistry.

Results and Discussion

Our methods allow the separation of three distinct populations of cells: luminal epithelium, glandular epithelium, and stroma (see Figure 1 for location of cells before digestion). This report concerns only the glandular and stromal cells. Observations of the characteristics of uterine cells in culture are summarized in Table 1. Specific immunostaining for cytokeratin is detected in epithelium, and staining for vimentin is detected in stroma. These general characteristics confirm the identity of the cells in our cultures. In addition, the glandular cells secrete uteroferrin, the main protein in uterine milk secreted by uterine glands. The cells also differ in the relative amounts of the two prostaglandins (Figure 2). PGE is known to stimulate uterine blood flow, and the relatively larger amount of PGE produced by stromal cells may enhance blood flow to the endometrium. In that regard, the ability of 4-

hydroxyestradiol to stimulate PG production by the stroma could represent a mechanism by which embryos enhance uterine blood flow to their locations within the uterus.

As we continue to study mechanisms by which hormones and other factors affect uterine cells, we hope to discover methods to enhance embryo survival and, thereby, increase litter size.

Table 1. Characteristics of Stromal and Epithelial Cells in Culture

Cell type	Characteristic	Prostaglandin secretion in response to:	
		Progesterone	4-hydroxyestradiol
Epithelial ^a	Produce uteroferrin Stain for cytokeratin PGE:PGF < 1	inhibited	inhibited
Stromal	Stain for vimentin PGE:PGF > 1	inhibited	stimulated

^aFrom uterine glands.

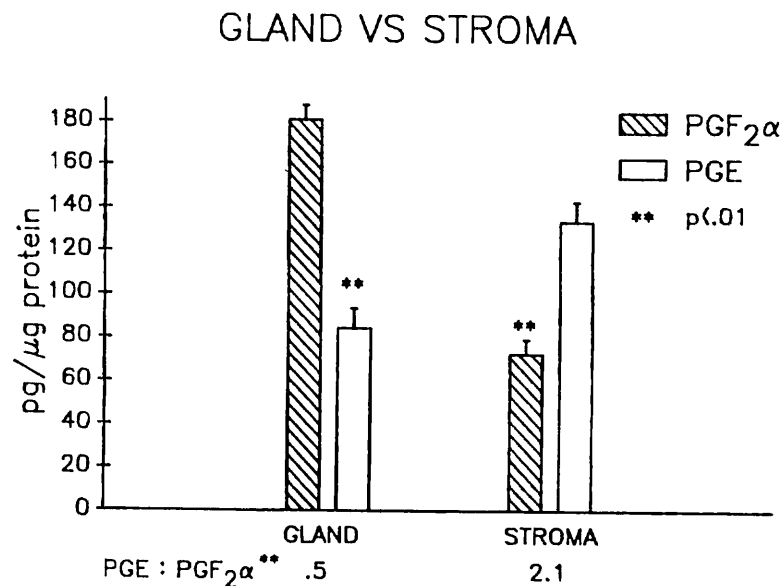


Figure 2. Prostaglandin E (PGE) and prostaglandin F₂α production by glandular and stromal cells in culture.