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C F. Rosenkrans

Duane L. Davis

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RESPONSES OF PIG EMBRYOS TO AMINO ACIDS

C.F. Rosenkrans, Jr. and D.L. Davis

Summary

Mixtures of vitamins and amino acids produced detrimental effects on the development of pig embryos. However, addition of three amino acids, phenylalanine, methionine, and isoleucine, enhanced pig embryo development.

(Key Words: Embryo Survival, Pregnancy, Amino Acids.)

Introduction

The first 2 wk of pregnancy are critical for pig embryos. After fertilization, the embryo grows slowly and its cells only divide about once a day. During the fifth day, the embryo is reshaped into a hollow ball of cells and its growth accelerates rapidly. Rapid growth would be expected to accelerate the embryo's utilization of nutrients, but essentially nothing is known of the embryo's nutritional needs. Although the embryo's nutrient demands could not be quantitatively great at this time, it may require specific nutrients in ratios not normally provided to the mother. It is also possible that young embryos are sensitive to excesses of certain nutrients and might be damaged or even killed by nutrient mixtures that are tolerated by the mother. For example, overfeeding in early pregnancy is believed to increase embryonic death. To study these questions, we have developed a system to culture pig embryos outside of the mother and are using that system to study the nutrient requirements of pig embryos.

Procedures

Embryos were collected surgically from anesthetized sows and gilts and cultured in .2 ml of culture medium and incubated at 101°F. Embryos from each female were assigned randomly to the treatments in the experiment. Embryos were observed and measured each day for 4 days, stained, and nuclei were counted using a phase contrast microscope.

Culture medium supplements were the vitamin/amino acid supplements for minimum essential medium and glutamine (1 mM), phenylalanine (.1 mM), isoleucine (.2 mM) and methionine (.05 mM). All media contained 10% (volume/volume) of heat inactivated fetal calf serum.

Results and Discussion

The vitamin and amino acid mixtures tested are used to culture other types of cells, but produced detrimental effects on the development of pig embryos. The results of testing the effects of the amino acids and vitamins in minimum essential medium (Table 1) are presented in Figure 1 (Experiment I). Pig embryos form a cavity and expand beginning on day 5 of pregnancy, and that expansion was not supported by the minimum essential medium supplements. These results indicate that pig embryos are particularly sensitive to the nutrient composition of their environment.

Next we tested the effects of amino acids that are known to affect hamster embryos. Results of one of those experiments are presented in Figure 1 (Experiment II). Addition of the three amino acids, phenylalanine, methionine, and isoleucine, enhanced pig embryo development, particularly on the third and fourth days of culture. Other experiments (data not shown) indicate that the beneficial effect is attributable to methionine supplementation. In other experiments, when serum was not included in the culture medium, glutamine improved development.

Interpretation

We are using in vitro culture of pig embryos to study the nutrient needs of the young embryos separate from the sow's requirements. We are also initiating studies of the effects of diet composition on the composition of the uterine secretions. Using those approaches, we hope to develop diets that improve embryo survival and avoid the negative effects of high feed intake on embryo survival and litter size.

Table 1. Minimum Essential Medium Supplements^a

Essential Amino Acids	mg/ml	Vitamins	mg/ml
L-Arginine HCL	.126	D-Ca Pantothenate	1.00
L-Cystine	.024	Choline Chloride	1.00
L-Histidine	.420	Folic Acid	1.00
L-Isoleucine	.525	i-Inositol	2.00
L-Leucine	.524	Nicotinamide	1.00
L-Methionine	.151	Pyridoxal HCL	2.00
L-Phenylalanine	.330	Riboflavin	.10
L-Threonine	.476	Thiamine HCL	1.00
L-Tryptophan	.102		
L-Tyrosine	.360		
L-Valine	.468		

^aGIBCO Laboratories, Grand Island, New York, 14072.

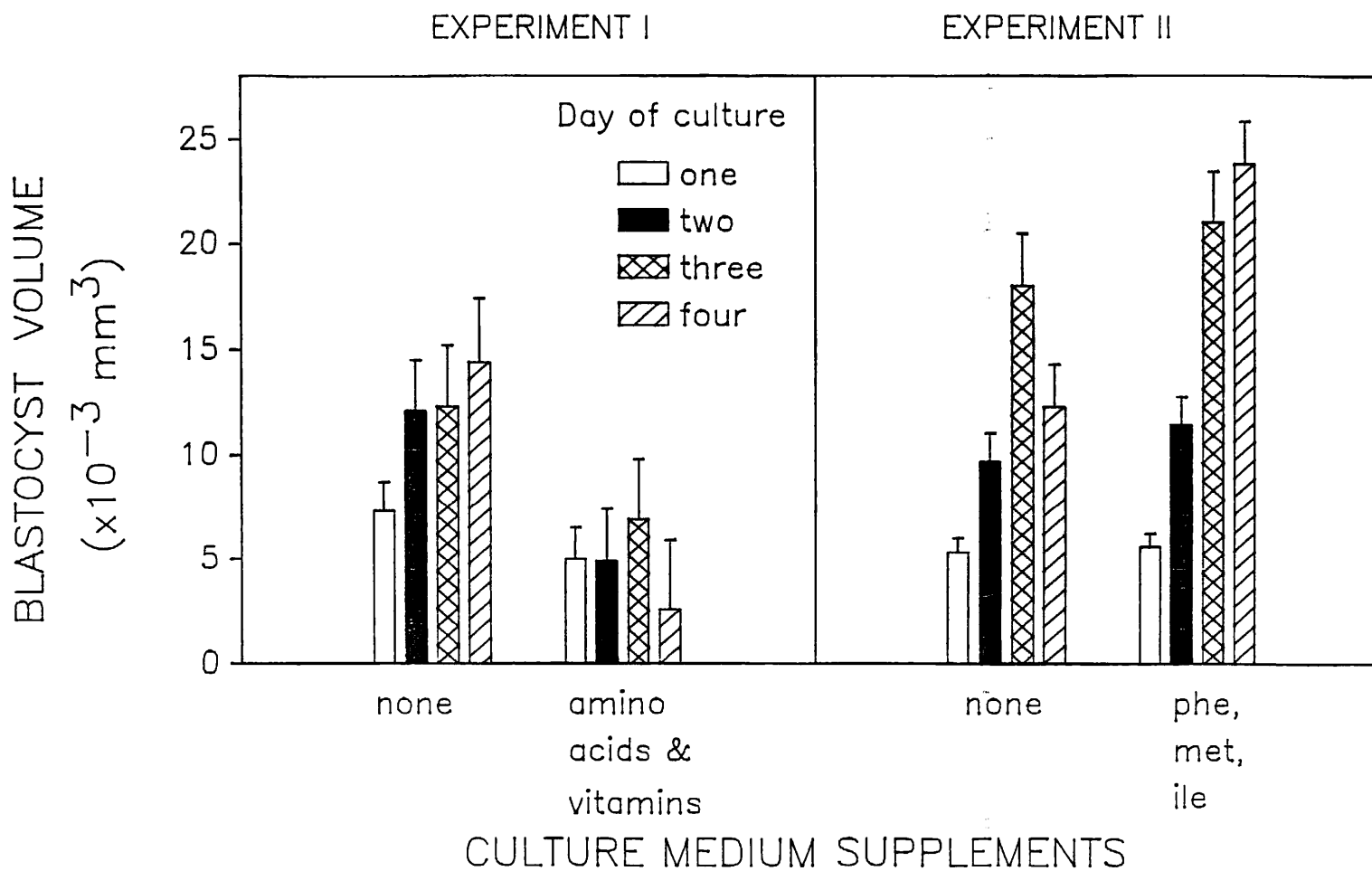


Figure 1. Development of day-5 pig embryos in culture. Minimal Essential Medium—amino acids and vitamins were used in Exp. I. Phe=phenylalanine, met=methionine, ile=isoleucine.