Preliminary Attempts to Terminate Pregnancy by Immunological Attack on Uterine Protein*

KRISHNAN[1] has recently reported that the administration of a chicken antiblastokinin serum to the rabbit during the preimplantation period of pregnancy results in either abnormal development of the young or complete cessation of the pregnancy. This paper describes some preliminary work with rabbit antiserum to swine uterine protein that, in part, substantiates KRISHNAN's observations.

Methods and materials. Pig uteri, obtained fresh from a local meat packing plant, were flushed with physiological saline solution; the flushings dialysed for 24 h against cold distilled water and then lyophilised to a dry powder according to the method of KRISHNAN and DANIEL[2]. The powder was dissolved in buffered saline solution and the protein content determined by use of the LOWRY procedure[3]. Sufficient saline solution was then mixed with the protein solution to give a final protein concentration of 1%. After filter sterilization the 1% protein solution was mixed with FREUND's adjuvant until a homogeneous suspension was produced having a final concentration of 4 mg of uterine protein per ml. 3 kinds of inoculations were made, one with proteins collected from sows whose ovaries contained corpora lutea (luteal phase protein), another with proteins from sows whose ovaries had developing follicles, but no corpora lutea (follicular phase protein) and the 3rd with pig serum proteins. To inoculate the rabbits 0.25 ml of the protein-adjuvant mixture was injected into the right foot pad; 3 weeks later a 2nd injection of 0.25 ml was given into the left foot pad and again 3 weeks later 0.25 ml of the protein solution along was given i.v. into the left ear vein. Blood samples were taken at the beginning and before each injection in the series and also one week after the last injection, to be used in comparison testing for the presence and progressive formation of antibodies. All bleeding was done from the marginal ear veins. Ouchterlony tests[4] were run on each rabbit using the original antigen against each of the 4 blood samples to establish the presence of antibodies.

All of the serum samples taken from the rabbits described above were pooled according to their type. Thus, one batch represented antisera against pig luteal phase uterine fluid protein, one batch was against follicular phase protein, and one batch was against porcine serum protein. These pooled antisera were then used to inject pigs in an effort to terminate or prolong their pregnancies.

9 Hormel miniature pigs[5] were bred for us at Colorado State University through the courtesy Dr. DONALD WILL. 3 of the animals were given the rabbit antiserum to luteal protein, 3 antiserum to follicular protein and 3 antiserum to pig serum proteins. Injections were made via the ear vein on the 5th, 7th and 9th days post coitum; with 5 ml

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Effect of rabbit serum on pig pregnancy

Animal number	Gestation period	No. young born	Of which were dead	Comments
Controls-antiserum to Freund's adjuvant plus pig serum				
1	113	12	2	
2	112	7	0	
3	113	8	0	1 had CNS problem and was euthanized
Experimental-antiserum to follicular phase pig uterine proteins				
1	122	7	0	
2	114	7	0	
3	115	8	0	
Experimental-antiserum to luteal phase pig uterine proteins				
1	–	–	–	Did not recycle until 173 days.
2	114	7	0	Possible loss of some of last inoculum from ruptured vein.
3	–	–	–	Had not recycled at time of writing which was 237 days.
Colony average as calculated from 100 untreated 'first time' gilts				
	113.4 ± 1.6[a]	7.25 ± 2.5[a]	0.049 ± 0.21[a]	The incidence of pregnancy failure is nil. Rarely an animal will require a repeat breeding at a second estrus.

[a] Standard deviation.

of antiserum being given on day 5, 10 ml on day 7 and 15 ml on day 9. These times were selected to coincide with the early stages of blastocyst development, which, we felt, were the stages most likely to be influenced by antisera to uterine protein. After the last injection the sows were retained in their respective pens and observed daily until about 110 days post coitum. At this time they were removed to the farrowing shed and kept until parturition had occurred, or it was obvious it was not going to occur. Number of young and lengths of gestation periods were recorded, as well as the time expired before the animal returned to estrus if she had not given birth.

Results. In the Ouchterlony tests for precipitating rabbit antibody against porcine uterine proteins, up to 8 bands can be detected against luteal phase protein whereas only 4 (at maximum) against follicular phase protein each with its corresponding antiserum. All share some serum antigens in common. Some of the bands appear with serum taken after 1 injection, others are not obvious until after 2 or 3 injections.

The results of the breeding experiments are shown in the Table. The 3 control animals had essentially normal litters within normal gestation periods, but the pregnancies of some members of the experimental classes were obviously influenced by the treatment. 2 of the 3 animals getting antiserum to luteal proteins did not give birth and did not recycle until 173 and 237 + days, respectively, after breeding. The 3rd animal had a normal pregnancy. Of the 3 animals getting antiserum to follicular protein 2 had normal gestation periods, but the 3rd one had a gestation period prolonged by 9 days. For comparison, it will be noted that in this herd, gestation does not normally vary by more than a few days from 113 days with an average of 7.2 piglets/litter.

Discussion. The one prolonged gestation period in a pig getting antiserum to follicular phase protein is highly unlikely by chance alone. The longest gestation period ever recorded in this herd was 116 days; 6 days less than this experimental animal. All of the young were of normal size at birth and one is therefore tempted to interpret this as a possible delayed implantation. Although not a common phenomenon in pigs, some rare cases of prolonged gestation or superfetation in other animals have been interpreted as explainable by delayed implantation (BURROWS[6]).

The complete failure of pregnancy in the 2 animals receiving luteal phase protein antiserum could happen by chance, but considering that pregnancy did not fail in any of the 100 animals used to establish the colony average, it seems to be an unplausible explanation. Even if fertilization had failed with the first service, the animals would normally have recycled in 21–30 days. One of these animals recycled after 173 days and the other one had not come into estrus again at the time of this writing which was 237 days. The swineherd at Colorado State University, a man of extensive experience, claims that these 2 animals showed typical signs of early pregnancy in that they had 'bellied down' (shown enlargement of abdominal region) about 3 weeks after breeding and then regressed slowly thereafter. Assuming the accuracy of this observation, one can only conclude that a resorption must have occurred at a time coincident with early post-implantation.

The failure of these animals to return to estrus after early termination of pregnancy leads one to suspect that they were retaining a progesterone domination. Hysterectomy of gilts after estrus has been shown to cause retention of the corpora lutea with subsequent production of progesterone for prolonged periods; typically 120–200 days elapse before the animals return to estrus, an observation commonly interpreted as a block to normal luteolysis[7,8]. Dr. F. BAZER of the University of Florida has suggested that the rabbit antisera used on the pigs in the experiments described here may contain antibodies against a luteolytic factor. Immunological blockage of this factor might cause the retention of the corpora lutea and account for the failure to recycle. This question will be examined[9].

Zusammenfassung. Der Einfluss von heterologen Antiseren gegen Uterusantigene auf die Schwangerschaft wird am Modell des Schweines und des Kaninchens studiert. In diesem Versuchstier werden Anhaltspunkte für eine beeinträchtigende Wirkung der Antiseren gefunden.

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[9] The author is grateful to Dr. DONALD WILL for providing the pigs used in this study and for their breeding and maintenance.

Pineal Gland Changes of Rats Exposed to Heat

Photic stimuli, i.e. continuous light and darkness, may act on the endocrine glands through the pineal gland since the removal of this organ abolishes the effect of environmental lighting on the endocrine system[1]. It has been suggested that the pineal gland might also mediate environmental influences other than light[2]. For example, exposure of rats to low temperature was found to bring about a decrease in weight of ovaries and uteri[3] and to produce hypertrophy and hyperplasia of the pineal gland[4]. The effects of cold on the female gonads, however, were not erased by pinealectomizing the animals[3]. High temperatures (34–36 °C), too, have been found to cause changes in rat endocrine function, these being reflected in weight reduction of the hormone excreting glands[5,6]. The total body weight of rats exposed to heat has also been found to be reduced[6]. No reports, however, on the effect of heat on the pineal gland have been found in the literature.

Materials and methods. Female rats of the Hebrew University 'Sabra' strain, 21 days old and weighing 36–45 g each, were divided into 2 groups: one being exposed to constant heat of 33 ± 1 °C and the other, which served as control, was kept in a room with controlled temperature of 23 ± 1 °C. The rats were housed 6 to a cage and fed ad libitum. Light was provided by overhead fluorescent tubes which were automatically switched on at 07.00 h and off at 19.00 h each day.