MAINTENANCE OF CORPORA LUTEA AND PREGNANCY IN UNILATERALLY PREGNANT GILTS BY INTRAUTERINE INFUSION OF EMBRYONIC TISSUE*

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(Received 7th September 1971, accepted 10th September 1971)

Summary. Pregnancy was not maintained by the infusion of saline or saline-soluble extracts of the freeze-dried porcine liver tissue into the sterile uterine horn of unilaterally pregnant gilts. Pregnancy rate at slaughter was 16·7% when the saline extract of the complete conceptus (Days 20 to 25) was infused into a restricted area of the sterile uterine horn. Increased pregnancy rates of 57·1% and 50·0%, respectively, were obtained when saline extracts of the complete conceptus (Days 20 to 25) and embryonic membranes (Days 35 to 40) were infused twice daily into the complete sterile uterine horn. The infusion of the saline extract of freeze-dried embryonic membranes into the uterus of non-pregnant gilts did not extend the life-span of the corpora lutea.

INTRODUCTION

It has been shown that placental extracts will inhibit the oestrous cycle in the rat (Ray, Averill, Lyons & Johnson, 1955) and mouse (Cerruti & Lyons, 1960). In the rabbit (Klein, 1938), guinea-pig (Macht, Stickets & Seckinger, 1929; Davies, Amoroso & Dempsey, 1959) and the hamster (Klein, 1938), placental extracts and the remaining placenta *in utero* have been reported to exhibit luteotrophic activity.

Repeated infusions of embryonic tissue homogenate into the uterus of the non-pregnant ewe have been shown to exert a luteotrophic effect. Rowson & Moor (1967) reported that homogenates from frozen sheep embryonic membranes collected on Days 14 and 15 of pregnancy prevented luteal regression when infused daily into the uteri of non-pregnant ewes. It was suggested that the substance derived from embryonic tissue probably acts on the endometrium in an anti-luteolytic manner.

The objective of this study was to determine if freeze-dried porcine embryos

* Contribution from Missouri Agricultural Experiment Station Journal Series No. 7163. Approved by the Director.

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and embryonic membranes exert a luteotrophic action when infused into the sterile uterine horn of unilaterally pregnant gilts.

MATERIALS AND METHODS

A total of forty-four Duroc, Hampshire, Poland China and crossbred gilts were used. Oestrus was checked daily with a sexually mature boar, and the first day on which a gilt was prepared to accept the boar was defined as Day 1 of the oestrous cycle or, if mated, of pregnancy.

Unilateral pregnancy was achieved by flushing one uterine tube with 20 ml of physiological saline 2 days after mating. The uterine tube adjacent to the ovary with the least number of ovulation points was occluded at the uterotubal junction by ligation and a cannula was inserted into the ampulla and tied in place. The flushings were collected in a glass bowl and an evaluation of the eggs was made with the aid of a dissecting microscope. Only those gilts having fertilized eggs were used in the experiment. The uterine horn adjacent to the flushed oviduct was occluded close to the uterine body by double ligatures with silk thread.

Freeze-dried embryos and embryonic membranes were introduced into the sterile uterine horn twice daily by infusion. This was accomplished by inserting and suturing a silicone rubber tube (outer diam. 2 mm) attached to a nylon insert with collar (outer diam. 2 mm, length 19 mm) into the uterine horn about 6 cm from the uterotubal junction. The tube was passed through the abdominal wall in the paralumbar spinal region, using a stainless steel trochar and cannula. The tubing was confined externally with surgical adhesive glue and tape.

The collection of embryos was made within 30 min following slaughter of donor gilts. Days-20 to -25 embryos, placental membranes and embryonic fluids were collected, stored at -20°C and later freeze-dried. Only placental membranes were collected at later stages of gestation. After freeze drying, the embryonic tissue was ground into a fine powder and the weight of the tissue collected from each pregnant animal was determined.

The amount of material required for each injection was weighed and added to paper packets, along with 1575 units penicillin and 2 mg dihydrostreptomycin. The packets were sterilized for a period of 5 to 10 hr with ethylene oxide gas and stored in a desiccator. At the time the material was to be used, the powder was placed in a sterile bottle, 5 ml of sterile saline was added and the mixture was allowed to settle for a period of 3 to 5 min. The supernatant of the sample was withdrawn from the bottle into a sterile syringe. For the control animals, only the antibiotics were added to the packets, which were subsequently handled as described above.

At the time of injection, the external surface of the infusion tube was thoroughly cleaned with 70% ethanol. A 20-gauge needle was inserted into the tube and attached to the syringe containing the infusion material. Following the infusion, the tube was tied anterior to each injection site to avoid contamination.

The forty-four gilts were assigned to seven treatment groups. Twice daily

intrauterine infusions were made, in unilaterally pregnant gilts, of saline (Group 1), or the saline-soluble extract of 200 mg freeze-dried liver tissue (Group 2), the complete conceptus collected on Days-20 to -25 (Groups 3 and 4) and Days-35 to -40 embryonic membranes only (Group 5). The saline-soluble extract of freeze-dried liver tissue (400 to 800 mg/day) and embryonic tissue collected on Days 26 to 40 of pregnancy (400 mg to 1.6 mg daily) were infused into the uterus of non-pregnant gilts, having an infusion tube located in each uterine horn. Four gilts received liver tissue (Group 6) and nine gilts were infused with embryonic material (Group 7).

The infusions were started on Day 9 of pregnancy, or of the oestrous cycle, and continued until the animals were slaughtered between Days 19 and 25. Corpora lutea, present on the ovaries at slaughter, were collected, stored in distilled water at -20°C and then analysed for progesterone by the procedure of Neill (1966) with thin-layer chromatography modifications as described by Armstrong, O'Brien & Greep (1964).

RESULTS

Complete pregnancy failure occurred in all gilts made unilaterally pregnant on Day 2 and infused with either saline or saline-soluble extracts of porcine liver tissue (Table 1). When the infused products of conception were restricted to the anterior 20 cm of the sterile uterine horn, only one of the six gilts remained pregnant. In this gilt, the CL adjacent to the pregnant uterine horn were maintained, but complete luteal regression had occurred on the contralateral ovary before slaughter.

In contrast to the results obtained in Groups 1, 2 and 3, 57.1% of the gilts were pregnant at slaughter when the saline extract of freeze-dried embryos and embryonic membranes was infused into the unrestricted sterile uterine horn (Group 4, Table 1). Three of the four pregnant gilts had CL present on both ovaries at autopsy, and three of six gilts were pregnant at slaughter, following the infusion of saline-soluble extract of 200 mg freeze-dried embryonic membranes (Group 5, Table 1).

The average weights of individual CL in gilts pregnant at slaughter are shown in Table 2. There was no apparent difference in the weight of the CL present on the ovary adjacent to the infused uterine horn and those adjacent to the gravid uterine horn.

The CL maintained unilaterally in the one gilt pregnant in Group 3 had a progesterone concentration of $50.3\text{ }\mu\text{g/g}$. In Group 4, the average content of progesterone per CL on the experimental side was 65% less than that of the CL adjacent to the pregnant uterine horn (Table 3).

Two of the three gilts that were pregnant in Group 5 had CL on both ovaries at the time of slaughter. However, one of these gilts had partial regression of the CL in the ovary adjacent to the infused uterine horn. There were six CL on the ovary at the time of laparotomy, but only two were present at the time of slaughter. Both average progesterone concentration and content of the CL were consistently lower when present in the ovary adjacent to the sterile uterine horn (Table 3).

TABLE 1

THE EFFECT OF FREEZE-DRIED PORCINE LIVER, EMBRYOS AND EMBRYONIC MEMBRANES INFUSED INTO THE STERILE UTERINE HORN OF UNILATERALLY PREGNANT GILTS ON SUBSEQUENT MAINTENANCE OF PREGNANCY

Group	Type of infusion	Amount of sterile horn infused	No. of gilts	Stage of pregnancy (days) Laparotomy	Slaughter	Pregnancy rate at slaughter (%)	Average no. of embryos	Pregnant gilts with bilateral maintenance of the CL (%)
1	Saline	Complete	6	3	20 to 24	0.0	0.0	0.0
2	Liver	Complete	6	3	19 to 20	0.0	0.0	0.0
3	Complete conceptus*	Partial†	6	3	21 to 25	16.7	7.0	0.0
4	Complete conceptus*	Complete	7	3	20 to 23	57.1	3.8	75.0
5	Embryonic membranes‡	Complete	6	3	19 to 25	50.0	5.7	66.7

* Tissue collected from donor gilts following slaughter on Days 20 to 25 of pregnancy.

† Infusion made into the isolated anterior 20 cm of the sterile uterine horn.

‡ Tissue collected from donor gilts following slaughter on Days 35 to 40 of pregnancy.

All of the unilaterally pregnant gilts that were non-pregnant at autopsy had either shown oestrus or signs of pro-oestrus before the time of slaughter.

The length of the oestrous cycle was considered to be normal in these gilts with the exception of one gilt in Group 3 which did not show oestrus. When slaughtered on Day 25, all the follicles were less than 5 mm in diameter. There was no explanation for this finding, since no embryonic remains were found in the intact uterine horn.

TABLE 2
AVERAGE WEIGHTS OF CORPORA LUTEA ON INTACT
AND EXPERIMENTAL OVARIES FOLLOWING INFUSION
INTO THE STERILE UTERINE HORN OF UNILATERALLY
PREGNANT GILTS

Group	No. of gilts	Av. weight of individual CL (g)	
		Intact side	Experimental side
3	1	0.506	—
4	2*	0.340	0.346
5	3*	0.371	0.323

* Includes only pregnant gilts with bilateral maintenance of CL.

TABLE 3
PROGESTERONE LEVELS IN CORPORA LUTEA FOLLOWING THE INFUSION INTO
THE STERILE UTERINE HORN OF UNILATERALLY PREGNANT GILTS

Group	No. of gilts*	Intact side		Experimental side	
		Concentration (µg/g)	Content/CL (µg)	Concentration (µg/g)	Content/CL (µg)
4	3	40.4	16.3	29.3	10.6
5	2	33.9	12.9	25.9	10.8

* Includes only pregnant gilts with bilateral maintenance of CL.

The results from two additional groups of gilts (Groups 6 and 7) included in this experiment are not shown in tabular form. Four gilts (Group 6) were infused with saline-soluble extracts of liver tissue and nine animals (Group 7) were infused with saline-soluble extracts of products of conception. Two to eight times more freeze-dried embryonic membranes were infused than in the unilaterally pregnant gilts. Regression of the CL was not affected in any of these gilts since all had normal oestrous cycles of 18 to 22 days in length.

DISCUSSION

In previous studies considered by du Mesnil du Buisson (1961), Anderson, Rathmacher & Melampy (1966), Niswender, Dziuk, Kaltenbach & Norton

(1970) and Longenecker & Day (1972) and in the present study, it has been found that the presence of a sterile uterine horn causes failure of pregnancy in the pig. It was also shown in the present study that when the products of conception were infused into a 20-cm isolated segment of the sterile uterine horn, the detrimental effects of unilateral pregnancy were not overcome. However, when the infused material had access to the entire sterile uterine horn, pregnancy rate was increased and, in some cases, the CL were maintained bilaterally. These results indicate that the freeze-dried products of conception contain an unknown factor that prevents failure of pregnancy in gilts with a sterile uterine horn. In unilaterally pregnant gilts, this factor must have access to the entire non-gravid uterine horn to exert its luteotrophic action.

The life-span of the CL was not extended in the non-pregnant gilt following the infusion of the products of conception. This contrasts with the results obtained in sheep since Rowson & Moor (1967) reported that the infusion of homogenates of frozen sheep embryos and embryonic membranes into non-pregnant sheep extended the life-span of the CL.

The present findings are interpreted to be in agreement with the conclusions of du Mesnil du Buisson (1961) and Anderson *et al.* (1966) that pregnancy failure in unilaterally pregnant gilts is due to the production of a luteolysin by the endometrium of the non-gravid uterine horn. Further consideration of the results of the present study suggests that the mechanism by which the living embryo and attached membranes negate a luteolytic effect of the uterus can be at least partially duplicated by twice daily intrauterine infusions of a saline-soluble extract of the complete conceptus or of the embryonic membranes.

Although the infusion of the saline-soluble extract of the freeze-dried conceptus was found to be a means of maintaining pregnancy in unilaterally pregnant gilts, maintenance of the CL adjacent to the sterile uterine horn was not achieved in all pregnant animals.

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