FERTILITY AND CORPUS LUTEUM CHARACTERISTICS IN PIGS WITH PLASTIC DEVICES IN THE UTERINE LUMEN

R. J. GERRITS, H. W. HAWK AND F. STORMSHAK

*Animal Husbandry Research Division, U.S. Department of Agriculture,
Beltsville, Maryland 20705, U.S.A.*

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Summary. Polyethylene spirals (IUDs) were inserted by laparotomy on the 1st day of oestrus into one or both uterine horns of cyclic gilts; sham-operated gilts served as controls. Immediate post-operative oestrous cycles averaged 20.0 days for twenty-seven gilts with IUDs and 19.8 days for fourteen controls. Eleven gilts with IUDs were mated at nineteen later oestrous periods; all returned to oestrus after mating, with cycles averaging 21.2 days.

Gilts were autopsied at 3, 8 to 10 or 14 days after mating. Ova were fertilized in IUD gilts and most embryos were still alive 8 to 10 days after mating. A high rate of embryonic mortality occurred between 8 and 14 days. Leucocytes were present in the uterine lumen of IUD gilts at 8 to 10 and 14 days after mating.

In twenty gilts killed 9 or 14 days after mating, weights of corpora lutea were significantly less in IUD gilts than in controls; luteal progesterone levels, however, did not differ significantly. In the IUD gilts there was no evidence that corpora lutea were smaller on the side of the IUD than on the contralateral side.

INTRODUCTION

Inert objects placed in the uterine lumen interfere with reproduction in all species in which their action has been studied. They inhibit sperm transport and ovum fertilization in sheep (Hawk, 1967) and interfere with survival of blastocysts and embryos in rats (Doyle & Margolis, 1964) and rabbits (Adams & Eckstein, 1964). They also affect ovarian function, apparently inhibiting ovulation in Indian water buffaloes (Buch, Shukla & Hawk, 1964), and interfering with development or maintenance of corpora lutea in sheep, cattle and guinea-pigs (Ginther, 1967).

This study was conducted to examine the effects of intra-uterine plastic devices on the following aspects of reproduction in the pig: oestrous cycle length, ovum fertilization and embryo survival, and corpus luteum weight and progesterone content. Inflammatory responses associated with the presence

of the devices were also examined. Part of the data has been reported briefly (Gerrits & Hawk, 1966).

MATERIALS AND METHODS

Intra-uterine devices were made from polyethylene plastic strips 75 cm long and 3 mm in diameter. The plastic strips were wrapped spirally around glass rods of appropriate diameter, fastened in place, immersed in boiling water for about 5 min and cooled in cold water, after which the plastic retained a cylindrical spiral shape. The finished spirals (IUDs) were each about 18 cm long and 2 cm in outside diameter, with about 1 cm between individual turns of the spiral.

The gilts were Duroc, Yorkshire and crossbreds about 8 months of age. Each gilt assigned to an experimental group had an oestrous cycle of 19 to 22 days' duration immediately preceding the insertion of spirals. Spirals were inserted into the uterus on the 1st day of oestrus (Day 0). Gilts were anaesthetized with pentobarbital injected into the anterior vena cava, laparotomized mid-ventrally and one or both uterine horns were punctured with a scissors' point. One to six plastic spirals were inserted into one or both horns of each gilt. All surgery was performed aseptically.

With the first three gilts, three spirals were inserted end-to-end into each uterine horn; this number of spirals was used in order to obtain irritation or stimulation of most of the length of both uterine horns for studying any effect on oestrous cycles. Oestrous cycle lengths were obtained for the cycle immediately after insertion and for the next three cycles of each gilt, during which time the gilts were not mated. After that, these gilts were each mated at each of the next three oestrous periods and then autopsied.

With each of twenty-four additional gilts, 26 to 28 cm of spiral was inserted into one uterine horn; one complete spiral plus one-half the length of another spiral were inserted end-to-end. Four of these twenty-four gilts expelled their IUDs at the first oestrus following insertion and were thereafter excluded from the study. The other gilts were mated at the second and any subsequent oestrous periods following insertion, then autopsied at an assigned day after mating.

Control gilts were laparotomized in the same way as IUD gilts. One or more plastic spirals were inserted into the uterine lumen and removed immediately.

Gilts were checked for oestrus daily by the use of vasectomized boars. Gilts to be bred were mated to fertile boars on the 1st day of oestrus and again the next day if the gilts were still in heat.

Gilts were autopsied at various days after mating. Some gilts were killed 3 days after mating to determine the incidence of ovum fertilization; the oviducts were removed and flushed with physiological saline solution, and ova were examined at a magnification of 200 by phase microscopy for cleavage and for the approximate number of spermatozoa in the zona pellucida. Other gilts were killed at 8 to 10 or at 14 days after mating to determine embryo survival rates at these intervals. The uterine horns were flushed with saline

solution and the number and condition of individual embryos were noted. At 14 days after mating, the embryos were not yet firmly attached to the endometrium.

Tissue specimens from each uterine horn of each gilt were fixed in Bouin's solution, sectioned at 9 μ , stained with haematoxylin and eosin and examined microscopically.

Corpora lutea (CL) from twenty gilts were analysed for progesterone. CL were excised or peeled from the ovarian stroma and all extraneous connective tissue was removed. The CL from each ovary were weighed to the nearest mg and analysed separately. Luteal tissue was stored in 95% ethanol at 10° C, then homogenized and extracted with 95% ethanol. Extracts were further purified for quantitative determination of progesterone as described by Stormshak, Inskeep, Lynn, Pope & Casida (1963), except that paper chromatograms were equilibrated overnight instead of 1 hr. [4-¹⁴C]Progesterone was added to each tissue sample extraction to correct for procedural losses of steroid. Data on CL weight and progesterone content and concentration were analysed statistically by analysis of covariance.

RESULTS AND DISCUSSION

Length of oestrous cycle

Plastic spirals were inserted into both uterine horns of three gilts and into one uterine horn of twenty-four gilts. Immediate post-operative oestrous cycles (from the day of surgery until the next oestrus) averaged 20.0 days for these twenty-seven gilts and 19.8 days for fourteen sham-operated controls (Table 1).

TABLE 1
EFFECT OF INTRA-UTERINE DEVICES ON MEAN
OESTROUS CYCLE LENGTH IN PIGS

Group	No. of pigs	Immediate post-operative cycle length* (days)	Subsequent cycle length after mating (days)
Control	14	19.8 $\pm$ 0.2†	—
IUD	27	20.0 $\pm$ 0.3	21.2‡ $\pm$ 0.6

* IUDs inserted on the first day of oestrus.

† Standard error.

‡ This figure is the mean of nineteen cycles of eleven gilts: nine cycles of three gilts with IUDs in each uterine horn and ten cycles of eight gilts with IUDs in one horn.

For the three gilts with IUDs in both horns, nine cycles subsequent to the immediate post-operative cycles averaged 20.2 days in length. There was thus no evidence that as many as six of these IUDs in one uterus could affect the length of the oestrous cycle. The lack of effect of intra-uterine devices on oestrous cycle length agrees with results obtained by Anderson (1962) after the insertion of a variety of objects into the pig uterus.

Gilts retaining their IUDs were mated at each oestrous period. Some of the gilts were autopsied at an assigned day of the cycle immediately after the first mating, but eight gilts with spirals in one uterine horn and the three gilts with spirals in both horns were mated at a total of nineteen oestrous periods and allowed to complete the following cycle. Each mating was infertile, with the infertile cycles averaging 21.2 days in length (Table 1). Four of the nineteen cycles were 25 to 27 days in length, accounting for the slightly greater mean length and greater variability of cycles after mating as compared to immediate post-insertion cycles. Each of the lengthened cycles occurred in gilts with IUDs in one horn only.

Ovum fertilization and embryo survival

At 3 days after mating, each of twelve ova recovered from one control gilt was cleaving normally (Table 2). Each of forty-nine ova recovered from five IUD gilts, including two of the gilts with IUDs in both horns, was also cleaving; none of fourteen ova recovered from one gilt was cleaving. Each cleaving ovum contained numerous spermatozoa in the zona pellucida. The uncleaved ova had no spermatozoa in the zonae.

TABLE 2
EFFECT OF INTRA-UTERINE DEVICES ON OVUM FERTILIZATION
AND EMBRYO SURVIVAL

	<i>Days after mating</i>		
	3	8 to 10	14
CONTROL GROUP			
Pregnant pigs/total pigs	1/1	5/7	6/6
Embryos/CL in pregnant pigs	12/12	67/67	55/80
IUD* GROUP			
Pregnant pigs/total pigs	5/6	8/10	4/7
Embryos/CL in pregnant pigs	49/63	78/93	14/56

* IUDs were present in both uterine horns in each of three gilts and one uterine horn in each of twenty gilts. Two gilts with spirals in both horns were autopsied at 3 days and one at 8 days.

At 8 to 10 days after mating, two of seven control gilts and two of ten IUD gilts were not pregnant. One of the non-pregnant IUD gilts was the last of the three gilts with spirals in both horns. The five pregnant control gilts had sixty-seven CL and sixty-seven embryos; the eight pregnant IUD gilts had ninety-three CL and seventy-eight embryos. Of the fifteen potential embryos that were missing from pregnant IUD gilts, fourteen were missing from horns containing IUDs and only one was missing from a horn not containing IUDs. A localized detrimental effect of IUDs on embryos was apparently being expressed at this time.

At 14 days after mating, each of six control gilts was pregnant and fifty-five of eighty CL (68%) were represented by embryos. Four of seven IUD gilts were pregnant, but only fourteen of fifty-six CL (25%) in the four pregnant gilts were represented by embryos. Only one of the fourteen embryos recovered

from IUD gilts was flushed from a uterine horn that contained IUDs. It appeared that embryos were able to survive somewhat longer in uterine horns contralateral to those containing IUDs than in the IUD horns themselves. However, the embryos present in the contralateral horns at 14 days would presumably have been lost shortly because the longest cycle length in any mated IUD gilt was 27 days. The comparative numbers of embryos present in uteri of IUD pigs at 8 and 14 days indicated that a high rate of embryonic loss occurred between these two periods.

In contrast to the complete failure of embryo survival in either uterine horn of these IUD pigs, Rathmacher, Anderson, Kawata & Melampy (1967) found living embryos in the uterine horn contralateral to the horn containing smooth plastic tubing in three pigs killed at 24 to 26 days after mating and in one pig killed at 55 days. The different results of the two experiments may have been due to the different types of intra-uterine devices used.

Corpora lutea

Corpora lutea from ten gilts with IUDs in one horn and from ten control gilts were weighed and analysed for progesterone. Five gilts of each group were killed at 9 days and five gilts at 14 days after mating (Table 3). Neither

TABLE 3

EFFECT OF PLASTIC SPIRALS IN ONE UTERINE HORN ON CL WEIGHT AND PROGESTERONE LEVELS IN GILTS AT TWO STAGES OF THE OESTROUS CYCLE

Item	Day 9		Day 14	
	Control gilts	IUD gilts	Control gilts	IUD gilts
No. gilts	5	5	5	5
Av. no. CL	15.4	13.9	13.8	13.8
Av. no. embryos	9.4	5.0	9.4	2.8
Av. CL wt (mg)*†	476 ± 34	408 ± 32	449 ± 32	346 ± 32
Av. progesterone content (µg)*	246 ± 49	256 ± 47	362 ± 47	268 ± 47
Av. progesterone concentration (µg/g)*	37 ± 7	44 ± 7	57 ± 7	52 ± 7

* Values adjusted by covariance to remove effects of variation in numbers of CL.

† Average CL weights were significantly lower in IUD gilts than in control gilts over both days ($P < 0.05$).

progesterone concentration nor content differed significantly between control and treated gilts. However, CL were significantly smaller ($P < 0.05$) in the ten IUD gilts than in the ten control gilts. Of the ten control gilts, nine had CL averaging more than 400 mg in weight; of ten IUD gilts, only four had CL averaging more than 400 mg. It appears that objects inserted into the uterine lumen of the pig can affect CL size without affecting oestrous cycle length.

Seven of the ten IUD gilts and eight of the ten controls used for progesterone assay were pregnant at autopsy (Table 3), but uteri of the IUD gilts contained fewer embryos than did uteri of controls, both at 9 and 14 days. The small number of embryos in IUD gilts as compared to controls could conceivably have been associated in some way with the smaller CL in IUD gilts.

Intra-uterine devices shorten the lifespan of CL in sheep, cattle and guinea-pigs. The effect tends to be unilateral, an IUD in one uterine horn inhibiting development or maintenance of CL in the adjacent ovary more frequently than in the opposite ovary (Ginther, 1967). Consequently, CL taken from pig ovaries adjacent to the IUD were weighed and analysed for progesterone separately from CL taken from the opposite ovary. The pigs were the same ones considered in Table 3. At the two intervals studied, Days 9 and 14 after mating, there was no evidence that IUDs in one uterine horn affected CL weight or progesterone content or concentration differently in the two ovaries (Table 4). However,

TABLE 4
EFFECT OF PLASTIC SPIRALS IN ONE UTERINE HORN OF GILTS ON CORPORA LUTEA IN THE ADJACENT AND OPPOSITE OVARIES AT TWO STAGES OF THE OESTROUS CYCLE

Item	Day 9		Day 14	
	CL opposite to IUD	CL adjacent to IUD	CL opposite to IUD	CL adjacent to IUD
No. gilts	5		5	
Av. no. CL	7.5	6.4	8.8	5.0
Av. no. embryos	4.2	0.8	2.4	0.4
Av. CL wt (mg)*	416 ± 19	409 ± 19	340 ± 22	358 ± 23
Av. progesterone content (µg)*	124 ± 36	123 ± 36	105 ± 43	156 ± 44
Av. progesterone concentration (µg/g)*	42 ± 11	41 ± 11	44 ± 13	50 ± 14

* Values adjusted by covariance to remove effects of variation in numbers of CL. Average CL weight differed significantly between days ($P < 0.05$). Other sources of variation were not statistically significant.

Rathmacher *et al.* (1967) reported a lower progesterone concentration in CL adjacent to an IUD than opposite to an IUD in one pig killed 16 days after mating.

Inflammatory responses

Cellular debris, composed largely of leucocytes, was always present in uterine flushings from IUD gilts killed at 8 to 10 days or 14 days after mating. The debris ranged in amount from 0.3 to 1.0 ml of packed sediment per uterine horn; it was greater in amount in 14-day gilts than in 8- to 10-day gilts and was greater in amount in uterine horns containing IUDs than in the opposite horns. Only trace amounts of debris were present in flushings from control gilts.

Microscopic observations for inflammatory responses were made in tissue specimens from several areas of each pig uterus. At 3 days after mating, polymorphonuclear neutrophilic leucocytes in considerable numbers were seen immediately beneath the basement membrane of the endometrial epithelium. The concentration of neutrophils was generally slightly greater in the area of IUDs than elsewhere. Although uterine tissue from unmated control pigs 3 days after oestrus was not available for comparison purposes, it is possible that the major part of this leucocytic infiltration at 3 days after mating was due neither

to the presence of IUDs nor to mating; the concentration of neutrophils in the endometrium of these 3-day gilts was similar to that seen in unmated oestrous gilts, both with and without IUDs, in another study made concurrently with this one.

At 8 to 10 or 14 days after mating, few if any neutrophils were found in the endometrium, even in tissue around the IUD, but eosinophils, often in high concentrations, were scattered throughout the stratum spongiosum. The presence of eosinophils was apparently normal for the mid- and late-luteal phases of the cycle because eosinophils were present in both uterine horns of all control and IUD gilts. Oedema was often seen histologically in the endometrium in the area of IUDs, and plasma cells were sometimes present in these areas.

The specific cause of IUD-induced embryonic mortality in these gilts is not known, but several possibilities can be noted. The physical presence of IUDs might prevent the movement and positioning of embryos. Possible depression of CL function by IUDs could, theoretically, be involved in embryonic mortality. However, changes in the uterine environment were more likely causes of embryonic death. Cellular debris, consisting mostly of both intact and disintegrating white blood cells, had begun to accumulate in the uterine lumen by 8 days after mating, at a time when most embryos were still alive. Changes in endometrial function probably accompanied the inflammatory response. Embryonic survival may have been impossible under such conditions.

A mild leucocytic response to the presence of foreign objects in the uterine lumen occurs in females of several species (e.g. Hawk, 1967), but the degree of leucocytic response in these gilts was greater than that usually seen. Whether bacterial infection elicited the response is not known, but the ejaculation by boars of large quantities of semen and gel directly into the uterus at mating may have provided an opportunity for infection to occur. The spiral IUDs may have trapped part of the ejaculate, which was undoubtedly contaminated with bacteria, and prevented its drainage from the uterus. Any subsequent development of infection could account for the leucocytic response and death of embryos in both uterine horns. It is perhaps relevant that cellular debris was not present in appreciable amounts around IUDs in unmated 14-day gilts in a concurrent study.

The anti-fertility effect of IUDs in the pigs of this study might be partially analogous to an experimental situation in rats, where a thread in the uterine lumen causes disappearance of blastocysts as they enter the uterus from the oviducts. Inflammation, associated with bacterial infection, has been implicated in the anti-fertility effect of the threads (Parr, Schaedler & Hirsch, 1967).

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