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Early Embryonic Environment and Early Embryonic Death in Dairy Cattle

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Early Embryonic Environment and Early Embryonic Death in Dairy Cattle.

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A - Abstract

Previous work has shown that the contrasting fertility of repeat breeders (RB) and dairy cows with normal fertility (NB) is due mainly to differences in the incidence of early embryonic death (EED), which becomes evident on day 7 post-breeding, when the morula is differentiating into the blastocyst. EED was accompanied by changes in the embryonic environment and uterine flushings had lowered total protein and higher ion concentrations in EED than when a normal embryo was found. The present investigation was meant to clarify the question: are the differences in EED, between RB and NB, due to factors operating before or after the embryo enters the uterus? The question was examined by transferring normal appearing embryos, collected on day 6 after breeding from RB or NB donors and transferring them to recipients furnishing the best uterine environment (virgin heifers - VH) or the worst uterine environment (RB).

In VH recipients, pregnancy rates, 6 weeks post-estrus were significantly higher than in RB recipients (15/39 - 38.5% vs 3/29 - 10.3%; $p < 0.02$). No significant differences were found within groups of recipients regarding the source of the donor embryos: for VH recipients, pregnancy rate was 9/21 (43%) from RB donors, and 6/18 (33%) from NB donors; for RB recipients, pregnancy rate was 1/15 (6%) from RB donors and 2/14 (14%) from NB donors.

Further evidence for the importance of the uterine environment for embryonic survival was found in comparing progesterone receptor (PR) concentrations in the endometrium. In inseminated cows, the presence of a normal embryo on day 6 or 7 post-breeding, was associated with lower levels of nuclear PR than when a degenerated embryo was found, both for RB and NB cows.

In non-inseminated cows, RB had significantly higher ($p < 0.02$) nuclear PR concentrations on day 6 post-estrus, than did NB cows, but not on day 7.

Electronmicroscopic studies on day 6 post-breeding revealed no significant differences in the ultrastructure of the endometrium, between the two types of cows, except for a smaller number of ciliated epithelial cells in RB cows. Also, the state of the embryo, normal or degenerated, was not reflected in differences in the ultrastructure of the luminal epithelium.

Uterine flushings of the cornua ipsilateral to the corpus luteum on day 6 post-breeding showed significant differences between concentrations of total protein, which were associated with the state of the embryo found: normal embryo - 5.48 mg% vs degenerated embryo - 2.91 mg% ($p < 0.02$). Also, differences in total protein concentrations were not significant in flushings containing a normal embryo as compared with flushings containing an unfertilized ovum (5.48 mg% vs 4.54 mg%) thus strengthening the impression that an abnormal embryo seems to interfere with normal levels of uterine proteins. Isoelectric focusing and electrophoresis in polyacrylamide gel of the uterine proteins failed to reveal significant differences connected with the type of cow or type of egg or embryo found.

Daily plasma progesterone concentrations were similar between the two types of cows and were not correlated with the presence of a normal or abnormal embryo.

Results of a preliminary field trial on the influence of supplementary progestin (intravaginal sponges, containing 250 mg. medroxy progesterone acetate, applied for the period 6-17 days post-insemination) - indicated that supplementary progestin may be of value in improving fertility (conception rate in treated cows: 18/11 (58%) vs 15/43 (35%); $p < 0.05$ in untreated controls).

It is concluded that: (1) there is clear evidence that the uterine environment is the major factor for embryonic survival at the transition period between morula and blastocyst stages. (2) The presence of an abnormal embryo may interfere with the attainment of normal levels of total proteins. (3) preliminary results suggest that supplementary progestin may be of value in improving fertility.

B. Scientific Report

B1. Introduction

In countries where the dairy cattle industry is well-developed, the widespread use of artificial insemination, together with eradication and preventive programs, have largely eliminated specific contagious disease causing infertility in cattle. This has exposed an economically-important group of cows which have difficulty in conceiving but which have no obvious pathology of the reproductive tract. These cows are known as "repeat-breeders" and constitute an important cause of economic loss to dairy farmers. Such animals return to estrus repeatedly after each insemination, at intervals between heats which are usually of normal duration. In parous animals, reports published to date (Hawk et al, 1955, Boyd et al, 1969 and Ayalon, 1972) have demonstrated that early embryonic death(EED) is the most important aspect of the problem. It has also been shown (Ayalon, 1978) that this EED is associated with significant differences in levels of some constituents of the embryonic environment (oviducal and uterine fluids) as compared with levels of these substances in the reproductive tract fluid accompanying normal embryos.

Attempts to diminish the severity of the repeat-breeder problem through selection have not been successful. Neither have hormonal treatments ameliorated losses due to this condition and this is not surprising since these treatments have been applied empirically, without knowledge of the type(s) and dosage(s) of the hormone(s) needed and without reference to the effect of such treatment on hormonal levels and on the composition of the reproductive tract fluid which nurtures the early embryo.

B₂-Scientific Background and Results of Work to Date

The fact that infertility in cows without clinical pathology (repeat-breeders) is a source of heavy economic losses in dairy cattle the world over, is too well-known to require emphasis. In Israel, with its high-producing, intensively-kept dairy cattle, an annual culling rate of 28% has been recorded (Annual Report - Hasherut, 1972), with infertility usually figuring as the chief cause of disposal. A recent survey of local dairy herds with low fertility showed an average incidence of 27% repeat-breeders (Francos et al, 1977). In Sweden an incidence of up to 20% repeat-breeders was found in herds having more than 20 cows (Hewett, 1968).

Despite the evident importance of the problem, surprisingly little research has been carried out on repeat-breeders (Ayalon, 1978). Published reports of these investigations have all emphasized the importance of early embryonic death (EED) as the chief characteristic of repeat-breeders (Tanabe, 1949; Hawk et al, 1959; Ayalon, 1972; Boyd et al, 1969; Ayalon, 1978; Linares, 1981; O'Farrel et al, 1983).

We have carried out previous research on this subject over a period of 7 years, with the aid of grants from the U. S. Dept. of Agriculture (P.L. 480, FG-IS-159 and 290). The investigation was based upon a comparison of various parameters, in repeat-breeders and cows with normal breeding histories. Data was accumulated on estrus duration, ovulation, ova recovery and fertilization rates, and EED incidence for different intervals after breeding. Basic information was also gathered on peripheral blood concentrations of progesterone and estradiol during the estrus cycle and after breeding. In addition, analyses of oviducal and uterine flushings were made and related to fertility findings after cows were slaughtered.

More than 550 cows were investigated and slaughtered during this study. Detailed results have been presented in the Annual Reports submitted within the framework of the PL 480 grants, and in a number of publications. A brief summary of the finds would include the following points:

- (1) No marked differences were found between repeat-breeders and normal cows with regard to estrus duration, ovulation time, and the incidence of anovulation and "silent" estrus.
- (2) Findings at slaughter showed no significant differences between the two types of cows regarding the following (repeat-breeders vs normal cows): oviducal ova recovery rates (81% vs 80%), fertilization rate (71% vs 83%), normal embryos at 4-5 days post-breeding (80% vs 88%).
- (3) Significant differences in fertility findings became apparent soon after the embryo enters the uterus, on day 7 post-breeding. There was no further increase in fertility losses in repeat-breeders from day 7 until at least 17-19 days post-breeding. In normal cows, major losses due to EED were not found until at least 16 days post-breeding. Table 1 summarises the findings (Ayalon, 1973; Ayalon, 1978).

Table 1: EARLY POST-BREEDING FERTILITY FINDINGS IN FRIESIAN COWS

Ova or Embryo Location	Time of slaughter (Days Post-Breeding)	Repeat Breeders		Normal cows	
		Normal embryos/ Cows slaughtered		Normal Embryos/ Cows slaughtered	
		No.	%	No.	%
Oviduct	2-3	12/17	71	10/12	83
	4-5	20/25	80	22/25	88
Uterus	6-7	25/44	57**	36/41	87**
	8-10	9/18	50	13/18	72
	11-13	9/18	50	16/18	89
	14-16	10/20	50	16/20	80
	17-19	9/21	43	12/21	57
	35-42	8/23	35	9/13	69

** = Differences at this period are highly significant ($P < 0.01$).

- (4) Analyses of oviducal and uterine flushings. These were carried out for the period 4-8 days post-breeding, with the following findings (PL 480, Annual Report No. 2, 1973, Ayalon, 1978):

- a) Ions - Calcium, zinc and potassium have been measured. Similar values were found in flushings from repeat-breeders and normal cows up to day 6 post-breeding, in parallel with the similar findings for fertility losses for the two types of cows up to then. On day 7 post-breeding, when fertility losses become significantly different, concentrations of calcium and zinc were also significantly different - reaching in the case of calcium for cows with degenerated embryos, an average concentration 12 times higher ($P < 0.001$) than that found in flushings from cows with normal embryos. Potassium and calcium ions are involved in the action of cyclic AMP (2). Calcium, in particular, is important in the regulation of many hormonal actions, and is a necessary ion for the action of cyclic AMP-dependent protein kinase (Robison et al, 1971).
- b) Total protein - Both oviducal and uterine flushings contained higher levels of total protein in normal cows, 4-7 days post-breeding, regardless of whether a normal or degenerated embryo was present. This would indicate a difference in the basic mechanism controlling the protein content of the genital secretion of repeat-breeders and normal cows. Preliminary studies of fractionation of the protein component of the endometrium indicate differences with regard to the pattern of peaks obtained with spectrophotometric evaluation of the fraction. It is worth noting that small amounts

of uterine-specific proteins have been found in the uterine fluid of cows both during the luteal phase of cycling cows and during early pregnancy (Roberts and Parker, 1974). Also a pregnancy-specific protein has been isolated from the endometrium of cows by no later than 15 days of pregnancy (Laster, 1977).

- (5) Cyclic AMP - With a view towards examining the possible role of cyclic AMP in changes occurring in the uterus of repeat-breeders and normal cows, biopsy samples of endometrium were collected during contrasting periods of the estrous cycle. Highest cyclic AMP values were found at the time of peak estradiol levels.
- (6) Peripheral Blood Hormone Levels - The patterns of levels of progesterone and estradiol during the estrous cycle of normal, cycling cows were established (Shemesh et al, 1971, Shemesh et al, 1972). Concerning hormone levels after breeding (PL 480 Annual Report No. 2), no significant differences in plasma progesterone levels were found, from day 0 (estrus) to day 7 post-breeding, between cows with normal or degenerated embryos. However, the importance of progesterone for EED cannot be dismissed since peripheral plasma concentrations may not reflect what is occurring in the uterus, and it has been shown, in the rat and in the rabbit, that pregnancy maintenance was more closely correlated with levels of uterine progesterone than with those of plasma progesterone (Csapo and West, 1969).

Results for plasma estradiol indicated different patterns of fluctuation during this period, between cows with normal or degenerated embryos. It has

been shown that the reproductive status of the female, or hormonal treatment can influence the concentration of ions in uterine flushings (Heap and Lanning, 1962) or in uterine secretions (Schultz et al, 1971). In mated animals, it has been found, in rats, that potassium levels in uterine fluid in early pregnancy increased following injections of high doses of progesterone (Clemetson et al, 1971).

The timing of peak losses due to EED occurs at the period when the morula is undergoing differentiation of cells, concomitant with the formation of the blastocyst. This period has also been found to be the time of severe embryonic loss in several other species: man, monkey, rabbit, rat and swine (Hertig, 1967).

No reports aside from ours have so far appeared concerning the cow with regard to daily post-breeding concentrations of ions and of protein analysis of the uterine fluid around the time of EED.

In summary, the results of investigation until now have shown that the major differences between repeat-breeders and normal cows were not connected with estrus, ovulation, or fertilization rate, but rather with EED, which became apparent at 7 days post-breeding. During the first week of post-breeding, repeat-breeders had plasma progesterone levels which were similar to those of normal cows, but a contrasting pattern of plasma estradiol was found. The embryonic environment also differed: repeat-breeders had lower total protein levels throughout the period 4-7 days post-breeding, whereas on the critical day 7, the concentration of calcium and zinc ions in oviducal and uterine flushings rose significantly in cows with degenerated embryos.

B₃ - Research Plan

The purpose of the present investigation was to extend the studies on the differences between repeat breeders and normal cows and to clarify the basic question whether EED occurs due to factors operating before the embryo has entered the uterus (lowered viability of the embryo due to genotype and/or environmental factors operating during the oviducal period) or after the embryo has entered the uterus (uterine environment). An additional aspect of the investigation was to carry out a preliminary trial (if it was established that EED was due to the uterine environment) on the influence of hormonal supplementation on EED.

Materials and Methods

1. Animals and Conditions

Repeat-breeders and cows with normal breeding histories were used. Normal cows were non-pregnant animals which had received not more than 2 inseminations since last calving and had conceived from not more than 2 inseminations during previous breeding periods. "Repeat breeders" were non-pregnant cows which had received 4 or more inseminations or else have had three inseminations and a negative pregnancy examination. The following criteria applied to both types of cows:

- 1 - History of normal calving
- 2-- Absence of clinically detectable endometritis or abnormal uterine discharge.
- 3 - Absence of other pathological changes in the uterus or ovaries
- 4 - Age less than 8 years
- 5 - Milk production of at least 10 liters daily

All animals were checked by a veterinarian from the Dept. of Reproduction before purchase.

The cows were brought in groups of 10 or 20 to the fertility clinic of the Veterinary Institute. Upon arrival they were given a 2-week environmental adjustment period, during which no examinations were made. Thereafter they were examined twice a day for estrus with the aid of a teaser bull or teaser cow. In order to equalize the male factor, all cattle in a given year were inseminated from a single ejaculate of frozen semen from a highly fertile bull. We have demonstrated in the past that changing the environment under such conditions does not significantly change fertility findings at slaughter as compared with cows slaughtered directly from their home herds (Final Research Report - Grant FG-IS-159, 1969, p. 24).

2. Embryo Collections and Transfers

Non-surgical embryo collections and transfers were carried out 6 days post-estrus. This day was chosen because there was no significant difference found in the incidence of normal embryos, between repeat breeders and normal cows, up to and including day 6 after breeding (Ayalon, 1973, Ayalon, 1978). Donors were either repeat breeders or normal cows. Recipients were chosen to present the best uterine environment (virgin heifers, who, in Israel, are highly fertile (Annual Report, Hasherut A. I. Center, 1972-74) or else the worst uterine environment (repeat breeders).

The techniques involved in embryo recovery and transfer were based upon our own experience (Ayalon et al, 1965, Ayalon et al, 1976) and that of others (Rowson et al, 1969, Elsdon et al, 1976). Cows intended for use as donors and recipients were no more than 12 hours apart in timing after estrus onset. The embryo transfer combinations entailed the following:

(1) repeat-breeders to virgin heifers, (2) normal cows to virgin heifers, (3) repeat-breeders to repeat breeders, and (4) normal cows to repeat breeders. Only normal appearing embryos were used, based upon the system of evaluation used by us previously (Ayalon 1977) as well as those described by Shea (1981) and Wright (1981). A total of 69 transfers was carried out.

B_{4I} Results - Results of these transfer trials and of other aspects of this investigation are presented below.

THE RELATIONSHIP BETWEEN UTERINE ENVIRONMENT AND EARLY EMBRYONIC MORTALITY (EEM) IN NORMAL (NB) AND REPEAT-BREEDER (RB) FRIESIAN COWS (Almeida et al, 1984)

I N T R O D U C T I O N

Embryonic mortality has been emphasized by several authors as one of the main causes of infertility in cattle. Laing (1949) and Tanabe & Casida (1949) demonstrated the occurrence of embryonic death in 30% of repeat-breeder cows (infertile cows with no apparent abnormalities). In the past two decades the importance of early embryonic mortality (eem) within the whole picture of cattle infertility has been pointed out by several reports, which have linked the problem with the repeat breeding syndrome (Boyd, 1969; Ayalon, 1972; Diskin & Sreenan, 1980; Linares, 1981). Repeat-breeder cows constitute a serious cause of economic loss to dairy farmers (Pelissier, 1976). Previous studies have demonstrated that these cows had normal estrous cycles and fertilization rates and that the incidence of abnormal embryos was not significantly different between NB and RB cows up to, and including, day 6 post-breeding. In these studies the "infertility" was shown to be due to very early embryonic mortality which, in Israeli Friesian cattle, became evident on day 7 post-breeding (Ayalon, 1972; Ayalon, 1978). Findings of eem in RB cows has been cited elsewhere (Hawk et al., 1963; Boyd, 1969; Linares, 1981). On the same day 7, when differences between the percentage of normal

embryos found either in NB or RB cows became significant. Ayalon (1978) also reported significant changes in ionic contents of uterine flushings depending on whether a normal or abnormal embryo was found. These results suggested the importance of the uterine fluids on the normal morula-blastocyst development, as also reported elsewhere (Newcomb & Rowson, 1980).

In the present study, the purpose was to test whether the uterine environment is indeed responsible for eem in RB cows on day 7 after insemination. This was tested by embryo transfers carried out with normal embryos on day 6, to recipients with the potentially best uterine environment (mature, virgin heifers) or with the worst uterine environment (RB).

MATERIALS AND METHODS

Repeat-breeder cows were defined as non-pregnant females which have received four or more inseminations the previous years and at least three inseminations this year with a negative pregnancy examination. NB cows were defined as having not received more than one insemination this year and have conceived the previous years from no more than two insemination. Both types of cows had a history of normal calving, absence of clinically detectable changes in uterus and ovaries, aged less than 8 years and had a milk production of at least 10 liters daily.

Two embryo transfer trials were carried out. In trial I, estrus was synchronized in both RB and NB donors in recipient heifers using progestagen-impregnated (250mg Medroxy-Progesterone Acetate) intravaginal sponges for 7 days with an injection of 500 µg of cloprostenol on the day of removal. Donors were inseminated at standing-heat and had their uteri flushed either in vivo or after slaughtering on the morning of the 6th day after estrus for embryo

recovery. Embryos morphologically classified as excellent (Ayalon, 1972; Linares & King, 1981; Shea, 1981; Wright, 1981) were non-surgically transferred to recipient heifers (to the uterine horn ipsilateral to the corpus luteum - Schneider & Hahn, 1979). Recipients were in estrus within 12 hours before or after estrus onset in the donors (Wright, 1981). The same treatment conditions applied in trial II, in which recipients were RB cows and donors were either NB or RB cows. Results were based upon pregnancy rates 6 weeks post-estrus. Data were analysed for significance using the Chi-square test.

R E S U L T S

Results are shown in Table I. In trial I, thirty-nine embryos were transferred from either RB or NB donors to recipient heifers. The overall pregnancy rate was 38.5% (15/39). The difference in pregnancy rates of heifers which received an embryo from RB (42.9% = 9/21) or from NB (33.3% = 6/18) donors was not significant. In trial II, two of the 14 RB recipients which received an embryo from NB donors became pregnant (14.3%), compared with one of the 15 RB recipients (6.6%) which received an embryo from RB donors (no significant differences). The differences in pregnancy rates between trials as well as the differences found when an embryo from RB donors were transferred either to heifers or to RB recipients, were significant ($p < 0.02$ and $p < 0.05$ respectively).

TABLE I - Results of trials I and II of non-surgical embryo transfers
6 days post-estrus.

Trial	Donors	Recipients	No. of embryos transferred	Pregnancy Rate	
					<u>TOTALS</u>
I	NB	HEIFERS	18	33.3% (6/18)	
	RB	HEIFERS	21	*42.9% (9/21)	38.5% (15/19) (a)
II	NB	RB	14	14.3% (2/14)	10.3%
	RB	RB	15	* 6.6% (1/15)	(3/29) (a)

*p < 0.05

(a) differences between trials are significant (p < 0.02).

DISCUSSION

The overall pregnancy rate obtained after transferring 6 day embryos from either RB or NB donors to recipient heifers (38.5%) is in agreement with other authors who found a lowered pregnancy rate when 5 to 6-day embryos compared to 7 to 8-day embryos were transferred (Tervit et al., 1980; Schneider et al., 1980; Newcomb & Rowson, 1980; Wright, 1981). Embryos in both trials were recovered from donors on day 6 post-estrus, when the uterine environment and the incidence of normal embryos are similar for both RB and NB cows (Ayalon, 1978). Virgin heifers and RB cows were chosen as recipients because it was expected that the very high fertility rates found in virgin heifers in Israeli Friesians and the low fertility rates of RB cows, would be reflected in the uterine environment and in the pregnancy rates of these recipients following embryo transfer. The results of these trials show that the new uterine environment of the transferred embryos was of greater importance than the origin

of the embryos. Heifer recipients had significantly higher pregnancy rates ($p < 0.02$) than RB recipient. It was also found in this study that for RB donors, embryos transferred on day 6 to virgin heifers had as good pregnancy rates as from NB donors and significantly higher possibility of pregnancy than embryos transferred to RB recipients ($p < 0.05$).

Further studies are needed to understand the relationship between embryo and uterine environment at early pregnancy as well as the mechanisms by which changes in this environment cause the embryo death.

A C K N O W L E D G E M E N T S

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R E F E R E N C E S

- AYALON N (1972) - Proc. VII Intl. Cong. Anim. Reprod. Artif. Insem.,
Munich, I. pp. 741-4.
- AYALON N (1978) - J. Reprod. Fert. 54, 483-93.
- BOYD H (1969) - Bri. Vet. J. 125, 87-97.
- CASIDA LE (1961) - J. Dai. Sci. 44, 2323-9.
- DISKIN M, SREENAN JM (1980) - J. Reprod. Fert. 59, 463-8.
- HAWK, HW, BRINSFIELD TH, TURNER, GD, WHITMORE, GE, NORCROSS MA (1963) -
J. Dai. Sci. 46, 1397-1401.
- LAING JA (1949) - J. Comp. Pathol. Therap. 59, 98-108.
- LINARES T (1981) - Theriogenology 15, 128 abstr.
- LINARES T, KING WA (1980) - Theriogenology 14, 123-33.
- NEWCOMB R, ROWSON LEA (1980) - Theriogenology 13, 41-9.
- PELISSIER CL (1976) - Theriogenology 6, 575-83.

SCHNEIDER HJ, CASTLEBERRY RS, GRIFFIN JL (1980) - Theriogenology 13, 73-85.
SCHNEIDER, U, HAHN J (1979) - Theriogenology 11, 63-80.
SHEA BF (1981) - Theriogenology 15, 31-42.
TANABE TY, CASIDA LE (1949) - J. Dai. Sci. 32, 237-246.
TERVIT HR, COOPER, MW, GOOLD PG, HASZARD GM (1980) - Theriogenology 13, 63-71.
WRIGHT JM (1981) - Theriogenology 15, 43-56.

B₄E Laboratory studies

A number of laboratory studies were carried out during the course of the investigation and they are discussed below:

a) Influence of Water Quality on in vitro Embryo Development

In the early stage of testing the influence of uterine environment on embryonic survival, ten morulae were transferred to heifers whose heats were synchronized with those of the donors, within a range of ± 24 hours. Only two of these recipients became pregnant (20%). Although there have been a number of reports of poor success following non-surgical embryo transfer, with results ranging from 18%-31% (2-5) - still there are several reports of better results in recent years, with pregnancy rates of 40-47% (6-8), and even one report of 63% pregnancies after transfer (9). Since the technique employed by us in the transfer was carried out with no difficulty whatsoever, using the equipment employed by the most successful transfer group - a search was made for other possible reasons for the poor rate of success. Our media was the routinely used Dulbecco's Modified Phosphate Buffered Saline (PBS) (10). Since all solid materials used in preparing the media were from fresh bottles of Analar or Aristar purity, it was decided to examine the quality of the water used to dissolve the ingredients of the flushing media. The distilled water used was deionized at the start and then triple-distilled over glass.

The influence of distilled water of various degrees of purity was examined, using the same chemical ingredients in the PBS formula. The influence of these solutions on embryo survival was tested by observing the development of mouse morulae to the blastocyst stage after incubation for 24 and 48 hours at 37°C with saturated humidity and a gas mixture of 5% CO₂ in air.

Table 1 shows the incidence of development of morulae to blastocysts in two trials, totaling 361 mouse morulae. Since no significant differences occurred between trials or the groups with the same type of water, the results were pooled for the two trials.

Table 1: The influence of quality of water on in vitro embryonic development of mouse morulae in phosphate-buffered saline (PBS) medium.

Type of water	No. of morulae	Development to blastocyst stage after 48 hr.	
		No.	%
1. Deionized	93	63	67.7%
2. Deionized and Distilled over Glass-3X	88	58	65.9%
3. Tap Water- Distilled over Glass-5X	100	84	84.0%
4. Commercial PBS Medium for Bovine Ova	80	65	81.2%

Several additional treatment groups await completion, but the differences between the present groups were highly significant ($P < 0.005$).

A search of the literature revealed that observations on the influence of the purity of the distilled water used in the culture medium on embryo development had been made before. Whittingham (11) starting with two-celled mouse ova, found that when single glass-distilled water was used as the solvent in the culture medium the incidence of development to the blastocyst stage was zero;

when double glass-distilled water was used 36% developed to the blastocyst stage and when triple glass-distilled water was used 92% developed to this stage.

The importance of the quality of distilled water in media has never been noted in literature dealing with embryo transfer in cattle. Indeed one publication (12) states specifically that ingredients for the PBS should be dissolved in deionized distilled water. While it is not clear how the deionization step interfered with embryo survival, it is clear that the quality of distilled water used for media must be considered for embryo transfer work.

b) Estrogen production by 6-day morulae

To examine the possibility of estrogen production by morulae, 7 trials were carried out with a total of 142 morulae from superovulated cows. Normal morulae (usually 3-5, and in one trial 8 embryos) were incubated in TCM 199 supplemented with 10% calf serum and 100 μ l of gentomycin per ml. For comparison, tubes containing 3-4 degenerated morulae were included in each trial. The incubator was maintained at 37°C and continuously flushed with 5% CO₂ in air. The capacity of the morulae to synthesize estrogen was tested by adding 30 ng of testosterone (T) to the incubated morulae and the amount of estrogen produced was compared with control normal morulae, incubated without T. Estrogen measurement in the media was by radioimmunoassay.

Results: The amount of estrogen found in the media with control normal morulae was similar to that found in the media of the treated groups (2 pg E₂/morula). However, there was a slight elevation of estrogen produced in the presence of T (from 2 to 3 pg E₂/morula). It is difficult to interpret this elevation as a net synthesis since it would be expected to find relatively large quantities of estrogen in the presence of large amounts of T in the medium if the

aromatase enzyme is present in morulae at this stage. Larger numbers of morulae per tube would clarify this question considerably. In the media from degenerated morulae, the amount of estrogen was completely undetectable ($<1\text{pg}/\text{morula}$).

c) Electronmicroscopy of the endometrium

Due to personnel changes, our American counterpart could not carry out the planned electronmicroscopy studies. This was carried out by Dr. Almeida of our staff. Details of his findings are presented below.

ELECTRON MICROSCOPIC FINDINGS IN REPEAT-BREEDER AND NORMAL COWS ON DAY 6 POST-BREEDING.

I N T R O D U C T I O N

The normal interaction between the uterine environment and the developing embryo within the uterus is essential for the establishment of pregnancy. This fact is particularly noticed in the bovine, whose blastocysts have a relatively late process of implantation (King, Atkinson & Robertson, 1981). In repeat-breeder cows, the embryo-endometrium relationship is apparently disturbed. Ayalon (1978) demonstrated that infertility in repeat-breeders is due to a very early embryonic mortality, which becomes evident on days 6-7 post-breeding. Changes in the uterine environment appear to be related to the embryo death in repeat-breeder cows (Ayalon, 1978). In the present study, endometrial epithelium of cows 6 days post-breeding has been examined in both scanning (SEM) and transmission electron microscopy (TEM) in order to establish the extent of correlation between our microscopic findings and the type of cow, whether a repeat-breeder or a cow with normal fertility.

MATERIALS AND METHODS

The definition of repeat-breeder (RB) and normal-breeding (NB) cows is given in our paper "The Relationship Between Uterine Environment and Early Embryonic Mortality in NB and RB Friesian Cows", in these proceedings.

SEM Observations

Two areas were clearly defined on the endometrial surface, the caruncular and the intercaruncular areas, which differed only by the absence of gland openings in the former. Glandular openings were round or slightly elliptical apertures (27 μm in diameter), randomly distributed on the epithelial surface (Fig. 1). Secretory vesicles and cellular detritus sometimes protruded from those apertures. Ciliated and secretory cells constitute the endometrial epithelium. Ciliated cells were fewer in number and appeared to concentrate around gland openings, in an area of about 50 μm around the center of the opening (Fig. 1). The average number of ciliated cells per square millimeter in the luminal epithelium of RB cows was lower than of NB cows (23.8 ± 17.2 and 41.8 ± 20.9 , respectively), but these differences were not highly significant ($p < 0.10$). Secretory cells were columnar in shape and their average cross-section diameter was 3.0 μm (Fig. 2). They had typical microvilli on their apical surface (Fig. 2). There was no difference between RB and NB cows as far as secretory cells were concerned.

TEM observations

Both luminal and glandular cells had the same basic structure. They were columnar in shape and rested in a basement membrane. Adjacent cells were tightly packed and desmosomes were observed (Fig. 3). In the apical part of all epithelial cells, a net of tight junction zone ($0.4 \pm 0.1 \mu\text{m}$ in depth) was present (Figs. 3,4). It followed the cell cross-section shape with a width of $0.12 \pm 0.03 \mu\text{m}$ adjacent to each cell, and were located $8.8 \pm 0.5 \mu\text{m}$ of the basement membrane. Anterior to the tight junctions, there were microvilli. The tight junction zones were located at the same plane of the basal bodies of

the ciliated cells (Fig. 4). Both types of cells had basal nuclei with the heterochromatin usually attached to the nuclear envelope. A rough-endoplasmic reticulum was present in their apical two-thirds (Figs. 3,4).

The cytoplasm of the secretory cells were more electron-dense than ciliated cells. They had typical apical microvilli, large supra-nuclear mitochondria and few Golgi apparati, usually related to smooth-surfaced vesicular endoplasmic reticulum and to free ribosomes (Fig. 3). There were large amounts of smooth surfaced vesicles and few glycogen granules and lipid droplets in the cytoplasm (Fig. 3). Ciliated cells had typical 9:1 kinocilia (Fig. 4). Their mitochondria were long-filamentous and electron dense (Fig. 4). There was no difference between RB and NB with regards to the TEM observations.

D I S C U S S I O N

The basic endometrial structures described in this paper have been reported before during the normal reproductive cycles of several species. However, we have observed few details which are not cited in the literature. The tight junction zone, common to all endometrial epithelial cells has been considered to function as a barrier for the intercellular space. Our findings that it surrounds completely the cell and that the basal bodies in ciliated cells are located at the same plane, suggest that they may also function as an anterior support for the columnar epithelial cells and for the ciliary movement. The different types of mitochondria described are probably related to different energetic needs of each cell type. It is difficult to explain our findings of a slight decrease in the number of ciliated cells in the luminal epithelium of RB cows. Ciliogenesis is known to be under strict hormonal control

(Ferenczy & Richart, 1973; Tachi et al., 1974). It is possible that some hormonal imbalance in RB cows may cause this decrease.

In summary, this study has not shown any major difference between RB and NB in the ultrastructure of the endometrium on D 6 post-breeding. This agrees with previous findings on the incidence of normal embryos in both types of cows on Day 6.

A C K N O W L E D G E M E N T S

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R E F E R E N C E S

- AYALON N (1978) - J. Reprod. Fertil. 54, 483.
BARTOOV B., ELTES F, WEISSENBERG, R, LUNENFELD B (1980) - Arch. Antrol. 5, 305.
FERENCZY A, RICHARD RM (1973) - J. Clin. Endocrinol. Metabol. 36, 999.
FISHER J, BARTOOV B (1980) - Arch. Androl. 4, 157.
KING GJ, ATKINSON BA, ROBERTSON HA (1981) - J. Reprod. Fert. 61, 469.
TACHI S, TACHI C, LINDNER HR (1974) - Biol. Reprod. 10, 391.

FIGURES

Figs. 1 and 2 = SEM of endometrium; glandular opening (fig. 1, x660) and secretory and ciliated cells (fig. 2, x6000) are seen.

Figs. 3 and 4 = TEM of endometrium. Apical region of secretory (fig. 3, x10000) and ciliated (fig. 4, x6600) cells. TJ = tight junctions, longitudinal (l) and sagittal (s) cross sections.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

d) Relationship between protein levels in uterine flushings and the presence of normal embryos in dairy cattle

This was examined in a total of 41 RB and NB cows. Significant differences were not found between the two types of cows, but differences in concentrations of total proteins were significant when comparing flushings from cows with normal embryos as compared with flushings accompanied by an abnormal (degenerated) embryo. The details are presented below.

THE RELATIONSHIP BETWEEN PROTEIN LEVELS IN UTERINE FLUSHINGS AND THE PRESENCE OF NORMAL EMBRYOS IN DAIRY CATTLE.

INTRODUCTION

Uterine secretions are of major importance for normal reproductive processes. Since the implantation processes are relatively slow in the bovine (King, Atkinson & Robertson, 1981) uterine fluids must provide the embryo its needs for normal early development. Proteins in uterine secretions and their possible roles on the establishment of normal pregnancy have been extensively studied in several species. Special attention has been given to uteroglobin, which constitutes the major component of protein contents in pregnant rabbit uteri (Krishnan & Daniel, 1967; Beier, 1968; Daniel, 1968; Beier, 1982). Uteroglobin accounts for 50-70% of the total protein contents in uterine fluids at pre-implantation stages and its release is controlled by progesterone (Beier, 1968). It is known to bind progesterone (Beato & Baier, 1975; Kulangara, 1976) and appears to function as steroid-carriers between endometrium and blastocysts. A major uterine-specific protein has also been described in the pig (Murray et al., 1972). Total protein contents in bovine uterine fluids have been shown to vary in the estrous cycle (Schultz, Fahning & Graham, 1971; Mills et al., 1973; Bartol et al., 1979; Bartol et al., 1981).

A few reports have described uterine-specific-proteins in bovine, as minor components of total proteins in uterine secretions (Roberts & Parker, 1974a; Laster, 1977). No steroid-binding activity has been detected in those proteins (Roberts & Parker, 1974b). Laster (1977) reported the presence of a pregnancy-specific protein in uterus of D 15 pregnant cows, which constituted 1% or less of the total protein in uterine fluids. Indications of uterine-specific proteins in pregnant cows were also reported elsewhere (Roberts, 1977; Bartol et al., 1979).

There is no report in the literature dealing with the relationship between uterine protein contents at earlier stages of pregnancy and the developing embryo in cattle. Ayalon (1978) reported significant changes in uterine flushings on day 6-7 post-breeding which appeared to be related to early embryonic mortality. In the present study, an analysis of proteins in uterine flushings from cows on day 6 post-breeding, as well as their relationship to the condition of the embryo within the uterus are described.

MATERIALS AND METHODS

Forty-one cows were used in this study. Estrus was synchronized using progesterone-impregnated (250 mg. Medroxy Progesterone Acetate) intravaginal sponges for 7 days with an injection of 500 ug of cloprostenol at sponge-withdrawal. They were inseminated at standing-heat and slaughtered on the morning of the 6th day after insemination. Uteri were removed and placed on ice until arrival at the laboratory (30-50 minutes after slaughtering). Both ipsi- and contralateral uterine horns were flushed for embryo recovery. Total proteins (Lowry et al, 1951) and DNA (Burton, 1951) were measured in 5 consecutive flushings (20 ml. each). Protein contents from the first flushings (first 20 ml) were pooled according to the different groups for this study and con-

centrated in dialysis tubes (4°C), to a concentration of 1.5-2.0 mg of proteins/ml. Isoelectrofocusing (Awdeh et al., 1968; McGaughey & Murray, 1972) and electrophoresis in polyacrylamide gel 5.5% (Avtalion, Personal Comm.) were then carried out. Data were analysed for significance using the Student's t test.

RESULTS AND DISCUSSION

Results were grouped according to the condition of the ovum present in the uterus, i.e., normal or abnormal embryos and unfertilized ova. The major amounts of proteins were recovered in the first flushings (first 20 ml). Although small amounts of proteins were present in subsequent flushings, they did not increase significantly the figures obtained in the first flushings. Therefore, results of protein contents in the first flushings were used for further analysis. The possibility that our flushings were damaging the endometrial tissue was tested by measuring DNA contents in each flushing. DNA values varied in a range of 14-22 μ /ml. There was no significant difference in DNA contents among the different flushings. Electronic microscopy further confirmed that no major damage was caused to the endometrium (Almeida, Ayalon & Bartoov, in press).

Results of total protein contents in uterine flushings are shown in Table I. Mean total protein in uterine flushings from the ipsilateral uterine horn was significantly lower ($p < 0.02$) when abnormal embryos were present as compared to the normal embryo group (2.91 ± 0.61 mg% $\pm$ sem vs 5.48 ± 0.67). Mean total protein concentrations in the unfertilized ovum group was 4.54 ± 1.12 mg% $\pm$ sem and these are intermediate values between normal and abnormal embryo groups (no significant differences). In the contralateral uterine horn, protein

contents were significantly higher ($p < 0.05$) than in the ipsilateral horn when abnormal embryos were found ($8.08 \pm 2.22 \text{ mg\%} \pm \text{sem}$ vs 2.91 ± 0.61). There was no significant difference between groups in the contralateral uterine horns.

TABLE I - Mean Total Protein Contents in Uterine Flushings
(first 20 ml) mg % $\pm$ sem (No. of cows).

Uterine horn	Condition of the ovum		
	Normal Embryos	Abnormal Embryos	Unfertilized Ova
Ipsilateral to the C. L.	5.48 ± 0.67 (a) (17)	2.91 ± 0.61 (a) (13) (b)	4.54 ± 1.12 (11)
Contralateral to the C. L.	6.08 ± 1.37 (14)	8.08 ± 2.22 (b) (13)	5.93 ± 1.11 (10)

(a) $p < 0.02$

(b) $p < 0.05$

There was no difference in isoelectrofocusing patterns (pH 3.5-9.5) either between uterine horns or related to the condition of the ova. Thirty-seven protein bands were present in uterine flushings. One of them (pI 8.1) was uterine-specific, regardless of the condition of the ova. Preliminary observations of the PAG-electrophoretic (pH 8.3) patterns showed no clear differences related either to the uterine horn or to the condition of the embryo. The general patterns of results showed no differences between repeat-breeders or normal cows.

These findings suggest the existence of a system of synthesis and/or release of uterine proteins which is not related to the presence of a normal 6 day-old embryo within the uterus. On the contrary, the presence of an abnormal embryo seems to interfere with normal levels of uterine proteins. It appears to

be a local effect, since so such differences were observed in the contralateral uterine horns. Further analyses of these proteins are now being carried out.

REFERENCES

- ALMEIDA AP, Fo., AYALON N, BARTOOV B - in press.
- AWDEH ZL, WILLIANSOON AR, ASKONAS BA (1968) - Nature 219, 66-7.
- AYALON N (1978) - J. Reprod. Fertil 54, 483-93.
- BARTOL FF, BAZER FW, BASHA SMM, ROBERTS RM, THATCHER WW (1979) - J. Anim. Sci. 49, Suppl. 1, 280, abstr.
- BARTOL FF, THATCHER WW, BAZER FW, KIMBALL FA, CHENAULT JR, WILLCOX CJ, ROBERTS RM (1981) - Biol. Reprod. 25, 759-76.
- BEATO M, BAIER R (1975) - Biochem. Biophys. Acta 392, 346-56.
- BEIER HM (1968) - Biochem. Biophys. Acta 160, 289-91.
- BEIER HM (1982) - In: Proteins and Steroids in Early Pregnancy, Beier & Karlson eds., Springer-Verlag Berlin Heidelberg, NY, pp 39-72.
- BURTON K (1956) - Biochemistry 62, 315-23.
- DANIEL JC (1968) - Comp. Biochem. Physiol. 24, 297-9.
- KING GJ, ATKINSON BA, ROBERTSON HA (1981) - J. Reprod. Fertil. 61, 469-74.
- KRISHNAN RS, DANIEL JC (1967) - Science 158, 490-3.
- KULANGARA AC (1976) - J. Reprod. Fertil. 46, 189-94.
- LASTER DB (1977) - Biol. Reprod. 16, 682-90.
- LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ (1951) - J. Biol. Chem. 193, 265-75.
- MCGAUGHEY RW, MURRAY FA (1972) - Fertil. Steril. 23, 399-404.
- MILLS AC, WARNICK AC, BAZER FW, THATCHER WW (1973) - J. Anim. Sci. 36, 195, abstr.
- MURRAY FA, BAZER FW, WALLACE HAD, WARNICK HC (1972) - Biol. Reprod. 7, 314-20.
- ROBERTS GP (1977) - J. Reprod. Fertil. 50, 337-9.
- ROBERTS GP, PARKER JM (1974a) - J. Reprod. Fertil. 40, 291-303.
- ROBERTS GP, PARKER JM (1974b) - J. Reprod. Fertil. 40, 305-13.
- SCHULTZ RH, FAHNING ML, GRAHAM EF (1971) - J. Reprod. Fertil. 27, 355-67.

The lack of differences in protein levels in uterine flushings between RB and NB cows is contrary to our earlier findings (Ayalon, 1978) and we have no satisfactory explanation for these contrasting results.

f. Plasma progesterone levels

Peripheral plasma progesterone concentrations followed the same pattern as in our previous investigation (Ayalon, 1978) and again no significant differences were found between cows with normal or abnormal embryos in concentrations for the period: Day 0 (estrus) up to and including Day 7 post-breeding.

f. Ion Concentrations in Uterine Flushings

Samples were saved from both RB and NB cows, with normal and abnormal embryos. Unfortunately, problems of instrumentation were not surmounted in the third year, as expected, because of insufficient funds. However, it is intended to carry out this important part of laboratory studies at another institution.

g. Progesterone Receptor Levels in the Endometrium

While not considered in the original plan of research, the opportunity arose of carrying out determinations of cytosol and nuclear progesterone receptors in the endometrium of cows slaughtered during the last year. These examinations were carried out in view of the possibility that, despite lack of significant differences in peripheral plasma progesterone concentrations between RB and NB cows - differences of local progesterone concentrations at the target cell level, as reflected by progesterone receptor levels in the endometrium - might cause a local hormonal imbalance related to embryonic mortality. While this work was completed well after the formal end of this

research project, the findings are included in the final report because of their interest and importance for the aims of the investigation. The following report has been submitted for publication in the Journal of Reproduction and Fertility.

PROGESTERONE RECEPTORS IN THE ENDOMETRIUM OF NORMAL AND REPEAT-BREEDER COWS.

Almeida, A. P., Fo., Ayalon, N., Bartoov, B.

ABSTRACT

Progesterone receptors were measured in the cytosol and nuclear fractions of endometrial cells from repeat-breeder (RB) and normal (NB) cows on both days 6 and 7 post-breeding. Both types of cows were divided in two groups, namely, inseminated and non-inseminated animals. Results were related to both the type of cow and to the condition of the embryo present within the uterus in the inseminated groups. Cows carrying morphologically abnormal embryos showed higher concentrations of cytosolic progesterone receptors as compared to the normal embryo group, regardless of the type of cow. Within the normal embryo group, RB cows had higher concentrations of progesterone receptors than NB. The same tendency of higher concentrations of progesterone receptors in RB cows was observed in the non-inseminated groups. The results of this study suggest the existence of a local hormonal imbalance, which seems to be related to the repeat-breeding problem.

INTRODUCTION

Previous works have demonstrated that a higher incidence of abnormal embryos was found in repeat-breeder (RB) Israeli Friesian cows on day 7 post-breeding, as compared to normal-breeding (NB) cows (Ayalon, 1978). This very early embryonic mortality seemed to be related to changes in the uterine environment in RB cows (Almeida et al., 1984). Although cows carrying either normal or abnormal embryo have similar levels of progesterone in the blood

plasma during the first week after insemination (Ayalon, 1978; Linares, Larsson & Edqvist, 1982; Sreenan & Diskin, 1983), there is the possibility that variations at the progesterone receptor levels at their target cells may cause a local hormonal imbalance related to the embryonic mortality.

A great amount of work has been accumulated during the past 12 years dealing with the characteristics of progesterone receptors in the endometrium (Stormshak, 1979; Jungblat, 1982). In the present work, specific progesterone receptors in both cytosol and nuclear fractions of endometrial cells were analysed in cows 6 and 7 days post-estrus. Results were related to both the type of cow and to the condition of the embryo.

MATERIALS AND METHODS

The methods used in the present study were modifications of exchange assay techniques proposed by Vu Hai & Milgrom (1978a), Vu Hai & Milgrom (1978b), Walters & Clark (1978, Atkins, Kramer, Harms & Fleeger (1980) and Zelinsky, Noel, Weber & Stormshak (1982).

In order to determine the saturating concentration of ^3H -promegestone (17, 21-dimethyl-19-nor-4, 9-pregnadiene-3, 20-dione, also known as R 5020), six cycling cows were slaughtered between day 6 and 10 after estrus. Steroid specificity of progesterone receptors was examined by incubating endometrial cytosol and nuclear fractions from three of those cycling cows with 20 nM ^3H -promegestone (saturating concentration) either alone or with a 100-fold excess of the following unlabelled steroids: promegestone, progesterone, testosterone, estradiol-17-beta and cortisol.

Further assays were carried out based on the saturating concentration, using endometrial samples taken by biopsies of the ipsilateral uterine horn.

Forty-eight cows (26 NB and 22 RB) were used for this study. Repeat-breeder cows were non-pregnant females which had received four or more inseminations the previous years and at least three inseminations this year with a negative pregnancy examination. Normal-breeding cows were defined as having not received more than one insemination this year and have conceived the previous years from no more than two inseminations. All animals had normal reproductive records and no detectable changes in uterus and ovaries.

Both RB and NB cows had their estrus synchronized with intravaginal progestagen-impregnated sponges (250 mg. Medroxi Progesterone Acetate) for 7 days, followed by an injection of 500 ug prostaglandin-analog cloprostenol. At the time of standing-heat, the animals were divided in four groups, as follows:

- A - Inseminated and biopsy taken on day 6 (followed by uterine flushing for embryo recovery).
- B - Inseminated and biopsy taken on day 7 (followed by uterine flushing for embryo recovery).
- C - Non-inseminated and biopsy taken on day 6.
- D - Non-inseminated and biopsy taken on day 7.

A TEM buffer (10 mM Tris-HCl, 1.5 mM EDTA, 3 mM Monothioglycerol, 10% Glycerol v/v, pH 7.4 at 25°C) was used for the assays, which were always carried out at 4°C. A piece of endometrial tissue (50 mg endometrium/ml TEM buffer) was homogenized in TEM buffer and centrifuged at 800 xg for 10 minutes. The supernatant was then centrifuged at 105,000 xg for 60 minutes to yield the cytosol fraction in the supernatant. The 800 xg-precipitate (nuclear pellet) was resuspended in TEM buffer to a tissue concentration of 20 mg/ml.

Aliquots of 500 μ l from both cytosol and nuclear fractions were then taken for the exchange assay. In order to determine the saturating concentration, they were incubated with 50 μ l of increasing concentrations of ^3H -promegestone (86 Ci/mmol; 2.5, 5, 10, 20 and 40 nM) or with labelled ligand plus a 100-fold excess of unlabelled promegestone. Further assays were carried out using the saturating concentration. Incubation was carried out for 20 hours at 4°C.

Following incubation, 500 μ l charcoal-dextran (250 mg charcoal and 25 mg dextran in 100 ml PBS, pH 7.0) were added to each tube of the cytosol fraction. After vortexing, the tubes were centrifuged at 1,500 xg for 10 minutes and 500 μ l of the supernatant were added to 10 ml of scintillator fluid.

After incubation, the nuclear-fraction tubes were centrifuged at 3,000 xg for 10 minutes. The precipitate was washed twice with 2 ml TEM buffer which contained 10 mg BSA/ml and once more with TEM buffer alone, before being re-suspended in 500 μ l TEM buffer. The suspension was then added to 10 ml scintillator fluid. The counting of all samples had 63% efficiency.

Blood samples were collected on either day 6 or 7 and progesterone concentrations were determined by RIA (Shemesh, Ailenberg, Mileguir, Ayalon & Hansel, 1978). The DNA contents of the nuclear pellets were determined according to the method of Burton (1956). The specificity of the assay was tested by Scatchard plot (Scatchard, 1949; Chamness & McGuire, 1975). The difference between total binding (samples incubated with ^3H -promegestone) and non-specific binding (samples incubated with ^3H -promegestone plus a 100-fold excess of unlabelled promegestone) represented the specific binding of promegestone to the progesterone receptors. Data were analysed for significance using the Student's t test.

RESULTS

The saturation curves for the specific binding of ^3H -promegestone to progesterone receptors in both cytosol and nuclear fractions of the endometrium are shown in Text-figure 1. A concentration of 20 nM labelled ligand was sufficient to saturate all binding sites for both fractions and, therefore, this concentration was used for further assays. The Scatchard plot for those assays showed a single, linear curve and K_d of 3.55 nM and 3.50 nM for cytosol and nuclear fractions, respectively. The specificity of the binding of promegestone to progesterone receptors, as compared to other steroids, is shown in Table 1. Only promegestone and progesterone had highly specific binding to progesterone receptors, whereas testosterone, 17-beta-estradiol and cortisol caused little or no displacement of labelled promegestone from progesterone receptors.

Table 2 shows the concentrations of progesterone receptors in both cytosol and nuclear fractions from endometria of RB and NB cows 6 days post-breeding. Results were grouped according to the condition of the embryo found within the uterus. Embryos were classified as normal or abnormal according to their morphological characteristics (Almeida et al., 1984). These data suggested a general tendency of having higher concentrations of progesterone receptors in the cytosol when an abnormal embryo was present. A comparison between RB and NB when a normal embryo was present showed higher concentrations of progesterone receptors in RB as compared to NB cows in both cytosol ($715.6 \text{ fmol/mg DNA} \pm 65.56 \text{ sem}$ vs 478 ± 79.76) and nucleus (666.6 ± 53.08 vs 415.2 ± 31.66).

In Table 3, the concentrations of progesterone receptors in cytosol and nucleus from non-inseminated RB and NB cows on days 6 and 7 after estrus, are

presented. There seemed to be a tendency of having higher concentrations of progesterone receptors in RB as compared to NB on both days 6 and 7. The differences found between day 6 and 7 within each group of cows did not appear to be related to the type of cow, since a slight increase in receptor concentrations was present in both RB and NB cows.

The progesterone concentrations in peripheral plasma from both RB and NB cows on days 6 and 7 post-estrus are shown in Table 4. Since no differences were found between the inseminated and non-inseminated groups or between normal and abnormal embryos, results were pooled together. Plasma progesterone levels did not differ significantly between RB and NB cows either on day 6 or 7 after estrus.

DISCUSSION

Two recently published works by King & Greene (1984) and by Welshons, Lieberman & Gorski (1984) suggested that the classic definition of the steroid-receptor translocation is in error, since it was shown in their works that the unoccupied estrogen receptor was mostly present in the nucleus of target cells. Although the basic concept that steroid-receptor complexes are nuclear regulatory elements still remains, the results of those works suggest that the cytosolic localization of receptors represents an extraction artefact. Therefore, one should be careful in analysing results dealing with steroid-receptors in the cytosol, until more work is done to clarify this new model.

Nevertheless, our results on progesterone receptors in the bovine endometrium indicated a tendency of having higher concentrations of progesterone receptors, particularly in the cytosol, which appeared to be related to abnormal embryos and to repeat-breeder cows. High concentrations of progesterone receptors in endometrial cytosol have been related to either high estrogen or low progesterone plasma levels (Leavitt, Toft, Strott & O'Malley, 1974; Walters &

Clark, 1978; Zelinsky et al., 1982). Furthermore, progesterone administration reportedly caused an increase in progesterone receptors in the nuclear fraction (Walters & Clark, 1978).

The results of the present work as well as previous publications, have shown no difference between cows carrying normal or abnormal embryos, as far as plasma progesterone and estrogen levels were concerned (Ayalon, 1978; Linares Larsson & Edqvist, 1982; Sreenan & Diskin, 1983). Moreover, there seemed to be no difference in plasma progesterone levels between RB and NB cows on days 6 and 7 post-estrus (Table 4). The results of the present paper suggest the existence of a local failure of the progesterone-sensitive cells to respond to the hormonal stimulus. This fact would cause a local hormonal imbalance, which could be related to uterine environment changes and to embryonic mortality, as well as to the decreased number of ciliated cells found in RB cows reported in a previous paper (Almeida, Ayalon & Bartoov, 1984).

Based only on these results, it is quite difficult to state that the repeat breeding problem is solely due to that hormonal imbalance. However, data obtained from the inseminated groups suggested the existence of some interaction between the uterine environment and the developing embryo. Even when normal embryos were found, RB cows had higher concentrations of progesterone receptors than NB cows in both cytosol and nuclear fractions (Table 2). A comparison between the inseminated and the non-inseminated groups also suggested that the insemination itself might participate in such interaction, reducing the concentration of progesterone receptors in both cytosol and nucleus, regardless of the type of cow.

The non-inseminated groups were utilized in order to try to explain our previous results, in which embryo transfers on day 6 resulted in lower pregnancy

rates when RB cows rather than virgin heifers were used as recipients (Almeida et al., 1984). In another work, Tanabe, Hawk & Hasler (1984) transferred day 7 embryos to both RB and NB cows and obtained similar pregnancy rates. Results in the present study showed a slight increase in progesterone receptors between days 6 and 7, which was not related to the type of cow. However, RB cows seemed to have a tendency for higher concentrations of progesterone receptors in the endometrium (Table 3). It is important to point out that between days 6 and 7 the bovine embryo reaches the blastocyst stage. At the transition stage between morula and blastocyst, the embryo is reportedly very sensitive to uterine environment changes (Dickman, 1970; Newcomb & Rowson, 1980). Slight local hormonal imbalance could be related to the failure of day 6 embryos to develop in RB cows, as compared to day 7 transfers in which the morula-blastocyst stage has already occurred in the donor cows.

The results of this study suggest the existence of some local hormonal imbalance at the progesterone receptor level in endometrial cells, which seems to be related to the repeat-breeding problem.

REFERENCES

- Almeida, A. P., Fo., Ayalon, N., Faingold, D., Marcus, S., Lewis, I. & Bartoov, B. (1984) - The relationship between uterine environment and early embryonic mortality in normal and repeat-breeder Friesian cows. Proc. X Int'l Cong. Anim. Reprod. & A. I., Illinois, USA, III, 438.
- Almeida A. P., Fo., Ayalon, N. & Bartoov B. (1984) - Electron microscopic findings in repeat-breeder and normal cows on day 6 post-breeding. Proc. X Int'l Cong. Anim. Reprod. & A. I., Illinois, USA, III, 439.
- Atkins, D. T., Kraemer, D. C., Harms, P. G. & Fleeger, J. L. (1980) - Progesterone-receptor complex in the nuclear fraction of the bovine endometrium. Biol. Reprod. 23, 317-323.

Ayalon, N. (1978) - A review of embryonic mortality in cattle. J. Reprod. Fert. 54, 483-493.

Burton, K. (1956) - A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemistry 62, 315-323.

Chamness G. C. & McGuire W. L. (1975) - Scatchard plots: common errors in correction and interpretations. Steroids 26, 538-542.

Dickman, Z. (1970) - Effects of progesterone on the development of the rat morula. Fert. Ster. 21, 541-548.

Jungblut, P. W. (1982) - Steroid receptors in the genital tract and the monitoring of steroid hormone action. In: Proteins and Steroids in Early Pregnancy, Beier, H. M. & Karlson P. eds. Springer-Verlag, New York, pp. 147-156.

King, W. J. & Greene, G. L. (1984) - Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature 307, 745-747.

Leavitt, W. W., Toft, D. O., Strott, C. A. & O'Malley, B. W. (1974) - A specific progesterone receptor in the hamster uterus: physiologic properties and regulation during the estrous cycle. Endocrinol. 94, 1041-1053.

Linares, T., Larsson, K. & Edqvist E. (1982) - Plasma progesterone levels from estrus through day 7 after A. I. - heifers carrying embryos with normal or deviating morphology. Theriogenology 17, 125-132.

Newcomb, R. & Rowson, L. E. A. (1980) - Investigations of physiological factors affecting non-surgical transfer. Theriogenology 13, 41-49.

Scatchard, G. (1949) - The attraction of proteins for small molecules and ions. Annals NY Acad. Sci. 51, 660-672.

- Shemesh, M., Ailenberg, M., Mileguir, F., Ayalon, N. & Hansel W. (1978) - Hormone secretion by cultured bovine pre- and post-implantation gonads. *Biol. Reprod.* 19, 761-767.
- Sreenan, J. M. & Diskin, M. G. (1983) - Early embryonic mortality in the cow: its relationship with progesterone concentration. *Vet. Rec.* 112, 517-521.
- Stormshak, F. (1979) - Uterine estrogen and progesterone receptors. *In: Beltsville Symposia in Agriculture Research (3) Animal Reproduction.* Hawk, H. W., ed., Allanheld Osmum & Co., Montclair, NJ, pp 399-410.
- Tanabe, T. Y., Hawk, H. W. & Hasler, J. f. (1984) - Comparative fertility of normal and repeat-breeder cows as embryo recipients. *Proc. X Int'l. Cong. Anim. Reprod. & A. I.*, Illinois, USA, II, 247.
- Vu Hai, M. T. & Milgrom, E. (1978a) - Characterization and assay of the progesterone receptor in rat uterine cytosol. *J. Endocr.* 76, 21-31.
- Vu Hai, M. T. & Milgrom E. (1978b) - Characterization and assay of the progesterone receptor in rat uterine nuclei. *J. Endocr.* 76, 33-41.
- Walters, M. R. & Clark, J. H. (1978) - Cytosol and nuclear compartmentalization of progesterone receptors in the rat uterus. *Endocrinol.* 103, 601-609.
- Welshons, W. V., Lieberman, M. E. & Gorski, J. (1984) - Nuclear localization of unoccupied oestrogen receptors. *Nature* 307, 747-749.
- Zelinsky, M. B., Noel, P., Weber, D. W., & Stormshak, F. (1982) - Characterization of cytoplasmic progesterone receptors in the bovine endometrium during proestrus and diestrus. *J. Anim. Sci.* 55, 376-383.

Text - figure 1 - Saturation Curves for the Specific Binding of ^3H -Promegestone to Progesterone Receptors in Cytosol and Nuclear Fractions from Bovine Endometrium. Inset: Scatchard Plot.

TABLE 1 - Competition of Steroid Hormones with ^3H -Promegestone for Endometrial Cytosol and Nuclear Progesterone Receptors.

Unlabelled Hormone	Hormone-Receptor Binding in Cytosol fmol/mg DNA $\pm$ sem (% Displacement)	Hormone-Receptor Binding in Nucleus fmol/mg DNA $\pm$ sem (% Displacement)
None	8697 $\pm$ 1063.5	2865.5 $\pm$ 349.3
Promegestone	5720 $\pm$ 857.4 (34%)	1238.5 $\pm$ 224 (57%)
Progesterone	5800 $\pm$ 882.7 (33%)	1253 $\pm$ 227.5 (56%)
Testosterone	8352 $\pm$ 1076.7 (4%)	2674 $\pm$ 347.8 (6.7%)
Estradiol 17-beta	8493 $\pm$ 1075.3 (2.4%)	2771 $\pm$ 354.2 (3.3%)
Cortisol	8733 $\pm$ 1061.4 (0)	2822 $\pm$ 352.7 (1.5%)

TABLE 2 - Concentrations of Progesterone Receptors in the Cytosol and Nuclear Fractions of the Endometrium from Inseminated Cows, 6 Days Post-Breeding - fmol/mg DNA $\pm$ sem (number of cases).

Type of Cow	Condition of the Embryo	Cytosol	Nucleus
N B	Normal	478 $\pm$ 79.76 (a) (5) (c)	415.2 $\pm$ 31.66 (d) (5) (e)
	Abnormal	1014.3 $\pm$ 110.51 (6) (a)	483.2 $\pm$ 97.76 (5) (e)
R B	Normal	715.6 $\pm$ 65.56(b) (5) (c)	666.6 $\pm$ 53.08 (d) (5) (e)
	Abnormal	1507.8 $\pm$ 223.34 (5) (b)	801 $\pm$ 61.85 (5) (e)

(a) $p < 0.005$

(b) $p < 0.01$

(c) $p < 0.05$

(d) $p < 0.005$

(e) N. S.

TABLE 3 - Concentrations of Progesterone Receptors in the Cytosol and Nuclear Fractions of the Endometrium from Non-Inseminated Cows, 6 and 7 Days Post-Estrus - fmol/mg DNA $\pm$ sem (Number of Cases)

Day after Estrus	Type of Cow	Cytosol		Nucleus	
6	NB	886.5 $\pm$ 153.83	(b)	615.8 $\pm$ 124.23	(d)
		(6)	(f)	(6)	(e)
	RB	1222.5 $\pm$ 169.24	(c)	984.0 $\pm$ 63.34	(d)
		(6)		(6)	
7	NB	1291.3 $\pm$ 75.60	(a)	957.2 $\pm$ 66.40	(e)
		(9)	(b)	(9)	(f)
	RB	1671.5 $\pm$ 85.61	(a)	1185.6 $\pm$ 161.54	(f)
		(6)	(c)	(6)	
		(a) $p < 0.01$	(c) $p < 0.05$	(e) $p < 0.02$	
		(b) $p < 0.02$	(d) $p < 0.02$	(f) N. S.	

TABLE 4 - Peripheral Plasma Progesterone Concentrations in Cows 6 and 7 Days Post-Estrus.

Type of Cow	Day of the Cycle	Progesterone Concentration	
		ng/ml $\pm$ sem	(No. of cases)
N B	6	5.99 $\pm$ 0.79	(16)
	7	7.98 $\pm$ 1.76	(6)
R B	6	7.46 $\pm$ 0.70	(19)
	7	7.79 $\pm$ 0.73	(6)

B4III Field Trials - Improvement of fertility by supplementary progestin

Two preliminary field trials were carried out, to examine the possibility of improving fertility in dairy cows by furnishing supplementary progestin. The time of treatment start was on day 6 or 7 after insemination, and was chosen on the basis of our findings on the critical period for embryo survival in RB cows. Medroxy progesterone acetate (250 mg.) was applied, using intravaginal sponges for slow absorption, of the type developed and described by us previously (Ayalon and Marcus, 1975). Sponges were removed on day 17 after insemination and conception rates were based upon pregnancy diagnosis by rectal palpation, 45-60 days after insemination..

Trial I was carried out on 74 dairy cows including only a small number of RB cows. 31 of them received, on day 6 or 7 after insemination, intravaginal sponges impregnated with the progestin, and the remaining 43 were untreated controls. Results were as follows: pregnancy rate in progestin-treated group: 18 of 31 (58%) versus 15 of 43 (34.8%) in the control, untreated group. The difference in pregnancy rate was significant ($p < 0.05$).

Trial II was a small trial conducted in a dairy herd with poor fertility associated with high levels of phytoestrogens in the feed given to the cows. A total of 24 cows were assigned to one of two groups: Group A - On day 6 after insemination cows were given an intravaginal sponge containing 250 mg of MAP. Sponges were removed on day 17 after insemination. Group B - Control cows, receiving no treatment. Table 4 presents the results obtained.

Table 4: Influence of supplementary progestin on conception rate of cows
in a low-fertility herd

Treatment Group	No. of cows	Conception rate			
		After 1st insemination		After 1st and 2nd insemination combined	
		No.	%	No.	%
A-Supplementary progestin	13	4/13	31	7/13	54
A-Control (untreated)	11	2/11	18	2/11	18

While the number of cows involved are too small to allow for a valid evaluation, the continued trend of improved fertility associated with supplementary progestin after insemination, is encouraging. On the basis of these results, additional trials have begun, with normal and repeat breeders cows, using coded sponges, half of which are dummies and observations will include milk progesterone levels to evaluate the influence of the progestin on progesterone levels, in addition to its influence on fertility.

REFERENCES

- Almeida, A.P. Fo, Ayalon, N., Faingold, D., Marcus, S., Lewis, I. and Bartoov, B. (1984). The relationship between uterine environment and early embryonic mortality (EEM) in normal (NB) and repeat-breeder (RB) Friesian cows. Proc. Tenth Intl. Congr. Anim. Reprod. and A. I. Champaign- Urbana, Ill. Vol. III, 438.
- Almeida, A. P. Fo, Ayalon, N. and Bartoov, B. (1984a). Electron microscopic findings in repeat-breeder and normal cows on day 6 post-breeding. Proc. Tenth Intl. Congr. Anim. Reprod. And A. I. Champaign- Urbana, Ill. Vol. III, 439.
- Almeida, A. P. Fo., Ayalon, N. and Bartoov, B. (1984b). The relationship between protein levels in uterine flushings and the presence of normal embryos in dairy cattle. Proc. Tenth Int'l. Congr. Anim. Reprod. and A. I. Champaign- Urbana, Ill. Vol. III, 440.
- Annual Report - Hasherut A. I. Center (1972-74) p. 24.
- Ayalon, N. (1969) Final Research Report PL 480. Grant FG-IS-150 Kimron Veterinary Institute. - Beit Dagan.
- Ayalon, N. (1972). Fertility losses in normal cows and repeat-breeders. Proc. VIII Intl. Congr. Anim. Reprod. and A. I. Munich Vol. I, 74.
- Ayalon, N. (1973). PL 480 - Grant FG-IS-290 Annual Report No. 2 Kimron Veterinary Institute, Beit Dagan.
- Ayalon, N. (1977) Final Research Report. PL 480. Grant FG-IS-290. Kimron Veterinary Institute. Beit Dagan.
- Ayalon, N. (1978). A review of embryonic mortality in cattle. J. Reprod. Fert. 54, 483.

- Ayalon, N., Krieger, Y. and Lewis, I. (1976). Non-surgical recovery of late blastocysts in cows. Proc. VIIIth Intl. Congr. Anim. Reprod. and A. I. Krakow. Vol. III: 233.
- Ayalon, N. and Marcus, S. (1975). Estrus synchronization and conception rate in dairy cattle treated with progestin-impregnated vaginal sponges. Theriogenology 3: 95-99.
- Ayalon, N., Tadmor, A. and Lewis, I. (1965). Surgical recovery of ova in the cows. Refuah Vet. 22, 131.
- Ayalon, N., Weiss, Y. and Lewis, I. (1968). Fertility losses in normal cows and repeat breeders. VIIth Intl. Congr. Anim. Reprod. and A. I. Paris, Vol. I, 393.
- Bowen, J. M., Elsdén, R. P. and Seidel, G. E., Jr. (1978). Non-surgical transfer in the cow. Theriogenology 10: 89.
- Boyd, H., Bacsich, P., Young, A. and McCracken, J. A. (1969). Fertilization and embryonic survival in dairy cattle. Brit. Vet. J. 125, 87.
- Clemetson, C. A. B., Kim, J. K., Mallikarjuneswara, V. R. and Wilds, J. H. (1972). The sodium and potassium concentrations in the uterine fluid of the rat at the time of implantation. J. Endocr. 54, 417.
- Csapo, A. I. and Wiest, W. G. (1969). An examination of the quantitative relationship between progesterone and maintenance of pregnancy. Endocrinology, 85, 735.
- Daniels, J. C. (1971). Advances in the Biosciences 6. Pergamon Press, Oxford and New York, 191.
- Elsden, R. P., Hasler, J. F. and Seidel, G. E., Jr. (1976). Non-surgical recovery of bovine eggs. Theriogenology 6, 523.
- Frances, G., Davidson, M. and Mayer, E. (1977). The influence of some nutritional factors in the incidence of the repeat-breeder syndrome in high producing dairy herds. Theriogenology 7, 105.

- Greve, I., Lehn-Jensen, H. and Rasbech, N. O. (1978). Practical application of non-surgical collection of bovine embryos in Danish pedigree cattle. In: Control of Reproduction in the Cow. M. Nijhoff - The Hague pp. 363.
- Hahn, J., Moustafa, L. Q., Schneider, U., Hahn, R., Romanowski, W. and Roselius, R. (1978). Survival of cultured and transported bovine embryos following surgical and non-surgical transfers. In: Control of Reproduction in the Cow. M. Nijhoff - The Hague, 356.
- Hawk, H. W., Brinsfield, T. H., Turner, G. D., Whitmore, G. E. and Norcross, M. A. (1963). Embryo survival in first-service and repeat-breeder cattle after ovariectomy and hormone therapy. J. Dairy Sci. 46, 1397.
- Hawk, H. W., Wiltbank, J. N., Kidder, H. E. and Casida, L. E. (1955). Embryonic mortality between 16 and 34 days post-breeding in cows of low fertility. J. Dairy Sci. 38, 673.
- Heap, R. B. and Lamming, G. E. (1962). The influence of ovarian hormones on some chemical constituents of the uterine washings of rat and rabbit. J. Endocr. 25, 27.
- Hertig, A. T. (1967) in: Comparative Aspects of reproductive failure. Ed. K. Benirschke, Springer Verlag. Berlin and New York, p. 11.
- Hewett, C. D. (1968). A survey of the incidence of the repeat-breeder cow in Sweden with reference to herd size, season, age and milk yield. Brit. Vet. J. 124, 342.
- Heyman, Y., Renard, J. P., Ozil, J. P. and du Masnil du Buisson (1978). Cervical embryo transfer at different stages in cattle. In: Control of Reproduction in the Cow. Edited by Sreenan, J. R. Published by Martinus Nijhoff - The Hague, pp. 330.
- Johnson, K. R., Ross, R. H. and Fourn, D. L. (1958). Effect of progesterone administration on reproductive efficiency. J. Animal Sci. 17, 386.

Lampeter, W. W., Kruff, B., Rieger, J., Wagner, H. G. and Ertel, J. (1980).

Zuchthyg. 15: 173.

Laster, D. B. (1977). A pregnancy-specific protein in the bovine uterus.

Biol. Reprod. 16, 682.

Linares, T. (1981). Embryonic development in repeat breeder and virgin heifers seven days after insemination. Animal Reprod. Sci. 4, 189.

McFeely, R. A. (1966). A direct method for the display of chromosomes from early pig embryos. J. Reprod. Fert. 11, 161.

McFeely, R. A. (1967). Chromosome abnormalities of the pig. J. Reprod. Fert. 13, 579.

McFeely, R. A. and Rajakoski, E. (1968). Chromosome studies on the early embryos of the cows. Proc. VIth Intl. Congr. Anim. Reprod. and A. I. Paris. Vol. II, 905.

Newcomb, R. (1978). In: Control of Reproduction in the Cow. M. Nijhoff - The Hague 316.

O'Farrel, K. J., Langley, O. H., Hartigan, P. J. and Sreenan, J. M. (1983).

Fertilization and embryonic survival rates in dairy cows culled as repeat breeders. Vet. Rec. 112, 95.

Reynard, J. P., Heyman, Y. and DuMesnil du Buisson, F. (1977). Unilateral and bilateral cervical transfer of bovine embryos at the blastocyst stage. Results of transfer at different periods. Theriogenology 7: 189.

Robison, G. A., Butcher, R. W. and Sutherland, E. W. (1971) in: Cyclic AMP. Academic Press, New York, p. 222.

Roberts, G. P. and Parker, J. M. (1974). Macro-molecular components of the luminal fluids from the bovine uterus. J. Reprod. Fert. 40, 291.

- Rowson, L. E. A., Moor, R. M. and Lawson, R. A. S. (1969). Fertility following egg transfer in the cow: effect of method, medium and synchronization of oestrus. *J. Reprod. Fert.* 18, 517.
- Schultz, R. H., Fahning, M. L. and Graham, E. F. (1971). A chemical study of uterine fluid and blood serum of normal cows during the oestrous cycle. *J. Reprod. Fert.* 27, 335.
- Seidel, G. E., Jr., Seidel, S. M. and Bowen, R. A. (1978). Bovine embryo transfer procedures. Colorado State University Expt. Station General Series 975: 1.
- Shea, B. F. (1981). Evaluating the bovine embryo. *Theriogenology* 15, 31.
- Shemesh, M., Ayalon, N. and Lindner, H. R. (1972). Oestradiol levels in peripheral blood of cows during the oestrous cycle. *J. Endocr.* 55, 73.
- Shemesh, M., Lindner, H. R. and Ayalon, N. (1971). Competitive protein - binding assay of progesterone in bovine systemic plasma during the oestrous cycle. *J. Reprod. Fert.* 26, 16/7.
- Sreenan, J. M. and McDonagh, T. (1979). Comparison of embryo survival rate in heifers following artificial insemination, non-surgical blastocyst transfer or both. *J. Reprod. Fert.* 56: 281.
- Tanabe, T. Y. and Casida, L. E. (1949). The nature of reproductive failure of cows of low fertility. *J. Dairy Sci.* 32, 237.
- Whittingham, D. G. (1971). Survival of mouse eggs in vitro. *Nature (Lond)* 220: 592.
- Whittingham, D. G. (1971). Culture of mouse ova. In: *Experiments on mammalian eggs and embryos*. *J. Reprod. Fert. Suppl.* 114: 7.
- Whittingham, D. G. (1971a). Survival of mouse eggs in vitro. *Nature (London)* 220, 592.
- Wright, J. M. (1981). Non-surgical embryo transfer in cattle. Embryo-recipient interactions. *Theriogenology* 15, 43.

D. Evaluation of the Research Achievement with respect to the Original Research Proposal

There were two chief questions which this project was meant to clarify:

- 1) Are the differences in early embryonic death (EED) between repeat breeders and cows with normal fertility due to factors operating before or after the embryo enters the uterus?
- 2) Can the early embryonic environment and EED be influenced by hormonal treatment?

The answer to the first question came through clearly from embryo transfer trials and less clearly from the laboratory studies. Embryo transfers of normal embryos from RB and NB donors were carried out before the crisis for embryo survival in RB cows (on day 6 post-breeding). Transfers were made to synchronized recipients furnishing the best uterine environment (virgin heifers) or the worst uterine environment (repeat-breeders). Pregnancy rates 6 weeks post-estrus showed very clearly that the recipient uterine environment determined the degree of embryo survival, regardless of the donor source. Results of laboratory studies were more equivocal. There was a suggestive difference in the ability of normal morulae to synthesize estrogen on day 6 post-breeding and slightly less ciliated cells found by ultra microscopy in the endometrium of RB cows on day 6. Total protein levels in uterine flushings were higher when a normal embryo was found, with no significant differences between RB and NB cows and no significant differences in isoelectrofocusing patterns or polyacrylamide gel-electrophoretic patterns- between the two types of cows or in relation to normality of the embryo. As in our previous work, peripheral plasma progesterone levels were similar for cows with normal or abnormal embryos. One striking difference that was found in the laboratory

studies was that concerning progesterone receptor levels. Cows carrying abnormal embryos showed higher concentrations of cytosol progesterone receptors, as compared to the normal embryo group, regardless of type of cow. RB cows had significantly higher concentrations of progesterone receptors than did NB cows, on day 6 post-insemination, when a normal embryo was found. Regarding non-inseminated animals, RB cows had significantly higher nuclear progesterone receptor concentrations than did NB cows on Day 6 post-estrus, but not on day 7. These differences may be connected with the seemingly contradictory results of embryo transfers carried out to RB and NB recipients on Day 6 post-estrus where we found clear evidence of the importance of the uterine environment as reflected in differences in results between the two types of recipients (Almeida et al, 1984) whereas our cooperator, Dr. Hawk and his group in the USA, failed to find differences in pregnancy rates of RB and NB cows to whom embryos were transferred on day 7 (Tanabe et al, 1984). (It should not be overlooked however, that Tanabe et al transferred embryos which were mostly at the blastocyst stage - and were therefore in a sense "survivors" of the critical period of embryo development, from morula to blastocyst).

Altogether, the results of this study suggest that EED is primarily connected with the conditions of the local uterine environment at the critical period of embryo development, when the morula has to differentiate into the blastocyst, between 6-7 days post-insemination. It appears that there is some local hormonal imbalance occurring in RB cows, which is reflected in the nuclear progesterone receptor levels of the endometrium on day 6 post-estrus. Further work is needed to extend the preliminary observations that hormonal treatment at a critical period may improve fertility.

E. Description of Cooperation

The work that was planned to be carried out at the cooperating institute- Dr. Hawk's group at the Animal Reproduction Laboratory in Beltsville, Md. - was to include (1) analysis of protein content of flushings of uterine and oviducal fluids from our cattle, and (2) evaluation by electron microscopy, of the ultra-structure of the endometrium from our cattle. Both parts of this planned program fell through, the first due to the veto of the veterinary authorities in the USA on the importation of genital tract fluid from Israeli cattle and the second due to personnel changes which precluded carrying out the electron microscopy studies. Because of the importance of these topics, this work was being carried out by our laboratory.

By mutual agreement the plan of investigation was changed and Dr. Hawk's group investigated embryo-endometrium-corpus luteum interrelationships that influence the maintenance of the CL and pregnancy, with emphasis on steroid and prostaglandin production by the embryo and endometrium. Dr. Hawk's complete report on this aspect of the investigation has been sent out and will be presented to BARD, when received.

Dr. Hawk and coworkers carried out an important embryo transfer trial, using embryos from heifers, transferred to either repeat breeder or normal breeder recipients on day 7 after estrus (as compared with day 6 in our investigation). The findings were recently published (Tanabe, Hawk and Hasler - Proc. Tenth Int'l Cong. on Animal Reproduction and A. I., Urbana, Illinois Vol II, 247-248) and the results were as follows:

Table 1. Comparative Rates of Pregnancy in First-Service and Repeat-Breeder Dairy Cows Following Surgical Transfer of Embryos

Status of cow	No. of Cows	Pregnant		Not Pregnant	
		No.	%	No.	%
Normal	28	23	82	5	18
Repeat Breeder	23	16	70	7	30
Total	51	39	76	12	24

The pregnancy rates of the two types of recipients were not significantly different.

These results are quite at variance with our findings with transfers of embryos to repeat breeders or virgin heifers at day 6 post-estrus - where significantly lower pregnancy rates were found regardless of whether the donors were repeat breeders or normal breeders. Dr. Hawk's group was of the opinion that the high conception rate in the RB recipients could be explained by either an improvement in maternal environment by the prostaglandin treatment given to induce estrus before the transfer, or else because by 7 days post-estrus, the maternal environment in repeat breeders is satisfactory for maintaining normal pregnancy. The results in Israel would seem to rule out the former contention, since PGF2 alfa was used in estrus induction in our recipients too, with very contrasting pregnancy results.

The difference in the results of the two groups can be explained by our findings with the differences in levels of progesterone receptors found on days 6 and 7, as described in section 4IIg. Significantly higher levels of nuclear progesterone receptors were found in cases of abnormal embryos or in

non-inseminated RB recipients on day 6 but not on day 7 after estrus. That, together with the fact that on day 7, mostly blastocysts were transferred (meaning "survivors" who had passed the critical developmental period from morula to blastocyst) - both these conditions are in our opinion the explanation for the varying results found.

The results of both investigations again emphasize the key importance of day 6 for embryonic survival in repeat breeder dairy cows and of the importance of the uterine environment at this time. Apparently a local hormonal imbalance occurs at this time, reflected in significantly higher nuclear progesterone levels. Work in the future should attempt to clarify this phenomenon further and to see if this condition is amenable to hormonal treatment.

F. Benefit to Agriculture

The fact that infertility in cows without clinical pathology (repeat breeders) is a source of heavy economic losses in dairy cattle, the world over, is too-well known to require emphasis. Until relatively recently, basic data about the nature of the problem was woefully lacking. Attempts to diminish the severity of the repeat-breeder problem through constant selection have not been successful. Neither have hormonal treatments ameliorated losses due to this condition, and this is not surprising since these treatments have been applied empirically, without knowledge of when, what kinds, and what dosages of hormones are needed.

The present investigation has furthered our knowledge about the nature of early embryonic mortality. It has demonstrated that it is most probably a problem of the local uterine environment at a critical period for embryonic survival, when the morula differentiates into the blastocyst, between day 6 and 7 post-breeding. Results of preliminary hormonal treatment trials suggest that fertility may be improved by progestin treatment given at a suitable time after breeding.

In summary, this research has contributed to our knowledge and understanding of early embryonic mortality and may be of value in developing a more logical approach in attempts to diminish the losses due to this condition in cattle.

G - List of Publications (resulting from Project)

- 1) Marcus, S. and Ayalon, N.: Improving conception rate in dairy cattle, using progestin-impregnated intravaginal sponges. Refuah Vet. 38: 55 (abstract). 1981.
- 2) Ayalon, N.: Embryo transfer as a research tool. Israeli-French Symposium on the Reproductive Potential of Cattle and Sheep, Rechovot, Israel, 1984 (to be published).
- 3) Ayalon, N: The Repeat Breeder Problem. Tenth Int'l Congress on Animal Reproduction and Artificial Insemination. Urbana Illinois. Vol. IV, Section III pp. 41-50. 1984.
- 4) Almeida, A. P. Fo, Ayalon, N., Bartoov, B., Faingold, D., and Marcus, S. The importance of the uterine environment in early embryonic mortality in dairy cattle. Second Symposium on Advanced Topics in Animal Reproduction FCAV-unesp. Jaboticabal, SP, Brazil. 89-96. 1983.
- 5) Almeida, A.P. Fo, Ayalon, N., Faingold, D., Marcus, S., Lewis, I. and Bartoov, B: The relationship between uterine environment and early embryonic mortality (EEM) in normal (NB) and repeat-breeder (RB) Friesian cows. Proc. Tenth Int's. Congress on Animal Reproduction and A.I., Urbans, Illinois, Vol. III, 438. 1984.
- 6) Almeida, A. P., Fo, Ayalon, N. and Bartoov, B.: Electron microscopic findings in repeat breeder and normal cows on day 6 post-breeding. Proc. Tenth Int'l. Congress on Animal Reproduction and A. I. Urbana, Illinois, Vol. III, 439. 1984.
- 7) Almeida, A. P. Fo, Ayalon, N., and Bartoov, B.: The relationship between protein levels in uterine flushings and the presence of normal embryos in dairy cattle. Proc. Tenth Int'l Congress on Animal Reprod. and A. I. Urbana, Illinois. Vol. III, 440, 1984.
- 8) Almeida, A. P. Fo, Ayalon, N. and Bartoov, B: Progesterone receptors in the endometrium of normal and repeat-breeder cows. Submitted for publication.

Final Report
from the
Reproduction Laboratory
Beltsville Agricultural Research Center
Beltsville, Maryland USA

BARD Project I-88-79
Early Embryonic Environment and Early Embryonic Death in Dairy Cattle
September 1984

In several species of laboratory animals, prostaglandins (PG's) appear to be necessary for maintenance of early pregnancy. PG's seem to be needed for controlling uterine blood flow, blastocyst hatching and expansion, and in some cases, extending luteal function. We found that bovine blastocysts and endometrium recovered on days 16 and 19 of pregnancy produced and metabolized PG's in vitro, and, per unit of tissue, blastocysts produced PG's considerably more actively than did endometrium. Under BARD I-88-79, our initial in vitro study with bovine blastocysts was designed to determine if endometrial slices modulated arachidonic acid metabolism by bovine blastocysts. For this study, blastocysts and endometrium were recovered from cows on day 19 of pregnancy and assigned to four incubation groups. Group 1: blastocysts incubated alone (n = 4 incubates); Group 2: endometrial slices incubated alone (n = 21 incubates); Group 3: blastocysts incubated with endometrial slices (n = 5 incubates); Group 4: blastocysts incubated in a 1:1 mixture of fresh culture medium (MEM) and MEM from previous endometrial incubations (n = 3 incubates). Incubates from all groups received 5 μ Ci [3 H]-arachidonic acid ([3 H]-AA) and 200 μ g radioinert arachidonic acid (AA) and were incubated for 24 h at 37°C. Endometrial incubations from which MEM was taken for mixture in Group 4 did not receive [3 H]-AA. After incubation, tissues and MEM were separated, and [3 H]-AA and its metabolites were extracted from MEM and separated on columns of Sephadex LH-20.

RESULTS

The Sephadex LH-20 chromatographic separation of [^3H]-AA and its metabolites in extracts of MEM which contained bovine blastocysts (group 1) is shown in figure 1. The elution profile was divided into eight regions, and, based upon Sephadex LH-20 chromatography and HPLC, regions II, IV, V and VI migrated with [^3H]-AA, [^3H]-13,14-dihydro-15-keto-PGF $_2\alpha$ ([^3H]-PGFM), [^3H]-PGE $_2$ and [^3H]-PGF $_2\alpha$, respectively. These regions might have contained other compounds that were not resolved by the chromatographic methods used. The metabolites of [^3H]-AA in regions I, III, VII and VIII have not been identified.

Fig. 1. Sephadex LH-20 elution profile of metabolism of [^3H]-AA in incubation medium from day 19 bovine blastocyst incubates. Regions II, IV, V and VI migrated with [^3H]-AA, [^3H]-PGFM, [^3H]-PGE $_2$ and [^3H]-PGF $_2\alpha$, respectively.

The greatest ($P < .05$) percentage of total dpm in groups 1 (43.0 ± 6.0) and 4 ($38.0 \pm .7$) was recovered as [^3H]-PGF $_2\alpha$, while in group 3, [^3H]-PGE $_2$ ($25.9 \pm 7.4\%$) and [^3H]-PGF $_2\alpha$ ($29.4 \pm 6.7\%$) were recovered in approximately equal proportions (table 1). In group 2 and in the incubation controls, the majority ($P < .05$) of the dpm was recovered as [^3H]-AA ($64.8 \pm 3.6\%$ and $78.3 \pm 3.2\%$, respectively), with only small percentages of the total dpm in the other chromatographic regions.

Table 1. Distribution of percentage of total dpm in eluates from Sephadex LH-20 chromatography of extracts of MEM from incubates that contained bovine blastocysts and endometrium recovered on day 19 of pregnancy. a, b, c

Region	Group 1	Group 2	Group 3	Group 4	Group 1/4	Incubation control
I	2.0 ± .4 ^d	2.1 ± .2 ^d	1.4 ± .2 ^d	2.2 ± .6 ^d	2.1 ± .4 ^d	5.0 ± .7 ^d
II ([³ H]-AA)	5.1 ± .4 ^e	64.8 ± 3.6 ^e	8.9 ± 1.9 ^e	11.3 ± 4.3 ^e	7.8 ± 1.8 ^e	78.3 ± 3.2 ^e
III	7.1 ± .5 ^e	9.8 ± .8 ^f	10.4 ± .9 ^e	7.2 ± .8 ^e	7.2 ± .4 ^e	6.6 ± .7 ^d
IV ([³ H]-PGFM)	5.5 ± .4 ^e	4.8 ± .4 ^g	10.1 ± 1.2 ^e	8.0 ± 1.3 ^e	6.6 ± .6 ^e	1.9 ± .3 ^f
V ([³ H]-PGE ₂)	12.0 ± 1.4 ^f	4.8 ± .3 ^g	25.9 ± 7.4 ^f	20.4 ± 4.1 ^f	15.6 ± 1.9 ^f	1.6 ± .2 ^f
VI ([³ H]-PGF ₂ α)	43.0 ± 6.0 ^g	4.7 ± .4 ^g	29.4 ± 6.7 ^f	38.0 ± .7 ^g	40.8 ± 3.6 ^g	2.3 ± .5 ^f
VII	11.3 ± 2.3 ^f	5.0 ± .7 ^g	7.6 ± 1.3 ^e	6.2 ± 1.1 ^e	9.1 ± 1.4 ^e	2.5 ± 1.0 ^f
VIII	14.0 ± 4.1 ^f	4.0 ± .7 ^g	6.3 ± 1.9 ^e	6.7 ± 1.8 ^e	10.8 ± 2.5 ^e	1.8 ± .4 ^f

a Group 1; blastocysts incubated alone (n = 4).

Group 2; endometrium incubated alone (n = 24).

Group 3; blastocysts incubated with endometrium (n = 5).

Group 4; blastocysts incubated in 7.5 ml fresh MEM plus 7.5 ml MEM from an endometrial incubate that did not contain [³H]-AA (n = 3).

Group 1/4; data from groups 1 and 4 combined.

Incubation Control; [³H]-AA incubated in MEM without tissue.

b Means ± S.E.M. with different superscript letters within a column indicate differences (P < .05) among regions within the incubation group.

c See table 2 for statistical comparisons between incubation groups for each region.

To determine if endometrial slices or secretions altered metabolism of [^3H]-AA, percentages of total dpm within each region were compared among incubation groups (table 2). The percentage of total dpm in each region did not differ between groups 1 and 4, so the data were combined (group 1/4) and compared to group 3. Neither endometrial slices nor endometrial secretions altered the proportion of [^3H]-AA metabolized, i.e., the proportion of dpm recovered as [^3H]-AA did not differ significantly between groups 1/4 and 3. Group 3 produced more [^3H]-PGFM ($P < .05$) and region III ($P < .01$) and tended ($P < .10$) to produce less [^3H]-PGF $_2\alpha$ and more [^3H]-PGE $_2$ than did group 1/4. Endometrial slices in group 2 produced less ($P < .05$) [^3H]-PGFM, [^3H]-PGE $_2$ and [^3H]-PGF $_2\alpha$ than incubates that contained blastocysts.

Table 2. Statistical Comparisons between Incubation Groups for Each Region.^a

Region	Group 1 vs Group 4	Group 1/4 vs Group 2	Group 2 vs Group 3	Group 1/4 vs Group 3
I	NS ^b	NS	NS	NS
II ([^3H]-AA)	NS	** ^c	**	NS
III	NS	NS	NS	**
IV ([^3H]-PGFM)	NS	*	**	*
V ([^3H]-PGE $_2$)	NS	**	**	TD ^d
VI ([^3H]-PGF $_2\alpha$)	NS	**	**	TD
VII	NS	*	NS	NS
VIII	NS	**	NS	NS

^a See table 1 for the data and the descriptions of incubation groups.

^b Means did not differ significantly.

^c Means were different; * $P < .05$, ** $P < .01$.

^d Means tended to differ; $P < .10$.

DISCUSSION

In the present study, MEM that contained endometrial secretions did not alter the profile of metabolism of [^3H]-AA by blastocysts, and endometrial slices incubated with blastocysts did not alter the proportion of [^3H]-AA metabolized. Endometrial slices incubated with blastocysts appeared to modify the pattern of metabolism of [^3H]-AA, i.e., blastocysts and endometrial slices incubated together tended to produce more [^3H]-PGE₂ than did blastocysts incubated alone or blastocysts incubated in MEM from endometrial incubations. The increase in proportion of [^3H]-PGE₂ found when blastocysts and endometrial slices were incubated together did not appear to be an additive effect of the endometrial slices because endometrium incubated alone metabolized very little [^3H]-AA to prostaglandins. Alteration of the metabolism of [^3H]-AA appeared to require the presence of endometrial slices and not just endometrial secretions. The current study was in vitro, so it still is not known whether the endometrium can regulate AA metabolism by blastocysts in vivo.

The proportion of [^3H]-PGFM was increased and [^3H]-PGF₂ α was decreased in MEM from incubates that had contained blastocysts and endometrial slices compared to MEM from incubates that contained blastocysts alone or blastocysts incubated in MEM from endometrial incubations. This result indicates that endometrial slices metabolized a portion of the [^3H]-PGF₂ α that was produced by blastocysts. It has been shown that endometrial slices are capable of metabolizing considerably more PGF₂ α than are blastocysts, therefore it is likely that the endometrium has an important role in controlling the amount of PGF₂ α and probably PGE₂ that accumulates in the uterine lumen and the amounts and types of prostaglandins that leave the uterus during early pregnancy. Indeed, on day 17 postestrus, pregnant heifers had significantly higher basal concentrations of PGFM in jugular plasma than did nonpregnant heifers; part of this PGFM could have been produced by endometrium from PGF₂ α secreted by blastocysts.

The results of the present study indicate that the endometrium altered the metabolism of [^3H]-AA by blastocysts, i.e., the endometrium appeared to direct blastocyst metabolism of AA away from synthesis of $\text{PGF}_{2\alpha}$ and toward synthesis of PGE_2 , and the endometrium metabolized $\text{PGF}_{2\alpha}$ produced by bovine blastocysts in vitro. This indicates that the endometrium may have an important role in regulating the amounts and ratios of prostaglandins in the uterine milieu and in the uterine venous effluent during early pregnancy in cattle. Additional studies are needed to determine if the endometrium has similar influences on prostaglandins produced by blastocysts in vivo.

In a second in vitro study, we determined the effect of bovine endometrial slices on accumulation of prostaglandins and incorporation of [^{14}C]-leucine by bovine blastocysts. For this study, blastocysts and endometrial slices were collected from uteri of 9 Holstein cows superovulated with follicle-stimulating hormone and slaughtered on day 19 of pregnancy (day 0 = day of estrus). Tissues from each cow were incubated for 24 hr at 37°C in petri dishes containing 10 ml of modified minimum essential medium (GIBCO Labs, Grand Island, NY), 200 μg of radioinert arachidonic acid (Sigma Chemical Co., St. Louis, MO) and 2 μCi of L[1- ^{14}C]-leucine (New England Nuclear, Boston, MA). The incubation groups were: blastocysts (171 ± 32 mg) incubated alone ($n = 9$); blastocysts (223 ± 34 mg) incubated with endometrial slices (503 ± 2 mg; $n = 9$); and endometrial slices (502 ± 2 mg) incubated alone ($n = 9$). At the end of the incubation period, tissues and media were separated by centrifugation and frozen. Prostaglandins E_2 (PGE_2) and $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) and 13,14-dihydro-15-keto- $\text{PGF}_{2\alpha}$ (PGFM) were quantified in aliquots of incubation medium by radioimmunoassays. Macromolecules were precipitated from aliquots of medium with 0.5 M trichloroacetic acid, disintegrations per minute of [^{14}C] were determined, and picomoles (pM) of [^{14}C]-leucine incorporated into macromolecules were calculated. All data

were expressed as quantity/ μ g of DNA. Data were analyzed by least-squares analyses of variance using the General Linear Models procedure of the Statistical Analysis System.

Results

More ($P < .05$) $\text{PGF}_{2\alpha}$ and PGE_2 accumulated in medium in which blastocysts were incubated alone than in medium in which blastocysts were incubated with endometrial slices or in which endometrial slices were incubated alone (Table 1). Medium which contained blastocysts incubated with endometrial slices or endometrial slices incubated alone did not differ in accumulation of $\text{PGF}_{2\alpha}$ or PGE_2 (Table 1). Incubation medium which contained endometrial slices or blastocysts alone accumulated comparable amounts of PGFM, but more ($P < .05$) PGFM accumulated in medium in which blastocysts were incubated with endometrial slices than in medium from the other two treatment groups (Table 1). Blastocysts incubated alone incorporated more ($P < .05$) [^{14}C]-leucine into soluble macromolecules than did blastocysts incubated with endometrial slices, and blastocysts incubated with endometrial slices incorporated more ($P < .05$) [^{14}C]-leucine than did endometrial slices incubated alone (Table 1).

Discussion

Bovine blastocysts recovered on day 19 of pregnancy produced prostaglandins and macromolecules in vitro. Blastocysts were considerably more active, per microgram of DNA, in producing $\text{PGF}_{2\alpha}$, PGE_2 and macromolecules than were endometrial slices, but endometrial slices were just as active as blastocysts in metabolizing $\text{PGF}_{2\alpha}$ to PGFM and presumably PGE_2 to its metabolites. Endometrial slices significantly attenuated accumulation of PGE_2 and $\text{PGF}_{2\alpha}$ and incorporation of [^{14}C]-leucine when they were incubated with blastocysts. This is similar to but not in complete agreement with previous work from this laboratory. In previous work, endometrial slices reduced [^3H]- $\text{PGF}_{2\alpha}$ production but increased production of [^3H]- PGE_2 .

The results of this in vitro study indicate that endometrial slices can modulate the synthesis of macromolecules by bovine blastocysts. In addition, it appears that endometrial slices regulate accumulation of PGE₂ and PGF₂α by either metabolizing these prostaglandins as they are released, by suppressing their synthesis or perhaps by a combination of the two mechanisms. It is possible that the accumulation of PGE₂ and PGF₂α and incorporation of [¹⁴C]-leucine by blastocysts incubated alone is somewhat out of control, and the endometrium is required to regulate these biochemical processes. In vivo, the endometrium might play an essential role in regulating the rate of protein synthesis by blastocysts which may be important for maintaining the synchrony between the blastocyst and the uterus. Also, the endometrium may be involved in controlling the amounts and classes of prostaglandins of blastocyst origin that accumulate in the uterine lumen.

Because of the difficulties associated with using cattle for studies of blastocysts, we used sheep and pigs to study aspects of blastocyst metabolism that required more blastocysts than we were able to obtain from cattle. We conducted four experiments to examine the metabolism of [³H] arachidonic acid ([³H]AA) and prostaglandin synthesis by ovine conceptuses and endometrial slices collected on various days after mating.

TABLE 3. VALUES OF VARIABLES MEASURED IN INCUBATION MEDIUM

Item	PGF ₂ ^a (ng)	PGFM ^a (ng)	PGE ₂ (ng)	¹⁴ C-leucine ^b (pM)
	———— quantity/μg DNA ————			
Blastocysts incubated alone	23.8 ^d	0.8 ^d	7.3 ^d	3.6 ^d
Blastocysts incubated with endometrial slices	0.6 ^e	1.8 ^e	0.3 ^e	0.4 ^e
Endometrial slices incubated alone	0.03 ^e	0.1 ^d	0.02 ^e	0.1 ^e
Standard error of the mean ^c	3.5	0.2	0.8	0.2

^a PGFM = 13,14-dihydro-15-keto-PGF₂^a.

^b Incorporation of [¹⁴C]-leucine into macromolecules.

^c Standard error of the mean from the model used to analyze the data.

^{d,e} Values within a column with different superscript letters differed (P<.05).

MATERIALS AND METHODS

Experiment 1.

To determine if blastocysts and endometrial slices could metabolize arachidonic acid in vitro, multiparous ewes, predominantly of Rambouillet breeding, were mated at a spontaneous estrus during the breeding season. Ewes were killed 14 (n=3) or 16 (n=3) days after mating, and the reproductive tract was recovered from each ewe. Blastocysts were flushed from the uterus of each ewe with sterile, isotonic saline. The blastocyst (day 14, 50 ± 7 mg; day 16, 114 ± 11 mg) from each ewe was transferred immediately to a petri dish containing 15 ml of modified minimum essential medium (MEM). Endometrium was dissected from uterine horns ipsilateral and contralateral to the corpus luteum (all ewes ovulated unilaterally), sliced with scalpel blades into pieces approximately 10 mm^3 , and 510 ± 7 mg were transferred to separate petri dishes containing 15 ml of MEM. Five microcuries of [5,6,8,9,11,12,14,15(N)- ^3H] arachidonic acid (^3H]AA; 60 to 100 Ci/mmol; New England Nuclear, Boston, MA) in 50 μl ethanol and 200 μg radioinert arachidonic acid (AA; Sigma Chemical Co., St. Louis, MO) in 20 μl ethanol were added to each incubate. All incubates were rocked for 24 hr at 37°C in an atmosphere of 50% N_2 :45% O_2 :5% CO_2 .

At the end of the incubation period, tissues and MEM were separated by centrifugation at 4°C and stored separately at -20°C . Arachidonic acid and its metabolites were extracted from aliquots of MEM and separated on columns of Sephadex LH-20 as described by us previously. Disintegrations per minute (dpm) were determined for all fractions from each column, and percentage of the total

dpm was calculated for each fraction by dividing each dpm by the total dpm recovered from the respective column. Profiles of metabolism of [^3H]AA were evaluated by plotting percentages of total dpm against fraction number. Separate extracts of MEM were evaluated by reversed-phase high performance liquid chromatography (HPLC) and thin layer chromatography.

Based upon Sephadex LH-20 profiles of metabolism of [^3H]AA by blastocysts, eight regions were discerned. Percentages of total dpm for all fractions within each region were totaled so that eight percentages were derived from each column. Data from endometrial incubates were reduced in this same manner. Percentages among regions within day postmating were compared by the General Linear Models (GLM) procedure of the Statistical Analysis System. Statistical comparisons were not made between days or between blastocysts and endometrial slices.

Experiment 2.

To quantify accumulation of prostaglandins in vitro, ewes were mated during the breeding season and killed 14 ($n=4$) or 16 ($n=4$) days later. Blastocysts (day 14, 32 ± 13 mg; day 16, 184 ± 30 mg) and endometrial slices (502 ± 2 mg) from each ewe were incubated as described for experiment 1 except that [^3H]AA was not added to the incubates. Prostaglandins were quantified in aliquots of MEM using radioimmunoassays for $\text{PGF}_2\alpha$, PGE_2 , 13,14-dihydro-15-keto- $\text{PGF}_2\alpha$ (PGFM) and 6-keto- $\text{PGF}_{1\alpha}$ (the stable metabolite of PGI_2) that have been described previously by Pexton et al. (1975), Lewis et al. (1978), Guthrie and Rexroad (1981), and Silvia et al. (1984), respectively. Prostaglandin values were corrected to the total volume of MEM. Total

quantities of each prostaglandin were compared for each tissue-type between days of pregnancy. Quantities of prostaglandins were not compared between incubates that contained blastocysts and those that contained endometrial slices because the total blastocyst from each ewe was incubated, whereas only a portion of the endometrium from each ewe was incubated. In addition, the quantities of prostaglandins were expressed as ng/mg tissue. Data for each prostaglandin per milligram of tissue were analyzed as a 2x2 factorial (day postmating and tissue type were the main effects). Accumulation of the individual prostaglandins (on a total quantity and a ng/mg tissue basis) was compared for each tissue type for each day of pregnancy. All analyses were by the GLM procedure of SAS.

Experiment 3.

Ewes were killed 16 (n=12), 20 (n=12) or 24 (n=12) days after mating during the breeding season to determine the pattern of metabolism of arachidonic acid by conceptuses and slices of endometrium in vitro and to evaluate the effects of indomethacin on metabolism of arachidonic acid by these tissues. From the ewes killed on day 16 of pregnancy, blastocysts (181 ± 34 mg) were recovered and transferred to MEM as described for experiment 1. On days 20 and 24 of pregnancy, conceptuses were recovered by dissecting open the uterine horns and transferring the conceptus with membranes intact to a petri dish. Membranes then were ruptured, embryos were removed and membranes (chorion on day 20, 257 ± 57 mg; chorioallantois on day 24, 1806 ± 269 mg) were transferred to MEM. Endometrial slices (511 ± 3 mg) were recovered, and all tissues were incubated as described for experiment 1 except that tissues from one-half of the ewes killed on each day were incubated with 4×10^{-4} M indomethacin (Sigma Chemical Co.).

At the end of the incubation period, tissues and MEM were separated, stored at -20°C and then analyzed as described for experiment 1. Percentages of total dpm for each chromatographic region were compared between indomethacin-treated and control incubates within each day by the GLM procedure of SAS.

Experiment 4.

To characterize the pattern over time of arachidonic acid metabolism by conceptuses, ewes were killed 16 ($n=6$), 20 ($n=5$) or 24 ($n=6$) days after mating during the breeding season. Blastocysts (day 16, 202 ± 55 mg) and conceptuses (day 20, 281 ± 75 mg; day 24, 728 ± 174 mg) were recovered and incubated as described for experiment 3. However, in this experiment, indomethacin was not added to any of the incubates, and MEM was sampled (0.5 ml/sample) at 0, 2, 4, 6, 12 and 24 hr from the onset of incubation. Samples of MEM were frozen immediately after collection. All incubations commenced within 5 min of the time of addition of $[^3\text{H}]\text{AA}$ to the incubates. Based upon the results of experiment 3, endometrial slices were not incubated because their metabolism of $[^3\text{H}]\text{AA}$ to prostaglandins was not sufficient for quantitation.

After incubation, tissues and MEM were separated and stored frozen. All samples of MEM were analyzed using Sephadex LH-20 as described for experiment 1. The only exception was that only three column fractions were collected; a fraction containing a compound(s) less polar than AA, the AA fraction, and a fraction containing the prostaglandins. Time trends for each fraction were analyzed within day using the GLM procedure of SAS.

RESULTS

Experiment 1.

Patterns of metabolism of [^3H]AA, as assessed by Sephadex LH-20 chromatography, by blastocysts recovered on days 14 and 16 of pregnancy are shown in figure 2. Eight chromatographic regions were discerned visually. Based upon calibration of Sephadex LH-20 columns with authentic radiolabeled standards, regions II, IV, V, VI and VII corresponded to AA, PGFM, PGE_2 , $\text{PGF}_2\alpha$ and 6-keto- $\text{PGF}_1\alpha$, respectively. Thin layer chromatography indicated that region I contained triglycerides. The HPLC profiles (figure 3) of AA and its metabolites indicated that some of the Sephadex LH-20 regions probably contained compounds in addition to the ones that migrated with authentic standards. However, the major HPLC peaks (I, II, III, IV and V) migrated with 6-keto- $\text{PGF}_1\alpha$, $\text{PGF}_2\alpha$, PGE_2 , PGFM and AA, respectively.

The percentages of the total dpm in each Sephadex LH-20 chromatographic region from extracts of MEM which contained blastocysts are shown in table 4. Day-14 blastocysts metabolized 64.8% of the [^3H]AA, and they produced approximately equal proportions of PGE_2 , $\text{PGF}_2\alpha$ and 6-keto- $\text{PGF}_1\alpha$. The percentage of the total dpm as PGFM could not be distinguished from background (5.0%). Background was defined as the average of the percentages of total dpm that had the smallest numerical values and that did not differ significantly from each other, i.e., in table 1 the background from the day-14 blastocyst incubates would correspond to the average of all values with a superscript of "c". Blastocysts recovered on day 16 of pregnancy metabolized 90.3% of the [^3H]AA. Prostaglandin $\text{F}_2\alpha$ and 6-keto- $\text{PGF}_1\alpha$ were produced in about equal

Figure 2. Sephadex LH-20 elution patterns of metabolism of [^3H] arachidonic acid by ovine blastocysts recovered on days 14 and 16 of pregnancy and incubated for 24 hr. Regions II, IV, V, VI and VII contained arachidonic acid, 13,14-dihydro-15-keto-PGF $_2\alpha$, PGE $_2$, PGF $_2\alpha$ and 6-keto-PGF $_1\alpha$, respectively. Each point used to construct the graphs was the average of percentages of total dpm from 3 blastocyst incubations per day of pregnancy. The vertical lines indicate the points at which the regions were divided.

Figure 3. Reversed-phase HPLC elution patterns of metabolism of [^3H] arachidonic acid by ovine blastocysts recovered on days 14 and 16 of pregnancy and incubated for 24 hr. Peaks I, II, III, IV and V contained 6-keto-PGF $_1\alpha$, PGF $_2\alpha$, PGE $_2$, 13,14-dihydro-15-keto-PGF $_2\alpha$ and arachidonic acid, respectively. Each point used to construct the graphs was the average of percentages of total dpm from 3 blastocyst incubations per day of pregnancy. The vertical line shows where peaks III and IV should be separated.

Table 4. Distribution of percentage of total dpm in extracts of medium from incubates that contained ovine blastocysts recovered on days 14 and 16 of pregnancy and incubated with [^3H] arachidonic acid for 24 hr.^a

Chromatographic region	Percentage of the total dpm	
	Day 14	Day 16
I	4.9 $\pm$ 0.6 ^c	7.3 $\pm$ 1.0 ^c
II (AA) ^b	35.2 $\pm$ 5.5 ^d	9.7 $\pm$ 0.4 ^c
III	7.1 $\pm$ 0.3 ^c	5.6 $\pm$ 0.2 ^c
IV (PGFM) ^b	3.7 $\pm$ 0.3 ^c	3.6 $\pm$ 0.1 ^c
V (PGE ₂)	11.2 $\pm$ 1.3 ^e	10.4 $\pm$ 0.3 ^c
VI (PGF ₂ α)	16.2 $\pm$ 1.9 ^e	24.9 $\pm$ 0.3 ^d
VII (6-keto-PGF ₁ α)	18.3 $\pm$ 2.2 ^e	31.0 $\pm$ 1.2 ^d
VIII	3.1 $\pm$ 0.4 ^c	7.1 $\pm$ 0.2 ^c

^a Day-14 blastocysts weighed 50 $\pm$ 7 mg and day-16 blastocysts weighed 114 $\pm$ 11 mg. The data were not adjusted for tissue weights.

^b AA = arachidonic acid; PGFM = 13,14-dihydro-15-keto-PGF₂ α .

^{c,d,e} Means within a column with different superscript letters differed (P<.05).

proportions (24.9 ± 0.3 and 31.0 ± 1.2 % of total dpm, respectively), and, together, they accounted for 55.9% of the total dpm. The percentages as AA, PGFM and PGE₂ were not different from background (7.3%).

Endometrial slices recovered on days 14 and 16 of pregnancy metabolized about 41% of the [³H]AA (table 5), but only a small proportion of the [³H]AA was recovered as identifiable prostaglandins. Day-14 endometrial slices synthesized (% of total dpm greater, $P < .05$, than background) PGF₂α ($8.7 \pm 0.4\%$) and region III ($9.7 \pm 0.2\%$; identity unknown). Endometrial slices recovered on day 16 synthesized PGE₂ ($5.5 \pm 0.2\%$), PGF₂α ($8.2 \pm 0.2\%$), 6-keto-PGF₁α ($6.2 \pm 0.03\%$), region I ($5.3 \pm 0.2\%$) and region III ($8.9 \pm 0.2\%$).

Experiment 2.

Quantities of prostaglandins accumulated in vitro per milligram of blastocyst or endometrial slices are shown in table 6. Blastocysts accumulated more ($P < .05$) PGF₂α during the 24 hr incubation than did endometrial slices (20.6 vs 0.5 ng/mg), but the accumulation of PGFM (3.8 ng/mg) was not affected by tissue type. Accumulation of PGE₂ and 6-keto-PGF₁α differed ($P < .05$) with tissue type and day of pregnancy, but there was an interaction ($P < .05$) of tissue type and day of pregnancy because day-14 blastocysts accumulated more PGE₂ and 6-keto-PGF₁α than did day 16 blastocysts (PGE₂; 12.3 ± 7.5 vs 0.9 ± 0.2 ng/mg and 6-keto-PGF₁α; 41.4 ± 4.8 vs 15.9 ± 2.7 ng/mg).

Day-14 blastocysts accumulated similar quantities (ng/mg) of the four prostaglandins assayed. However, day-16 blastocysts accumulated (ng/mg) more

Table 5. Distribution of percentage of total dpm in extracts of medium from incubates that contained ovine endometrial slices recovered on days 14 and 16 of pregnancy and incubated with [^3H] arachidonic acid for 24 hr.

Chromatographic region	Percentage of the total dpm	
	Day 14	Day 16
I	4.1 ± 0.3^b	5.3 ± 0.2^b
II (AA) ^a	58.1 ± 1.6^c	59.9 ± 0.3^c
III	9.7 ± 0.2^d	8.9 ± 0.2^d
IV (PGFM) ^a	5.1 ± 0.3^b	3.8 ± 0.1^e
V (PGE ₂)	7.2 ± 0.3^b	5.5 ± 0.2^b
VI (PGF ₂ α)	8.7 ± 0.4^d	8.2 ± 0.2^b
VII (6-keto-PGF ₁ α)	5.9 ± 0.1^b	6.2 ± 0.03^b
VIII	1.2 ± 0.04^b	2.2 ± 0.05^e

^a AA = arachidonic acid; PGFM = 13,14-dihydro-15-keto-PGF₂ α .

b,c,d,e Means within a column with different superscript letters differed ($P < 0.05$).

Table 6. Quantities per milligram of wet tissue of prostaglandins released into incubation medium by ovine blastocysts and endometrial slices collected on days 14 and 16 of pregnancy and incubated for 24 hr.

Prostaglandin	Blastocysts		Endometrial slices	
	Day 14	Day 16	Day 14	Day 16
	quantity (ng)/mg tissue ^a			
PGF ₂ ^a	32.1 ± 17.9 ^e	9.0 ± 4.1 ^e	0.6 ± 0.09 ^e	0.4 ± 0.05 ^e
PGFM ^c	8.4 ± 6.2 ^e	0.5 ± 0.08 ^f	3.2 ± 0.1 ^f	2.9 ± 0.2 ^f
PGE ₂ ^d	12.3 ± 7.5 ^e	0.9 ± 0.2 ^f	0.1 ± 0.04 ^e	0.03 ± 0.01 ^e
6-keto-PGF ₁ ^a	41.4 ± 4.8 ^e	15.9 ± 2.7 ^e	5.0 ± 0.4 ^g	3.1 ± 0.2 ^f

^a Quantities/mg tissue are means ± standard errors of the means. Average wet tissue weights for day-14 and -16 blastocysts were 32 ± 13 mg and 184 ± 30 mg, respectively, and was 502 ± 2 mg for endometrial slices.

^b Means differed (P<.05) between tissue types.

^c PGFM = 13,14-dihydro-15-keto-PGF₂^a.

^d Means differed between tissue types (P<.05) and between days (P<.05), but there was a tissue type by day interaction (P<.05).

^{e,f,g} Means within a column with different superscript letters differed (P<.05).

($P < .05$) $\text{PGF}_2\alpha$ (9.0 ± 4.1) and 6-keto- $\text{PGF}_1\alpha$ (15.9 ± 2.7) than PGFM (0.5 ± 0.08) or PGE_2 (0.9 ± 0.2). Endometrial slices collected on day 14 of pregnancy accumulated (ng/mg) more ($P < .05$) 6-keto- $\text{PGF}_1\alpha$ (5.0 ± 0.4) than PGFM (3.2 ± 0.1). Prostaglandin $\text{F}_2\alpha$ and PGE_2 were accumulated in similar quantities (0.35 ng/mg), and these quantities were less ($P < .05$) than those of 6-keto- $\text{PGF}_1\alpha$ and PGFM. On day 16, endometrial slices accumulated comparable amounts (ng/mg) of 6-keto- $\text{PGF}_1\alpha$ and PGFM (3.0), and these amounts were greater ($P < .05$) than those of PGE_2 and $\text{PGF}_2\alpha$ (0.2).

In incubates which contained day 14 and day 16 blastocysts, total quantities (ng/incubate) of $\text{PGF}_2\alpha$ (day 14, 408.8 ± 7.95 ; day 16, 1294.9 ± 493.8), PGFM (66.6 ± 9.2 ; 78.7 ± 3.4), PGE_2 (250.8 ± 132.3 ; 158.0 ± 40.4) and 6-keto- $\text{PGF}_1\alpha$ (1482.9 ± 638.3 ; 3012.5 ± 885.4) did not differ with day of pregnancy (table 7). Endometrial slices recovered on days 14 and 16 accumulated similar quantities (ng/incubate) of $\text{PGF}_2\alpha$ (281.4 ± 44.2 and 218.8 , respectively), PGFM (1613.6 ± 64.1 and 1447.9 ± 99.4 , respectively) and PGE_2 (49.7 ± 20.9 and 16.8 ± 5.0 , respectively) but endometrial slices recovered on day 14 of pregnancy accumulated more ($P < .05$) 6-keto- $\text{PGF}_1\alpha$ than did those recovered on day 16 (2498.9 ± 228.6 vs 1572.1 ± 127.3 ; table 7).

Regardless of day of pregnancy on which they were recovered, blastocysts accumulated more ($P < .05$; ng/incubate) 6-keto- $\text{PGF}_1\alpha$ than $\text{PGF}_2\alpha$, PGFM or PGE_2 (table 7). Endometrial slices recovered on day 14 of pregnancy produced more ($P < .05$; ng/incubate) 6-keto- $\text{PGF}_1\alpha$ than PGFM, and they accumulated more PGFM than PGE_2 or $\text{PGF}_2\alpha$ (table 7). On day 16, endometrial slices accumulated (ng/incubate) similar quantities of PGFM and 6-keto- $\text{PGF}_1\alpha$, which were accumulated in greater ($P < .05$) amounts than were $\text{PGF}_2\alpha$ and PGE_2 (table 7).

Table 7. Total quantities of prostaglandins released into incubation medium by ovine blastocysts and endometrial slices recovered on days 14 and 16 of pregnancy and incubated for 24 hr.^a

Prostaglandin	Blastocysts		Endometrial slices	
	Day 14	Day 16	Day 14	Day 16
	quantity (ng)/incubate ^b			
PGF ₂ α	408.8 $\pm$ 79.5 ^e	1294.9 $\pm$ 493.8 ^e	281.4 $\pm$ 44.2 ^e	218.8 $\pm$ 26.7 ^e
PGFM ^c	66.6 $\pm$ 9.2 ^e	78.7 $\pm$ 3.4 ^e	1613.6 $\pm$ 64.1 ^f	1447.9 $\pm$ 99.4 ^f
PGE ₂	250.8 $\pm$ 132.3 ^e	158.0 $\pm$ 40.4 ^e	49.7 $\pm$ 20.9 ^e	16.8 $\pm$ 5.0 ^e
6-keto-PGF ₁ α ^d	1482.9 $\pm$ 638.3 ^f	3012.5 $\pm$ 885.4 ^f	2498.9 $\pm$ 228.6 ^g	1572.1 $\pm$ 127.3 ^f

^a Quantities of prostaglandins were not compared between incubates that contained blastocysts and those that contained endometrial slices because the total blastocyst from each ewe was incubated, whereas only a portion of the endometrium from each ewe was incubated.

^b Quantities/incubate are means $\pm$ standard errors of the means. Average wet tissue weights for day -14 and -16 blastocysts were 32 $\pm$ 13 mg and 184 $\pm$ 30 mg, respectively, and was 502 $\pm$ 2 mg for endometrial slices.

^c PGFM = 13,14-dihydro-15-keto-PGF₂ α .

^d Means for endometrial slices differed (P<.003) between days.

^{e,f,g} Within columns, means with different superscript letters differed (P<.05).

Experiment 3.

The effects of indomethacin on metabolism of [^3H]AA by blastocysts and endometrial slices are shown in tables 8 and 9. Blastocysts recovered on day 16 of pregnancy produced significant (greater, $P < .05$, than a background of 10.5%) amounts of $\text{PGF}_2\alpha$ and 6-keto- $\text{PGF}_1\alpha$. Indomethacin reduced ($P < .05$) the percentage of total dpm recovered as $\text{PGF}_2\alpha$ (from 15.1 ± 0.5 to 1.9 ± 0.1) and as 6-keto- $\text{PGF}_1\alpha$ (from 20.1 ± 0.4 to 2.5 ± 0.2) without causing a significant reduction in overall metabolism of [^3H]AA. The major effect of indomethacin was an increase ($P < .05$) in the percentage of total dpm recovered in region I (from 10.9 ± 0.4 to 59.8 ± 1.6) and an overall reduction in the percentages of total dpm in regions III through VIII. Day-20 conceptus tissue produced significant ($P < .05$) proportions (background of 4.6%) of regions I and III, and of $\text{PGF}_2\alpha$. Indomethacin reduced ($P < .05$) the synthesis of $\text{PGF}_2\alpha$, but it did not reduce the percentages of total dpm in regions I or III and did not cause a significant change in utilization of [^3H]AA. Conceptus tissues recovered on day 24 of pregnancy produced significant ($P < .05$) amounts (background of 3.6%) of regions I and III, and of PGFM , PGE_2 , $\text{PGF}_2\alpha$ and 6-keto- $\text{PGE}_1\alpha$. However, $\text{PGF}_2\alpha$ was the only metabolic product of [^3H]AA that was present in striking quantities. Indomethacin did not alter the metabolic profile of [^3H]AA by the day-24 conceptuses.

Endometrial slices recovered on days 16 and 24 of pregnancy and incubated as controls did not metabolize [^3H]AA to detectable amounts of prostaglandins. Day-20 endometrial slices incubated as controls produced (% of total dpm) significant ($P < .05$) amounts of $\text{PGF}_2\alpha$ (9.2 ± 0.4) and region III (12.4 ± 0.7), and they metabolized a significant proportion of the [^3H] AA to region

Table 8. Effects of $4 \times 10^{-4}M$ indomethacin on the metabolism of [3H] arachidonic acid by ovine conceptuses collected on days 16, 20 and 24 of pregnancy and incubated for 24 hr.

Chromatographic region	Percentage of total dpm					
	Day 16		Day 20		Day 24	
	Control	Indomethacin	Control	Indomethacin	Control	Indomethacin
I						
II (AA) ^a	$10.9 \pm 0.4^{b,c}$	59.8 ± 1.6^c	26.1 ± 2.5^c	20.4 ± 2.8^c	13.6 ± 1.2^c	18.3 ± 1.4^c
III	12.6 ± 0.9^c	18.9 ± 0.8^d	28.9 ± 3.0^c	43.7 ± 2.8^d	17.2 ± 0.7^c	29.0 ± 1.2^d
IV (PGFM) ^a	10.0 ± 0.3^c	8.6 ± 0.5^e	12.8 ± 0.8^d	14.2 ± 0.6^d	12.5 ± 0.4^d	13.2 ± 0.4^e
V (PGE ₂)	9.7 ± 0.4^c	4.9 ± 0.3^f	5.4 ± 0.2^e	7.8 ± 0.9^e	12.5 ± 0.5^d	9.4 ± 0.4^f
VI (PGF _{2α})	$9.5 \pm 0.5^{b,c}$	2.6 ± 0.1^f	5.8 ± 0.3^e	3.7 ± 0.3^e	11.9 ± 0.4^d	7.2 ± 0.6^f
VII (6-keto-PGF _{1α})	$15.1 \pm 0.5^{b,d}$	1.9 ± 0.1^f	$13.6 \pm 1.4^{b,d}$	5.9 ± 0.4^e	16.3 ± 2.0^c	13.6 ± 0.6^e
VIII	$20.1 \pm 0.4^{b,d}$	2.5 ± 0.2^f	6.6 ± 0.9^e	3.3 ± 0.2^e	11.9 ± 1.3^d	7.5 ± 0.9^f
	$10.4 \pm 1.1^{b,c}$	0.5 ± 0.1^f	0.6 ± 0.06^e	0.4 ± 0.07^e	3.6 ± 0.8^e	1.4 ± 0.399

^a AA = arachidonic acid; PGFM = 13,14-dihydro-15-keto-PGF_{2α}.

^b Means for the chromatographic region differed ($P < 0.05$) between control and indomethacin-treated conceptuses within day of pregnancy.

c,d,e,f Means within a column without any of these superscript letters in common differed ($P < 0.05$).

Table 9. Effects of $4 \times 10^{-4}M$ indomethacin on the metabolism of [3H] arachidonic acid by ovine endometrial slices collected on days 16, 20 and 24 of pregnancy and incubated for 24 hr.

Chromatographic region	Percentage of total dpm					
	Day 16		Day 20		Day 24	
	Control	Indomethacin	Control	Indomethacin	Control	Indomethacin
I	6.8 ± 0.7^b	5.9 ± 0.1^b	23.6 ± 3.9^b	23.3 ± 4.1^b	8.5 ± 0.3^b	8.6 ± 0.2^b
II (AA) ^a	58.5 ± 1.8^c	59.8 ± 1.2^c	37.6 ± 3.1^b	39.7 ± 3.5^b	47.5 ± 0.9^c	46.2 ± 0.8^c
III	10.4 ± 1.0^b	11.9 ± 0.4^d	12.4 ± 0.7^c	12.4 ± 0.5^c	10.0 ± 0.3^b	12.6 ± 0.2^d
IV (PGFM) ^a	6.5 ± 0.1^b	8.0 ± 0.2^b	4.7 ± 0.4^d	6.5 ± 0.5^d	8.0 ± 0.2^b	8.1 ± 0.3^b
V (PGE ₂)	4.8 ± 0.3^b	4.4 ± 0.2^b	4.6 ± 0.1^d	4.3 ± 0.2^d	5.8 ± 0.2^b	6.2 ± 0.2^b
VI (PGF ₂ α)	4.6 ± 0.5^b	3.8 ± 0.2^b	9.2 ± 0.4^c	6.2 ± 0.3^d	7.2 ± 0.4^b	6.7 ± 0.3^b
VII (6-keto-PGF ₁ α)	5.6 ± 0.4^b	4.8 ± 0.4^b	6.4 ± 0.4^d	6.4 ± 0.3^d	10.2 ± 0.3^b	8.9 ± 0.4^b
VIII	2.8 ± 0.2^b	1.4 ± 0.2^b	1.5 ± 0.2^d	1.2 ± 0.2^d	2.8 ± 0.2^d	2.7 ± 0.2^e

^a AA = arachidonic acid; PGFM = 13,14-dihydro-15-keto-PGF₂ α .

^{b,c,d,e} Means within a column without any of these superscript letters in common differed ($P < 0.05$).

I (23.6 ± 3.9). Indomethacin essentially had no effect on metabolism of [^3H]AA by endometrial slices recovered on days 16, 20 and 24 of pregnancy.

Experiment 4.

The changes over time in percentages of total dpm recovered as [^3H] AA, region I and prostaglandins from extracts of incubation medium which contained conceptuses recovered on days 16, 20 and 24 of pregnancy are shown in figures 4, 5 and 6. With day-16 blastocysts, the changes over time in [^3H]AA and region I were best described by cubic equations (levels of significance from the models used to describe the data were $P < .04$ and $P < .005$, respectively), while the changes in prostaglandins were quadratic ($P < .04$). The percentage of total dpm recovered as [^3H]AA decreased essentially linearly from 56.8 ± 2.9 at time 0 to 28.2 ± 2.1 at 6 hr, a decrease of 38.6 percentage points. From 6 to 12 hr, the decrease was only 8.9 percentage points, and from 12 to 24 hr, the percentage remained constant. Region I (% of total dpm) increased from 6.7 ± 0.8 at time 0 to 50.6 ± 3.4 at 6 hr, remained constant to 12 hr (51.9 ± 3.8), then was decreased (36.7 ± 8.9) at 24 hr of incubation. The percentage of total dpm in the fraction which contained the prostaglandins changed very little during the first 6 hr of incubation. After 6 hr, the percentage as prostaglandins increased, i.e., at 6 hr the percentage was 21.2 ± 1.9 and at 24 hr it was 43.3 ± 8.0 .

Changes over time in the three fractions from the day-20 conceptus incubates were quadratic (region I, $P < .0001$; [^3H]AA, $P < .007$; prostaglandins, $P < .01$). The percentage recovered as [^3H]AA was 67.8 ± 4.9 at time 0 and it had changed by only 6.9 percentage points by 4 hr (60.9 ± 3.2). From 4 to 6

Figure 4. Time course for the metabolism of [^3H] arachidonic acid, and the appearance of region I (unidentified compound(s) less polar than arachidonic acid), and prostaglandins by ovine conceptuses collected on day 16 (n=6) of pregnancy.

Figure 5. Time course for the metabolism of [^3H] arachidonic acid, and the appearance of an unidentified compound(s) less polar than arachidonic acid (region I), and prostaglandins by ovine conceptuses collected on day 20 ($n=5$) of pregnancy.

Figure 6. Time course for the metabolism of [^3H] arachidonic acid, and the appearance of region I, and prostaglandins by ovine conceptuses collected on day 24 (n=6) of pregnancy.

hr, [^3H]AA (% of total dpm) decreased from 60.9 ± 3.2 to 49.6 ± 6.5 (11.3 percentage points), and by 24 hr it had decreased to 23.8 ± 1.8 (another 25.5 percentage points). Region I (% of total dpm) increased from 6.3 ± 0.4 at time 0 to 41.2 ± 6.1 at 6 hr (34.9 percentage points), and it had reached 58.9 ± 7.1 by 24 hr of incubation. The percentage of total dpm as prostaglandins changed very little throughout the incubation period.

The changes over time in [^3H]AA in extracts of incubation medium from day 24 conceptuses was linear ($P < .0001$), and the changes in region I and prostaglandins were quadratic ($P < .04$ and $P < .03$, respectively). The percentage of total dpm recovered as [^3H]AA was 68.8 ± 6.3 at time 0 and 22.6 ± 4.4 24 hr later, a reduction of 46.2 percentage points. Region I increased from 6.6 ± 1.0 at time 0 to 44.5 ± 5.5 at 12 hr (37.9 percentage points), and it was 63.8 ± 6.5 at 24 hr (another 19.3 percentage points). The changes in percentage of total dpm in the fraction which contained the prostaglandins were small throughout the incubation period.

DISCUSSION

Ovine conceptuses recovered on days 14, 16, 20 and 24 of pregnancy metabolized [^3H]AA to prostaglandins and other lipids in vitro. This is consistent with previous studies which demonstrated de novo synthesis of prostaglandins from radiolabeled AA by ovine, bovine, and porcine conceptuses. Ovine conceptuses produced mainly $\text{PGF}_2\alpha$ and 6-keto- $\text{PGF}_1\alpha$, while bovine blastocysts produced primarily $\text{PGF}_2\alpha$ and porcine blastocysts synthesized mostly PGE_2 .

Synthesis of prostaglandins appeared to be enzymatic because prostaglandin synthesis by day 16 and 20 conceptuses was inhibited by indomethacin. Because the incorporation of [^3H] AA into prostaglandins by day 24 conceptuses and by endometrial slices from days 16, 20 and 24 was low, indomethacin could not affect the quantitative production of prostaglandins by these tissues. A similar problem has been reported for human reproductive tissues. Indomethacin increased the synthesis by day 16 blastocysts of region I, a triglyceride-containing region that has been isolated previously. The percentage of total dpm as region I increased as [^3H] AA was metabolized, and the percentage of total dpm as region I began to decrease as the percentage of total dpm recovered as prostaglandins increased. This relationship was most evident with day-16 blastocysts because day-20 and -24 conceptuses did not synthesize prostaglandins as actively as did the day-16 blastocysts. Conceptuses in vitro likely synthesize prostaglandins from AA that has been esterified, stored and then used as required rather than directly from free AA added to incubation medium. Indeed, Bridges and Coniglio (1970) found that rat testes elongated [^{14}C]AA to less polar, storage forms of lipids, and Isakson et al. (1976) found that [^{14}C] AA was incorporated into phospholipids by intact kidney and heart tissues and subsequently metabolized to [^{14}C] prostaglandins.

Even though day-16 blastocysts weighed almost six times as much as day-14 blastocysts, radioimmunoassays indicated that day 14 blastocysts accumulated as much or more of each prostaglandin during the 24-hr incubation period as did day-16 blastocysts. Compared to day-16 blastocysts, perhaps day-14 blastocysts are more active metabolically (reflected by prostaglandin accumulation), and they expand at a faster rate, because fluid accumulation appears to be regulated by prostaglandins.

Day-14 and -16 blastocysts accumulated more, per milligram of tissue, PGE_2 , $\text{PGF}_{2\alpha}$ and 6-keto- $\text{PGF}_{1\alpha}$ than did endometrial slices; PGFM accumulation was comparable between tissue types. Similar results have been reported for ovine, porcine, and bovine blastocysts. Bovine endometrial slices metabolized [^3H] $\text{PGF}_{2\alpha}$ more actively than did blastocysts. It appears that the same is true for ovine endometrium because if catabolic capabilities were comparable, the amount of $\text{PGF}_{2\alpha}$ in medium from blastocyst incubations (as much as 60 times more per milligram of tissue as in endometrial incubates) should have resulted in more PGFM than was measured.

Prostaglandin $\text{F}_{2\alpha}$ of uterine-origin appears to be the luteolysin in sheep, but uterine secretion of $\text{PGF}_{2\alpha}$ during early pregnancy when the corpus luteum is maintained is not reduced. In fact, uterine secretion of $\text{PGF}_{2\alpha}$ often is increased during early pregnancy. It is likely that the conceptus makes a substantial contribution to the $\text{PGF}_{2\alpha}$ in uterine venous blood during early pregnancy. However, because ovine endometrium contains more $\text{PGF}_{2\alpha}$ and can release more $\text{PGF}_{2\alpha}$ in vitro than endometrium collected from nonpregnant ewes on comparable days after estrus, most of the $\text{PGF}_{2\alpha}$ in uterine venous blood probably originates in the endometrium.

In ewes, the corpus luteum maintains early pregnancy. A conceptus in the uterus by about day 12 postestrus maintains the corpus luteum of ewes through a local utero-ovarian mechanism. Intrauterine PGE_2 extends the lifespan of corpora lutea beyond the expected time of regression in cyclic ewes. Prostaglandin E_2 was found in uterine venous blood during early pregnancy and two studies indicate that pregnancy increases the concentration of PGE_2 in

uterine venous blood of ewes. Since the ovine conceptus synthesizes PGE_2 , one might speculate that PGE_2 of conceptus-origin maintains the corpus luteum of early pregnancy. However, this is difficult to reconcile because the amount of PGE_2 synthesized by the conceptus is small compared to the quantities of $\text{PGF}_{2\alpha}$ and 6-keto- $\text{PGF}_{1\alpha}$ (a stable metabolite of PGI_2) that are synthesized. Indeed, PGI_2 stimulated the secretion of progesterone by bovine corpora lutea, but uterine secretion of PGI_2 in ewes was not increased during early pregnancy. If endogenous prostaglandins are involved in luteal maintenance during early pregnancy in ewes, it is likely that changes other than or in addition to alterations in basal secretion by the uterus are required, i.e., changes in reception of prostaglandins and/or amplification of their message by luteal cells.

In addition to their potential effects on luteal function, prostaglandins probably have other roles during early pregnancy. Blood flow to the gravid uterine horn increased during early pregnancy in ewes. Prostaglandin I_2 is a potent vasodilator. Also, PGE_2 dilated the uterine vascular bed of ewes, but $\text{PGF}_{2\alpha}$ constricted the vascular bed. Perhaps prostaglandins that originate in the conceptus regulate local alterations in endometrial vasculature and result in the changes in blood flow to the gravid uterine horn of ewes. The increases in uterine blood flow during early pregnancy might increase the quantity of prostaglandins delivered to the ovary adjacent to the gravid uterine horn without changing the concentrations of prostaglandins in uterine venous blood.

During early pregnancy, the conceptus membranes (epithelial in nature) expand rapidly, and, as the amnion and chorion develop, there is a marked accumulation of fluids within the conceptus. Prostaglandins are involved in fluid transport across epithelia and it is possible the prostaglandins synthesized by the conceptus are required for the accumulation of fluids by the conceptus membranes.

In summary, ovine conceptuses and endometrial slices in vitro metabolized AA to prostaglandins and other lipids. Conceptuses were more active than endometrial slices per unit of weight in synthesizing prostaglandins. It appeared that AA was esterified to storage forms of lipids and then it was liberated from the esterified lipids for prostaglandin synthesis. Prostaglandins probably are required for survival of conceptuses and for maintenance of early pregnancy in ewes, but mechanisms and metabolic sequences remain obscure.

A study was conducted with pigs to determine whether porcine blastocysts and endometrial slices have an active arachidonic acid (AA) cascade and to determine the effect of various concentrations of radioinert AA on metabolism of radiolabeled AA (*AA) by blastocysts.

MATERIALS AND METHODS

Gilts were slaughtered and reproductive tracts were recovered 16 days after the first day of estrus. Five crossbred gilts were mated at approximately 12 h and again at 24 h after detection of estrus (day 0).

Blastocysts were flushed out of each reproductive tract with sterile, isotonic saline. Porcine blastocysts recovered 16 days after mating are elongated, become intertwined during the flushing procedure and are difficult to separate. Therefore, the mass of blastocysts from each gilt was divided with forceps into four portions with average wet weights of 216 ± 18 mg each (data are expressed as means $\pm$ standard errors). Each portion of blastocyst tissue was placed into a separate petri dish containing 15 ml modified minimum essential medium (MEM).

Endometrium was dissected from the midpoint of both uterine horns from each gilt, and the samples were placed into a petri dish and sliced with scalpel

blades into pieces with a volume of approximately 9 mm³. Duplicate portions (509 ± 3 mg each) of endometrial slices from each gilt were placed into separate petri dishes with 15 ml of MEM.

All incubates received 5 µCi of *AA in 50 µl ethanol. Tissues from two of the gilts received [5,6,8,9,11,12,14,15(N)-³H]-arachidonic acid (60 to 100 Ci/mmol; New England Nuclear, Boston, MA) and tissues from the other three gilts received [1-¹⁴C]-arachidonic acid (40 to 60 mCi/mmol; New England Nuclear). For incubation controls, 5 µCi of *AA were added to 15 ml MEM and incubated at the same time as tissues from each gilt. Blastocyst incubates from each gilt received either 25, 50, 100 or 200 µg AA (Sigma Chemical Co., St. Louis, MO) in 20 µl ethanol, and the endometrial incubates received 200 µg AA in 20 µl ethanol. All of the incubates were placed into a culture chamber (Bellco Glass Inc., Vineland, NJ) on a rocking platform (Bellco Glass, Inc.) for 24 h at 37°C in an atmosphere of 50% N₂:45% O₂:5% CO₂. At the end of the incubation period, tissues and MEM were separated by centrifugation at 4°C, and supernatants and tissues were transferred to separate plastic storage vials, frozen immediately and stored at -20°C. Incubation controls were handled in the same manner as the tissue incubates.

To evaluate metabolism of *AA, tissues were homogenized and *AA and its metabolites were extracted from the homogenates and from aliquots of MEM. Radiolabeled AA and its metabolites in the extracts were separated into 100 fractions using columns of Sephadex LH-20 (Sigma Chemical Co.) as described by us previously. The overall recovery of radioactivity was 75.0 ± 1.8%. Percentage of the total disintegrations per minute (dpm) for each fraction collected from each column was calculated by dividing dpm for each fraction by the total dpm recovered from the respective column. Percentages of total dpm were plotted against fraction number for visual evaluation of *AA metabolism.

Nine chromatographic regions were identified by visual inspection of Sephadex LH-20 profiles of metabolism of *AA by blastocysts. The identities of compounds within these regions were determined with Sephadex LH-20 columns and a high performance liquid chromatographic (HPLC) system calibrated with authentic prostaglandins and arachidonic acid. Percentages were derived for the nine chromatographic regions from each Sephadex LH-20 column by summing the percentage of total dpm for each fraction within each chromatographic region. These percentages were compared by analysis of variance and Duncan's multiple range tests when F-ratios were significant using the General Linear Models procedure of the Statistical Analysis System (18). Statistical comparisons were made among regions within each variable (incubation medium, blastocyst homogenate, etc.). The percentage of *AA metabolized was calculated as described previously.

RESULTS

The pattern of metabolites of *AA in extracts of MEM from porcine blastocyst incubations is shown in figure 7. The percentages of total dpm from all 20 blastocyst incubations were combined to construct this graph because the distribution of the percentages of compounds that were not resolved by the chromatographic procedures used. The metabolites of *AA in regions I, III, IV, VIII and IX have not been identified.

Blastocysts metabolized $79.8 \pm 1.8\%$ of the *AA added to MEM whereas endometrial slices metabolized only $55.8 \pm 5.1\%$ of the *AA added to MEM. Of the *AA that was not metabolized by blastocysts during the 24 h incubation period, $80.2 \pm 2.5\%$ was found in the blastocyst homogenates, and $37.0 \pm 8.5\%$ of the *AA that was not metabolized by endometrial slices was in the endometrial homogenates.

Fig. 7. Sephadex LH-20 elution pattern of metabolism of *AA by porcine blastocysts recovered 16 days after the onset of estrus. Regions II, V, VI and VII migrated with *AA, *PGFM, *PGE₂ and *PGF₂α, respectively. Each point used to construct the graph was the average of percentages of total dpm from 20 blastocyst incubations. The vertical broken lines indicate the points at which the regions were divided.

The greatest ($P < .05$) percentage of total dpm in extracts of MEM from blastocyst incubations was in the region that contained $*PGE_2$ (35.7 ± 1.8) followed by the regions that contained $*PGF_{2\alpha}$ (16.4 ± 1.5), $*PGFM$ (12.6 ± 1.7) and $*AA$ ($11.1 \pm .8$; table 1). The remainder of the percentages of total dpm were distributed fairly uniformly among the other regions. In extracts of blastocyst homogenates, $66.2 \pm 3.3\%$ of the dpm were in region I and $25.3 \pm 3.4\%$ of the dpm were recovered as $*AA$ (table 10). Regions III through IX each contained an average of 1.2% of the total dpm.

The majority ($P < .05$) of the dpm in extracts of MEM from endometrial incubates was recovered as $*AA$ ($59.0 \pm 3.6\%$; table 10). Radiolabeled PGE_2 and $*PGFM$ were approximately equal proportions ($7.7 \pm .9\%$) of the total dpm, and the remainder of the dpm were distributed fairly evenly among the other regions. In extracts of endometrial homogenates, region I contained $44.1 \pm 1.0\%$ of the total dpm (table 10), region II ($*AA$) contained $29.9 \pm 2.8\%$ of the total dpm, and the distribution of the remaining dpm did not differ among the other regions. In contrast to the distribution of total dpm in blastocyst and endometrial incubates, $77.8 \pm 5.4\%$ of the total dpm in the control incubations was recovered as $*AA$, each of the other eight regions contained no more than $4.4 \pm 1.2\%$ of the total dpm.

DISCUSSION

Porcine blastocysts recovered 16 days after mating produced at least eight metabolites of $*AA$ in vitro. Three of these metabolites migrated with $*PGFM$, $*PGE_2$ and $*PGF_{2\alpha}$, which is consistent with the reports on in vitro metabolism of $*AA$ by bovine and ovine blastocysts and with a report on in vitro metabolism

Table 10. Distribution of percentage of total dpm in extracts of homogenates of porcine blastocysts and endometrial slices recovered on day 16 postmating and incubated with *AA for 24 h and in extracts of medium from the incubates.^a

Region	Percentage of total disintegrations per minute				
	Blastocysts		Endometrial Slices		Incubation controls
	Incubation medium	Blastocyst homogenates	Incubation medium	Endometrial homogenates	
I	3.9 ± .5 ^b	66.2 ± 3.3 ^b	2.3 ± .3 ^b	44.1 ± 1.0 ^b	1.7 ± .3 ^b
II (*AA)	11.1 ± .8 ^c	25.3 ± 3.4 ^c	59.0 ± 3.6 ^c	29.9 ± 2.8 ^c	77.8 ± 5.4
III	3.8 ± .4 ^b	1.5 ± .2 ^d	4.6 ± .7 ^d	2.9 ± .2 ^d	3.1 ± .8
IV	4.1 ± .2 ^b	1.2 ± .1 ^d	4.4 ± .7 ^d	3.4 ± .3 ^d	3.3 ± .6
V(*PGFM)	12.6 ± 1.7 ^c	1.9 ± .1 ^d	7.8 ± 1.0 ^e	6.2 ± .6 ^d	4.4 ± 1.2 ^d
VI (*PGE ₂)	35.7 ± 1.8 ^d	1.0 ± .1 ^d	7.6 ± 1.0 ^e	3.1 ± .3 ^d	2.6 ± .8
VII (*PGF _{2α})	16.4 ± 1.5 ^e	.8 ± .1 ^d	5.3 ± .7 ^d	2.6 ± .3 ^d	2.1 ± .7
VIII	7.0 ± .3 ^b	.6 ± .2 ^d	4.7 ± .6 ^d	2.9 ± .3 ^d	2.5 ± 1.0 ^d
IX	5.3 ± .6 ^b	1.4 ± .5 ^d	4.3 ± .9 ^d	4.9 ± .3 ^d	2.5 ± 1.2 ^c

^a Different superscript letters within a column indicate differences (P<.05) among regions within the item. Data are expressed as means ± standard errors.

of $^*PGF_2\alpha$ by porcine blastocysts. The metabolites of *AA in five of the chromatographic regions have not been identified, but it is likely that the non-polar compound(s) in region I are esterified forms of *AA . With kidney and heart tissues, *AA was incorporated into phospholipids and subsequently metabolized to radiolabeled prostaglandins. Perhaps a substantial proportion of the prostaglandins produced by the blastocysts were synthesized from *AA liberated from an esterified *AA pool rather than being synthesized directly from free *AA .

The reason blastocyst metabolism of *AA was not altered by the concentration of AA in MEM is not clear. One possibility is that the rate of utilization of AA was not linear for each concentration in MEM, i.e., essentially all the substrate was metabolized regardless of its concentration, so the metabolism of *AA was not altered because the enzymes involved in utilization of AA were not saturated.

Endometrial slices appeared to produce *PGE_2 and *PGFM , but only in small amounts. The identification of *PGFM suggests that the endometrial slices produced $^*PGF_2\alpha$, but that the $^*PGF_2\alpha$ was metabolized before the end of the 24 h incubation period. Endometrial slices seemed to be less active in metabolizing *AA than were blastocysts; bovine endometrium also metabolized less *AA than did bovine blastocysts. In addition, pregnancy would be expected to reduce *AA metabolism by porcine endometrial slices, but effects of pregnancy were not tested in the present experiment.

Radiolabeled PGE_2 was the predominant product of *AA metabolism by porcine blastocysts. Although the function of prostaglandins produced by blastocysts during early pregnancy is not known, PGE_2 is a potent vasodilator. Production of PGE_2 by porcine blastocysts may cause endometrial vascular changes that would be involved in increasing the volume of blood that flows to the gravid uterine horn during early pregnancy. Also, PGE_2 might be involved in controlling fluid dynamics of blastocysts; blastocysts expand rapidly during this stage of early pregnancy.

Uterine secretion of $\text{PGF}_{2\alpha}$ is reduced during early pregnancy in gilts (23), and at the same time $\text{PGF}_{2\alpha}$ accumulates in the uterine lumen (24). It was proposed that during early pregnancy the endometrium in gilts changed from an endocrine to an exocrine organ (24). However, endometrium from pregnant gilts produced less $\text{PGF}_{2\alpha}$ in vitro than endometrium from nonpregnant gilts (20). The findings of the present experiment indicate that a proportion of the prostaglandins found in the uterine lumen during early pregnancy in gilts are of blastocyst origin.

The results of this study indicate that porcine blastocysts metabolize *AA to several compounds in vitro. Prostaglandins E_2 and $\text{F}_{2\alpha}$ produced by blastocysts may be involved in growth and development of blastocysts and in controlling local changes in uterine vascularity and blood flow known to occur during early pregnancy. Additional studies are needed to determine the quantities of prostaglandins produced by porcine blastocysts; and to determine the functions of prostaglandins during early pregnancy.

Summary

Studies with bovine, porcine and ovine blastocysts indicate that they can synthesize prostaglandins from exogenous arachidonic acid. The role of the metabolites of arachidonic acid in pregnancy is not known, but arachidonic acid metabolites probably are essential for the survival and development of blastocysts and for the maintenance of pregnancy. Additional studies are essential for determining whether there is a relationship between arachidonic acid metabolism and embryonal mortality.

- Lewis, G. S. Control of the quantity of prostaglandins produced by bovine blastocysts. Proc. 10th International Congress on Animal Reproduction and Artificial Insemination, Brief Communication 89, 1984.
- Lewis, G. S. and R. A. Waterman. Metabolism of arachidonic acid in vitro by ovine conceptuses recovered during early pregnancy. (Submitted to Prostaglandins). 1984.
- Lewis, G. S. and R. A. Waterman. Effects of endometrium on metabolism of arachidonic acid by bovine blastocysts in vitro. Prostaglandins 25:881. 1983.
- Lewis, G. S. and R. A. Waterman. Metabolism of arachidonic acid in vitro by porcine blastocysts and endometrium. Prostaglandins 25:871. 1983.