Assessment of sheep blastocyst effects on neutral lipids in the uterine caruncular epithelium

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Summary. Neutral lipids in the maternal uterine caruncular epithelium were studied by histochemical localization with Oil Red O. Results using a scoring system of 1 (negligible lipid) to 5 (maximal lipid) showed that intraepithelial lipid stores were minimal until Days 7–8 of the oestrous cycle and then increased to have a mean score of 4.4 on Days 14–15. In early pregnancy, although relatively high with a mean score of 3.2 at Day 15–16, such neutral lipids were significantly lower than those present at a comparable stage in the oestrous cycle. Thereafter, levels declined to a mean score of 1 on Days 21–23 of pregnancy. Such neutral lipid loss appears to be one of the first signs of the maternal response to the implanting embryo and precedes morphological evidence of transformation of either maternal or fetal tissues.

Introduction

The periodic appearance and disappearance of histochemically demonstrable lipids in the uterine epithelium of rats, sheep and cattle during the oestrous cycle is well known (Alden, 1947; Marinov & Lovell, 1968; Brinsfield & Hawk, 1973; Boshier & Holloway, 1973). However, there are relatively few studies of lipid levels in the endometrium in early pregnancy when the blastocyst is in close association with the uterine epithelium and the tissue re-organizations associated with the placental development are not yet evident or have just begun.

Bloch (1939) demonstrated that intra-epithelial osmiophilic granular secretions diminished or vanished when in close proximity with the implanted embryo in albino mice. Alden (1947) observed that osmiophilic lipid was slightly altered in its distribution at the implantation site in albino rats, but could deduce little about lipid levels during pregnancy. Boshier (1976) showed that, by the morning of Day 6 of pregnancy, all traces of intra-epithelial neutral lipids had disappeared from the rat implantation chamber as the result of enhanced esterase activity.

It is of interest to know whether such changes in intra-epithelial neutral lipids are found in other mammalian groups during implantation. This study was therefore undertaken to investigate changes in the patterns of neutral lipid distribution in the sheep uterine epithelium during the oestrous cycle and early pregnancy. Particular attention was given to studying lipid levels in the caruncular epithelium, as it is the site of blastocyst attachment and placental formation (Boshier, 1969).

Materials and Methods

All tissues examined were obtained from mature mixed-age New Zealand Romney ewes which were pasture-fed and run in the breeding season with teaser or entire rams fitted with brisket-mounted marking crayons. The day on which each ewe was marked during mating is Day 1 of the oestrous cycle. Ewes were studied at Days 3–4 ($N = 3$), 7–8

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(N = 4), 10–11 (N = 2) and 14–15 (N = 3) of the oestrous cycle. Additional ewes were studied at Days 15–16 (N = 6), 18–19 (N = 3) and 21–23 (N = 5) after a fertile mating. At the time of slaughter, the uteri of the mated ewes were flushed with saline for embryo collection to confirm pregnancy status. Blocks of uterine caruncular tissue were taken from freshly killed animals and fixed in Baker's formal calcium solution. The tissue blocks were soaked for 24–48 h in 25% aqueous sucrose at 4°C then frozen on the quick-freeze stage and sectioned at 6 µm in a microtome-cryostat (–25°C).

Three randomly selected blocks of tissue from each animal were sectioned to give in excess of 10 specimens per block which were then stained for liquid hydrophobic neutral lipids with Oil Red O (Bayliss High, 1977) and counter-stained with Mayer's haemalum. Ten sections from each block (donor ewe unknown) to give at least 30 sections per ewe were then randomly selected, examined, and scored 1–5, as judged by the following criteria: 1, little/no significant Oil Red O (ORO+) staining seen in the caruncular epithelial cells; 2, sparse ORO+ droplets in most cells (Fig. 1a); 3, some apical and few basal ORO+ droplets in all cells (Fig. 1b); 4, ORO+ droplets present apically and basally, some lateral to nuclei (Fig. 1c); 5, ORO+ droplets larger, numerous, and spread throughout the cytoplasm (Fig. 1d).

Statistical analysis of the data was based, after expert advice, on single classification analysis of variance followed by Snedecor's (1956) modification of Tukey's test for comparisons among means.

Results

As shown in Fig. 2, intra-epithelial neutral lipids were rarely demonstrable during the oestrous cycle until the 7th or 8th day, from which time their levels increased, reaching the maximum score of 4.4 ± 0.05 (mean $\pm$ s.e.m.) by the 14th and 15th days. Significant ($P < 0.01$) differences were shown between the mean scores of the 4 oestrous cycle groups.

Fig. 2. Intra-epithelial lipid scores during the oestrous cycle and early pregnancy in ewes. In statistical comparisons made using Tukey's test, day groups having the same alphabetic superscripts do not differ at $P = 0.05$. *Scores differ at $P < 0.05$.

Fig. 1. Representative sections of ovine caruncular epithelium, stained with Oil Red O, which demonstrate the basis of the scoring system used: (a) score = 2; (b) score = 3; (c) score = 4; (d) score = 5. When necessary, intermediate scores using gradations of 0.5 were allocated. $\times 650$.

During pregnancy, neutral lipid levels were still high (score 3.2 ± 0.12) 15–16 days after mating but decreased markedly during the next 8 days, scoring 1.8 ± 0.11 on Days 18–19 and reaching negligible levels by the 21st day of pregnancy. These decreases were of statistical significance ($P < 0.01$) between the 3 day groups. Neutral lipid scores on Day 15–16 of pregnancy were significantly lower than those found on Days 14–15 of the oestrous cycle ($P < 0.05$).

Discussion

While appreciating that the use of an ordinal scale for scoring the amount of lipid present in the uterine epithelia studied does involve subjective assessment, albeit within defined constraints, tissue scores are obtained relatively quickly and easily, and if sufficient assessments are made valid conclusions may be drawn from them.

Our demonstration that, during the oestrous cycle of the sheep, stored liquid hydrophobic neutral lipids are not demonstrable histochemically in the uterine caruncular epithelium until about Day 8; thereafter increasing to a maximum by the 14th or 15th day, confirms the findings of Brinsfield & Hawk (1973), although they did not distinguish between caruncular and intercaruncular epithelia. This cyclic increase and decrease in epithelial lipid storage has been shown to depend on prior plasma concentrations of progesterone and oestrogen in the laboratory rat and the sheep. Progesterone facilitates lipid storage and oestrogen increases neutral lipid loss via enhanced esterase activity (Boshier & Holloway, 1973; Brinsfield & Hawk, 1973; Boshier & Katz, 1975).

In early pregnancy in sheep, uterine intra-epithelial neutral lipids decline, despite the fact that plasma progesterone concentrations, normally supporting lipid storage, show no marked change in concentration and values remain similar to those in non-pregnant ewes at mid-cycle (Bassett *et al.*, 1969). Plasma oestradiol-17 β concentrations are also little changed in early pregnancy (Tsang, 1978). These hormone profiles contrast markedly with those in non-pregnant ewes, in which there is a dramatic decline in plasma progesterone values at luteolysis (Bassett *et al.*, 1969) and a sharp increase in the preovulatory plasma oestrogen concentrations (Baird *et al.*, 1976). These results suggest that ovarian hormones do not control the metabolism of lipids during early pregnancy as they do during comparable periods of the oestrous cycle.

The loss of neutral lipids at the implantation site in sheep when a blastocyst is present is similar to that in rats in which the presence of the blastocyst is also necessary since agar droplets comparable in size with the blastocysts do not give the same response (Boshier, 1976). By the 14th day of gestation in the ewe, the blastocyst has expanded sufficiently to fill the uterine horn (Rowson & Moor, 1966), and soon after shows the first evidence of attachment to the uterine epithelium (Boshier, 1969). As shown by the present results, the pregnant uterine horn containing the expanded blastocyst had a lower lipid score ($P < 0.05$) than a non-pregnant horn of the same age after oestrus. The stimulus to altered intra-epithelial neutral lipid metabolism therefore is the physical presence of trophoblastic tissue close to the uterine epithelium, a characteristic of the attachment phase of embryo implantation (Bloch, 1939; Alden, 1947; Ljungkvist & Nilsson, 1971; Boshier, 1976). The stimulus must also have been received before Day 15, because the cytochemical evidence of lipid loss reflecting altered intracellular metabolism was apparent by this time.

Suggestions as to the nature of the stimulus which induces these biochemical changes can as yet only be conjectural. The trophoblast may produce oestrogens which diffuse into the uterine epithelium and increase epithelial esterase activity (Boshier, 1976); or possibly the stimulus is the low molecular weight protein released by the trophoblast after Day 13 (Godkin *et al.*, 1982; Heyman *et al.*, 1984). Certainly by Day 21 the tissue transformations involved in the implantation response and placental formation have begun (Boshier, 1969, 1976). Catabolism of the neutral lipids could provide substrates for the cellular re-organization characteristic of this period, for the production of prostaglandins important in the implantation process (Kennedy, 1977; Boshier *et al.*, 1981) and for the metabolic use of the blastocyst.

These results in sheep confirm earlier findings in rodents, suggesting that altered neutral lipid metabolism in the uterine epithelium adjacent to the trophoblast may be one of the first signs of the morphological changes seen during the maternal response in the events of implantation in mammals.

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