Histological and Histochemical Changes in Bovine Endometrium Following Treatment with a Progestin

Abstract

Melengesterol acetate (MGA), a synthetic progestin, was fed to 15 beef heifers at 1.0 mg per animal per day for 14 days. Eight animals were started on the MGA feeding at day 4 (4-day animals) and 7 animals were started at day 15 (15-day animals) of the estrous cycle. Five control animals were not treated with MGA. The heifers were mated at the synchronized estrus and slaughtered 3 days later. Fertilization and the number of ova undergoing normal cleavage were reduced in heifers treated with MGA as compared to control heifers. The surface and glandular epithelial cells of the endometrium from control animals contained moderate to heavy concentrations of glycogen; however, there was little evidence of glycogen accumulation in the surface epithelial cells of 15-day animals. Although different, glycogen accumulation in the endometrial surface epithelial cells from 4-day animals was more like the controls than like the 15-day animals. These results suggest a diminished estrogen secretion or a failure of the endometrium of MGA treated heifers to respond to estrogen.

Introduction

The orally administered synthetic progestins, offer the farmer a relatively simple and potentially effective way of controlling estrous cycles in livestock. Generally, conception rates of the synchronized estrus have been lower than in control animals (3, 11). Many factors including failure of ovulation, ovum loss, failure of fertilization, embryonic death (5, 13) and follicular atresia (4) have been proposed to explain the lower conception rates at the synchronized estrus. The role of the uterine environment following estrus synchronization has not been studied extensively.

The objectives of this experiment were to utilize histology and histochemistry for partially describing the uterine environment at 3 days post mating in beef heifers treated with melengesterol acetate (MGA).2

Experimental Methods

Twenty Angus and Hereford heifers, which were part of a larger experiment (5), were utilized. Since undernutrition can change histology and histochemistry of the bovine endometrium (14), the animals were maintained on a ration which resulted in an average daily gain of .57 kg. Melengesterol acetate was added to the grabl ration and group fed at 1.0 mg per animal per day for 14 days. Eight animals were started on the MGA feeding at Day 4 of the estrous cycle (4-day animals) and 7 animals were started at Day 15 of the estrous cycle (15-day animals). Five control animals were fed a similar ration without the MGA.

After withdrawal of MGA, heifers were allowed to be mated by two different bulls at the "synchronized estrus" and slaughtered at 3 days postmating. Immediately after slaughter the reproductive organs were removed and the uterus and oviducts flushed with Ringer's solution to recover ova. The ova were located under a stereoscope at 12 ×. They were then observed at 50 ×, 100 ×, and 400 × and classified as cleaved or uncleaved.

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2 MGA—Registered Trademark of the Upjohn Company for melengestrol acetate.
Uterine tissue samples approximately 1 cm in thickness were obtained from the upper one-third of the uterine horn opposite the side of ovulation. The tissue samples were immediately placed in cold, buffered, 10% formalin and fixed for 12 hr. The tissue samples were processed in an Autotechnicon Tissue Processor containing Technicon standardized histological reagents, embedded in paraplast, and sectioned at 7 μ on an American Optical Spencer 820 rotary microtome.

Tissue sections were stained with periodic acid-Schiff (9), diastase-periodic acid-Schiff (7), and hematoxylin and eosin. Glycogen accumulation within the endometrial surface and glandular epithelial cells was recorded as none, light, moderate, or heavy. Surface and glandular epithelial cell heights were measured with an eyepiece micrometer and were analyzed statistically.

Results

Ova recovery and classification. A normal fertilized ovum was recovered from each of the five controls (100%). Eight ova were recovered from the 4-day animals with 4 ova (50%) being classified as fertilized and undergoing normal cleavage and 4 ova (50%) classified as unfertilized or fertilized but undergoing abnormal cleavage. Seven ova were recovered from 15-day animals with 4 ova (57%) being classified as fertilized and undergoing normal cleavage and 3 ova (43%) classified as unfertilized or fertilized but undergoing abnormal cleavage. All cleaved ova contained sperm within the zona pellucida.

Histology and histochemistry of control animals. The endometrial surface and glandular epithelial cells contained a neutral mucosub stance (14) concentrated in the apical portion of the epithelial cells and a moderate to heavy concentration of glycogen granules (Fig. 2 and 3). The histological characteristics of the endometrium at 3 days postestrus have been attributed to high estrogen at estrus (1, 10). In control animals slaughtered 3 days postmating, histological examination indicated edema and increased vascularity within the lamina propria and connective tissue stroma (Fig. 1), and low glandular epithelial cells.

Histology and histochemistry of 15-day animals. In 15-day animals there was little evidence of glycogen accumulation in the endometrial surface epithelial cells although the neutral area of mucosubstance (Fig. 4) was similar to controls. The glycogen content of the endometrial glandular epithelial cells ranged from none in some animals to moderate amounts in other animals. Histology indicated that both the lamina propria and the connective tissue stroma appeared more dense and less vascular than controls.

Histology and histochemistry of 4-day animals. In 4-day animals there was light to moderate accumulation of glycogen in the endometrial surface and glandular epithelial cells (Fig. 5 and 6). A neutral area of mucosubstance which appeared similar to that from the control animals was evident in the apical portion of the epithelial cells. The lamina propria and connective tissue stroma from the 4-day animals were more edematous and vascular than from the 15-day animals and were more similar to the controls.

Epithelial cell heights. The average surface and glandular epithelial cell heights of the control animals were 20.84 ± .66 and 18.10 ± .88 μ. The average surface and glandular epithelial cell heights of the 4-day animals were 24.88 ± .90 and 22.13 ± .71 μ and for the 15-day animals were 25.80 ± .58 and 20.57 ± .37 μ. Surface and glandular epithelial cells were greater in height (P < .05) in both of the MGA-treated groups than in the control group. There was no significant difference (P > .05) between the MGA treated groups.

Discussion

These results indicate differences in the histology and glycogen content of the endometrium of MGA-treated and control heifers 3 days postmating. Also, there was a reduction in fertilization rate and number of ova undergoing cleavage in the MGA-treated heifers.

Our results suggest a diminished estrogen secretion or a failure of the endometrium of MGA-treated heifers to respond to estrogen. This interpretation can only be speculative since blood estrogen levels were not available. The general histological appearance and the accumulation of glycogen granules in the endometrium from heifers fed MGA from Day 4 of the estrous cycle resembled the control animals more closely than did sections from the 15-day animals. Possibly the longer overall progestin influence (15 days of the cycle plus 14 days of MGA feeding) caused the endometrium to be less responsive to the estrogen secreted by the ovary.

It is necessary to synchronize the uterine environment with the developmental stage of the embryo (15). The glycogen content of uterine surface and glandular epithelial cells may provide energy for the "free floating" blastocyst and also for endometrial cell func-
Fig. 1. Section of endometrium from a control animal, 3 days postestrus showing presence of edema within the connective tissue stroma (CTS). Uterine glands (G) are indicated. H and E stain (282×).

Fig. 2. Section of endometrium from a control animal, 3 days postestrus with endometrial surface epithelial cells exhibiting moderate to heavy concentrations of glycogen granules (arrow). Uterine lumen (UL). Lamina Propria (LP). Periodic Acid-Schiff stain (245×).

Fig. 3. Section of endometrium from a control animal, 3 days postestrus, with glycogen granules in the cellular cytoplasm. Connective tissue stroma (CTS). Glandular epithelium (GE). Periodic Acid-Schiff stain (282×).

Fig. 4. Section of endometrium from MGA-treated animal showing absence of glycogen in surface epithelial cells but presence of apical area of neutral mucosubstance (arrow). Melengesterol acetate feeding was started at Day 15 of the estrous cycle. Periodic Acid-Schiff stain (611×).

Fig. 5. Section of endometrium from MGA-treated animal showing moderate concentration of glycogen in the surface epithelial cells. Melengesterol acetate feeding was started at Day 4 of the estrous cycle. Periodic Acid-Schiff stain (171×).

Fig. 6. Section of endometrium from MGA-treated animal showing moderate concentration of glycogen in the surface epithelial cells. Melengesterol acetate feeding was started at Day 4 of the estrous cycle. Periodic Acid-Schiff stain (611×).
TECHNICAL NOTES

Lions (6). Although it has been suggested that the uterine environment performs a vital function in sperm capacitation in some species (2), Mahajan and Menge (8) did not confirm the necessity for sperm capacitation in cattle. Smallwood and Sorensen (12) noted separation of the surface epithelium from the stratum compacetum and other morphological changes in the bovine endometrium after treatment with 180 mg per animal of Repromix 3 for 18 days. In our study utilizing 1.0 mg MGA no separation of the surface epithelium was seen.

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References


3 Repromix, Registered Trademark of the Upjohn Company for medroxyprogesterone acetate.