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HISTOCHEMISTRY OF DEHYDROGENASES IN THE SHEEP UTERUS DURING PREGNANCY[1]

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ABSTRACT

Histochemical localization and activities of succinate, malate, NAD-isocitrate, glucose-6-phosphate and lactate dehydrogenases were studied in gravid and non-gravid uterine horns of the ewe from days 10-80 of gestation. Enzyme activity in the sections was scored on a scale of 0-5.

There were no differences in the enzymatic activity of the gravid and non-gravid uterine horns. Enzyme activity was higher in the luminal epithelium and superficial glands followed by the maternal caruncles, circular myometrium and deep glands. In other uterine tissues, the enzymes either could not be localized histochemically or the activity was generally low.

In the luminal epithelium, activities of succinate and glucose-6-phosphate dehydrogenases decreased on days 25-30;

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خلاصـــه

فعالیت تعدادی از آنزیمهای گـــــروه دی هیدروژناز درشاخ رحم دارای جنیـــن وشاخ رحم بدون جنین از روزهای ۱۰ تـا ۸۰ آبستنی درگوسفنـــــد مطالعه گردید. فعالیت آنزیم‌ها براساس میزان تولیـد رنگ دربافتها از صفر تا پنج طبقه‌بنـــدی گردید.

بین شاخهای رحم دارای جنیـــــن وبدون جنین از نظر فعالیت آنزیمهـــا تفاوتی موجود نبود. فعالیت آنزیمها دربافت پوششی رحم وغدد سطحی رحـــم زیادتر از کارانکل ها، ماهیچه مدور رحم وغدد عمقی رحم بود. درسایر بافتهـای رحم بطورکلی یا آنزیمها فعالیتـــــی نداشتند یا اینکه فعالیت آنها خیلـــی کـم بود.

دربافت پوششی رحـم فعالیـــــت سوکسینات دی هیدروژناز و گلوکـز -۶- فسفات دی هیدروژنـــــاز در روز ۳۰-۲۵ آبستنی کاهش یافت. دراین بافـــت بعد از روز ۳۰ آبستنی فعالیت ایـــن آنزیمها وهم چنین فعالیت آنزیمهـای لاکتات دی هیدروژناز و مـــــــالات دی هیدروژناز بطور مشخصی افزایـــش یافت. درکارانکل های رحم فعالیـت لاکتات دی هیدروژناز در روز ۸۰ آبستنی

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after day 30, the activity of these enzymes and also of lactate and malate dehydrogenases significantly increased. In the maternal caruncles, lactate dehydrogenase activity increased on day 80, but the activity of other enzymes did not change during pregnancy.

Activities of succinate, isocitrate, and glucose-6-phosphate dehydrogenases in superficial glands of the uterus were constant during gestation, while malate dehydrogenase activity decreased slightly on day 20. Lactate dehydrogenase activity fell initially after day 15, but it rose again on day 80. In deep glands, activities of lactate and glucose-6-phosphate dehydrogenases decreased on days 25-30, but these as well as succinate, malate, and isocitrate dehydrogenase activities increased on day 80.

In the circular layer of the myometrium, activities of succinate and glucose-6-phosphate dehydrogenases decreased after day 30, malate dehydrogenase could not be localized histochemically, and the activity of the other enzymes was constant in this tissue.

افزایش یافت ولی فعالیت آنزیمهای دیگر در این بافت تغییری نکرد.

در غدد سطحی رحم فعالیت سوکسینات دی هیدروژناز، آیسوسیترات دی هیدروژناز وگلوکز ـ ۶ ـ فسفات دی هیدروژناز در دوران آبستنی ثابت بود در حالیکه فعالیت مالات دی هیدروژناز در روز ۲۰ آبستنی کاهش یافت. فعالیت لاکتات دی هیدروژناز در روز ۱۵ آبستنی کم شد ولی در روز ۸۰ افزایش یافت.

در غدد عمقی رحم فعالیت آنزیمهای لاکتات دی هیدروژناز وگلوکز ـ ۶ ـ فسفات دی هیدروژناز در روز ۳۰ـ۵۰ آبستنی کاهش یافت ولی فعالیت این آنزیمها وهمچنین آنزیمهای سوکسینات، مالات وآیسوسیترات دی هیدروژناز در روز ۸۰ آبستنی افزایش یافت. در ماهیچه مدور رحم فعالیت آنزیمهای سوکسینات وگلوکز ـ ۶ ـ فسفات دی هیدروژناز در روز ۳۰ آبستنی کاهش یافت. آنزیم مالات دی ـ هیدروژناز فعالیتی نداشت وفعالیت سایر آنزیمها ثابت بود.

INTRODUCTION

The basic function of the uterus is to provide an environment for implantation of the zygote and its subsequent growth and development, and to expel the products of conception when they are capable of independent existence. Since the major control of the earlier processes and even the latter rests with the endometrium, the metabolic processes in this organ deserve consideration (1). Changes in the activities of the uterine and placental enzymes may well indicate the significance of particular metabolic pathways as pregnancy proceeds. Even though measureable enzyme activity is but one factor indicating the rate of utilization of a substrate, it has been argued that knowledge of enzyme activities may reveal clues about the metabolic potential of an organ during development (10).

Enzyme histochemical techniques can be employed to study

changes in the activity of enzymes in the uterus. Enzyme histochemistry of the sheep uterus during the estrous cycle was recently reported (13). No information is available on the histochemistry of dehydrogenases in the sheep uterus during pregnancy, although activity of several enzymes in this organ has been determined by highly sensitive microfluorometric methods (12, 14, 15).

This paper describes the histochemical localization and semiquantification of succinate (SDH, E.C.1.3.99.1), malate (MDH, E.C.1.1.1.37), NAD-isocitrate (ICDH, E.C.1.1.1.41), glucose-6-phosphate (G6PDH, E.C.1.1.1.4.9) and lactate (LDH, E.C.1.1.1.27) dehydrogenases in the sheep uterus during the first half of pregnancy.

MATERIALS AND METHODS

Thirty-five Merino ewes (6-7 years old) were tagged and kept in a pen under natural lighting conditions. The animals were fed a mixture of lucerne and wheaten chaff; water was supplied through an automatic watering device.

The day of marking with a crayon by a ram of proven fertility was taken as day 0 of pregnancy after which the ewes were checked twice daily for return to heat. on days 10, 15, 20, 25, 30 and 80 of pregnancy, 4-5 ewes were slaughtered and the uterus or part of it was immediately frozen in liquid nitrogen. The tissues were then kept at -80°C until processed.

A small segment from the mid-part of each of the frozen uterine horns was dissected in a cold chamber (-25°C), placed on a specimen holder and then frozen in cold hexane kept on dry ice. Frozen sections (10µm) were cut in a Slee cryostat (-25°C), mounted on microscope slides, air-dried and used for the histochemical localization of dehydrogenases. All chemicals used for histochemistry were obtained from Sigma (St. Louis, Missouri, U.S.A.).

The optimal incubation media for dehydrogenases (with 0.3 mM Mg^{++}) were prepared according to Pearse (8) and

contained nitro-blue tetrazolium, substrate, sodium azide or cyanide, polyvinylpyrrolidone, phenazine methosulphate, and $NAD^+(P)$ in the appropriate buffer and in amounts as listed in Table 1. The optimal concentrations for each enzyme were determined by factorial experiments (11).

Fresh incubation medium (0.5 ml) was pipetted onto the sections which were incubated in an oven at 37°C for the specified time. Control sections were incubated in a medium containing all ingredients except the substrate. Following incubation the sections were rinsed in distilled water, fixed in formol saline (3 min), rinsed with running tap water (1 min), mounted in Farrant's medium and sealed with a coverslip.

To semiquantify enzymatic activity, tissue sections from different stages of pregnancy were randomly selected and scored (200 X) on a scale of 0-5, from no activity (0), very weak (1) to very strong (5) activity. At the time of scoring, the identity of sections was not known. The scores were then analyzed by the analysis of variance and the means were compared by the least significant difference method (9).

Enzyme activity was determined in the luminal epithelium, superficial glands, deep glands, circular myometrium, longitudinal myometrium, maternal caruncles, stroma, endometrial and myometrial blood vessels.

RESULTS

Control sections, incubated for non-specific staining, showed a very small amount of non-specific tetrazolium reduction. There was no difference between the gravid and non-gravid uterine horns; the results are therefore presented as mean values for both uterine horns (mean ± S.E.).

Succinate Dehydrogenase (SDH)

Moderate levels of SDH were found in the caruncles (3.2 ± 0.3) and superficial glands (3.4 ± 0.3) but the longitudinal layer of the myometrium, the stroma, and blood vessels were

Table 1. Optimal incubation media for dehydrogenase (DH) histochemistry.

Parameter	Succinate DH	Lactate DH	Glucose-6-P DH	Malate DH	NAD-Isocitrate DH
Buffer 100 mM	Tris-HCl	Tris-HCl	Tris-HCl	Phosphate	Tris-HCl
pH	7.4	7.2-7.4	7.4	7.4	8.0
Nitro-blue-tetrazolium (mg/ml)	0.5-1	1	0.5-1	1	1
Sodium azide (mM)	10	-	10	-	-
Sodium cyanide (mM)	-	10	-	10	-
NAD (mg/ml)	-	1	-	1	2
NADP (mg/ml)	-	-	1	-	-
Substrate (mM)	200	200	100	200	200
Polyvinylpyrrolidone (mg/ml)	-	150-300	150-300	-	-
Phenazine methosulphate (mg/ml)	0.5-1	1	0.5-1	1	-
Incubation time at 37°C (min)	25-30	10-15	25-30	50	120

devoid of SDH activity. The activity of this enzyme in the luminal epithelium and deep glands decreased between days 25-30 of pregnancy but by day 80, significantly higher levels were found in these structures (Table 2).

Table 2. Succinate dehydrogenase activity (mean score) in different uterine structures during ovine pregnancy.

Days from mating	Luminal epithelium	Deep glands	Circular myometrium
10	4.2bc	3.0ab	2.2a
15	4.0bc	3.0ab	2.4a
20	4.6ab	2.8abc	2.5a
25	3.7c	2.4bc	2.8a
30	3.6c	2.1c	2.2a
80	5.0a	3.2a	0.6b

Within each column, the means followed by similar letter(s) are not significantly different at the 5% probability level.

Malate Dehydrogenase (MDH)

The stroma and myometrium did not stain for MDH, but caruncles (1.9 ± 0.2) and endometrial blood vessels (2.1 ± 0.4) showed weak activity throughout pregnancy. In other structures, the activity decreased in early pregnancy, but it had increased significantly by mid-pregnancy (Table 3).

NAD-Isocitrate Dehydrogenase (ICDH)

The activity of ICDH during pregnancy was low in the stroma (1.8 ± 0.3) and moderate in the luminal epithelium (3.2 ± 0.4), superficial glands (2.9 ± 0.4), maternal caruncles (2.9 ± 0.4), circular myometrium (2.7 ± 0.4), longitudinal myometrium (2.5 ± 0.4), and endometrial (2.5 ± 0.3) and myometrial (2.6 ± 0.4) blood vessels. The activity in the

Table 3. Malate dehydrogenase activity (mean score) in different uterine structures during ovine pregnancy.

Days from mating	Luminal epithelium	Superficial glands	Deep glands
10	4.2ab	3.6a	2.4b
15	4.0ab	3.1abc	2.3bc
20	3.7bc	2.7c	2.0bc
25	3.6bc	2.9abc	1.6c
30	3.3c	2.8abc	1.7bc
80	4.6a	3.4ab	3.4a

Within each column, the means followed by similar letter(s) are not significantly different at the 5% probability level.

deep glands decreased ($P<0.05$) from a moderate level on day 15 (3.0 ± 0.4) to low levels on days 20-30 (1.7 ± 0.4), to increase ($P<0.05$) again on day 80 (2.8 ± 0.4).

Glucose-6-Phosphate Dehydrogenase (G6PDH)

The activity of G6PDH in the luminal epithelium and deep glands decreased significantly between days 25-30 of gestation but increased significantly on day 80 (Table 4). In the circular myometrium there was a slight decrease in activity on day 80. In the superficial glands (3.2 ± 0.4), maternal caruncles (3.2 ± 0.2), longitudinal muscle (2.0 ± 0.3), stroma (1.4 ± 0.2), and endometrial (1.5 ± 0.2) and myometrial (1.7 ± 0.3) blood vessels the activity of G6PDH did not change throughout pregnancy.

Lactate Dehydrogenase (LDH)

In the luminal epithelium the activity decreased on day 30 but increased significantly to very high levels on day 80 (Table 5). In the caruncles, the activity was constant from days 10 to 30; however, by day 80 the moderate staining had

Table 4. Glucose-6-phosphate dehydrogenase activity (mean score) in different uterine structures during ovine pregnancy.

Days from mating	Luminal epithelium	Deep glands	Circular myometrium
10	4.1ab	2.7ab	3.3a
15	4.0ab	2.7ab	3.1ab
20	3.5bc	2.1bc	3.4a
25	2.9c	1.4c	3.3a
30	2.9c	1.5c	3.2ab
80	4.7a	3.5a	2.4b

Within each column, the means followed by similar letter(s) are not significantly different at the 5% probability level.

Table 5. Lactate dehydrogenase activity (mean score) in different uterine structures during ovine pregnancy.

Days from mating	luminal epithelium	Superficial glands	Deep glands	Maternal caruncles
10	3.8bc	4.2ab	4.6a	3.1a
15	3.8bc	3.3c	4.1ab	2.7a
20	4.5ab	3.8bc	3.6b	3.4a
25	4.1ab	3.9bc	3.6b	2.8a
30	3.1c	3.2c	2.7c	3.0a
80	4.7a	4.8a	4.8a	4.6b

Within each column, the means followed by similar letter(s) are not significantly different at the 5% probability level.

increased to very strong staining. In the uterine glands, LDH activity decreased in early pregnancy but increased significantly by mid-gestation when similar levels were found in the caruncles, luminal epithelium, and in the superficial and deep glands. Blood vessels showed no LDH activity, but the activity in the stroma (1.5 ± 0.2) and circular (3.3 ± 0.4) and longitudinal (1.7 ± 0.3) myometrium did not change during pregnancy.

DISCUSSION

Histochemical data on SDH, MDH, ICDH, and G6PDH showed that the activities of Krebs cycle and pentose shunt in the caruncles and superficial glands remained constant at a moderate level, whereas in the deep glands and luminal epithelium, such activities decreased in the 4th week followed by a rise at mid-pregnancy. LDH activity in the luminal and glandular epithelia was lowest in the 4th week of pregnancy, but it increased to very high levels by day 80 in all epithelial and caruncular tissues. The increased Krebs cycle activity may be necessary in supplying intermediate metabolites in the histotrophe that fetal tissues can absorb and utilize (5). It is noteworthy that, in cyclic ewes, MDH activity (determined fluorometrically) was highest around days 8-9(15), corresponding to the period in pregnancy when the blastocyst was free in the uterine lumen and dependent on histotrophic nutrition. There seems to be a general agreement between the histochemical results reported here and the data obtained by quantitative methods for several dehydrogenases (15). Thus, qualitative histochemical methods for dehydrogenases may be used with some degree of confidence, particularly when the time factor is limiting, and when absolute values are not required.

A number of dehydrogenases have been studied in the uterus of rat (2) and rabbit (17) during implantation and in the pig placenta during pregnancy. If it is accepted that implantation in the ewe is accomplished by the 4th

week of pregnancy, then the histochemical changes of dehydrogenases in the sheep uterus correspond with those reported for the rat (2) and rabbit (1) during implantation.

In the present work, the activity of dehydrogenases was generally higher in the luminal epithelium than in the glandular epithelium, similar to the results reported by Christie (3); however, the reverse has been found in pig (4, 6). Histochemical studies showed a generally lower enzymatic activity in the myometrium and stroma than in epithelia and caruncles, and some enzymes could not be detected in these structures. Activity of SDH in the myometrium was lower during the luteal phase of the cycle (13), and in pregnancy it decreased. Whether this reflects the metabolic state of the quiet myometrium during the period of progesterone dominance is a matter for speculation.

The cell spectrum of enzymes determines its ability to metabolize substrates or synthesize products. In the case of some organs such as the uterus, function may not be completely realized in the absence of specific hormones. It is not clear which hormone(s) controls the activity of enzymes in the ovine uterus during pregnancy as enzyme activity in different structures varies and changes differently while all structures are under the same hormonal influences.

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