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Lactate Dehydrogenase Activity Changes During Peri-Implantation

K.A. Farley[1], J. Hornsby[1], K. Glass[2], G.K. Ballard[2], C.L. Moore2,
and M.A. Jones[1]
Department of Chemistry[1]
Department of Agriculture[2]
Illinois State University
Normal, Illinois 61761

ABSTRACT

The specific activity of lactate dehydrogenase was studied in the reproductive tissues of female ICR mice. The specific activity of the enzyme was determined for the uteri and oviducts of pregnant and pseudopregnant mice on Days 1-5 after ovulation as well as for normally cycling mice. The activity of lactate dehydrogenase in the oviducts was found to increase sharply on Days 2 and 3 of pregnancy but only if the embryos were flushed from the oviducts. In pseudopregnant mice, the specific activity of lactate dehydrogenase did not increase when ova were removed. This suggests that an inhibitor of lactate dehydrogenase activity may be present in the oviduct on Days 2 and 3 of pregnancy. In the uterus, the activity was found to increase on Days 1, 2, and 3 for pregnant mice and Days 2 and 3 for pseudopregnant mice. Lactate dehydrogenase activity in both groups was observed to decrease on Days 4 and 5.

INTRODUCTION

Following ovulation and fertilization, the biochemical factors which govern the transport and viability of the developing embryo are poorly understood. There have been a number of studies which address metabolic energy requirements and energy sources for embryos as well as the enzymes required. Embryos at the 1 to 2 cell stage require pyruvate and lactate as energy sources (Georgiev et al., 1970). Lactate dehydrogenase (LDH) is an enzyme which catalyzes the interconversion of lactate and pyruvate using nicotinamide adenine dinucleotide in the oxidized and reduced forms (NAD/NADH). Since the

substrates of LDH are of prime importance to the early embryo, regulation of their levels by LDH has received considerable attention. Spielmann et al., (1978) reported that embryonic LDH activity increased rapidly at implantation and may involve a release from an inhibitory factor. Other studies, also using electrophoresis, a qualitative technique, showed that LDH is present in mouse reproductive tissues (Rapola and Koskimies, 1967). A quantitative method developed by Reeves and Fimognari (1963) for the activity of LDH was later applied to reproductive tissue. The activity of LDH has been determined in the oviductal flushings of the mouse, rabbit, guinea pig, and rat during their estrous cycles (Gibson and Masters, 1970). Further characterization of the activity of LDH of oviducts was seen when estrous rabbits were given injections of progesterone, estradiol, or testosterone (Georgiev et al., 1970). All three hormones were found to increase the activity of LDH from that normally found in an estrous rabbit. In a similar study done with rat reproductive tissue, the uterus and oviduct both showed an increase in LDH activity several days after a progesterone injection was administered. These findings are of interest since the release of an egg into the oviduct normally coincides with a change in the peak level of circulating progesterone. Studies of LDH activity in pregnant animals have mainly been limited to the periods of mating and implantation. When LDH activity was monitored in the rabbit oviduct before and after mating, it was found to increase within 14 hours after copulation (Varma and Talwar, 1975). Another study compared the activity found in the rat uterus during the normal estrous cycle with that activity measured on Days 4 and 5 of pregnancy (Jelinek and Jelinkova, 1977). These authors report that activity of LDH decreased on Days 4 and 5 when compared with the values obtained for the cycling rat uterus. More recently, the effect of estradiol on LDH activity in the rat uterus has been probed (Holt and Rhe, 1987). Ovariectomized rats used in the study were given injections of estradiol at various concentrations. Of the three uterine cell types tested (surface epithelium, muscle, and stroma), the LDH activity in the surface epithelium cells was the only type affected. In these cells, the enzyme activity increased to a maximum on Day 4 of the estradiol injections. The activity in the other two cell types was found to remain constant. These findings suggest that the control of LDH activity by estradiol is limited to the epithelium of the endometrium. Although several studies have been published, changes in LDH activity which may be modulated by the presence of embryos have not been reported. Therefore, in the present study, we have undertaken a systematic study of LDH activity in mouse oviducts and uteri during the peri-implantation period. Since it is difficult to know the exact time at which an embryo associates, irreversibly with the uterus, peri-implantation may be considered to be that period of time during which the uterus is preparing to receive the embryo and some short, but unspecified time after the actual event. Pregnant and pseudopregnant animals were compared to estimate regulation of maternal LDH by the embryos.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of the Mice

All tissues were obtained from adult (21-24g) female ICR mice (*Mus musculus*) from Harlan Sprague Dawley, Inc. (Indianapolis, IN). For studies with pregnant mice, normally cycling mice were given intraperitoneal (ip) injections of 10 IU of pregnant mare serum gonadotropin (PMSG). After two days, the mice were injected with 5 IU of human chorionic gonadotropin (hCG) and placed with males of proven fertility. Pseudopregnant animals received only the PMSG and hCG as described above. In some experiments pseudopregnant mice were prepared by the same PMSG and hCG treatments before being placed with vasectomised males. In both cases the uteri from these animals increased in size suggesting a progesterone response and indicating that ovulation had occurred. In all cases, the presence of a vaginal plug, indicating mating, was observed. The females were then sacrificed on Days 1 through 5 after the hCG treatment, by cervical dislocation, followed by rapid removal of the oviducts and uterus.

Lactate Dehydrogenase Assays

LDH activity was analyzed spectrophotometrically at 340 nm by the method of Reeves and Fimognari (1963). The units of LDH which catalyzed the oxidation of umoles of NADH per minute were determined by dividing the rate (change in absorbance per minute) by 6.2. The specific activity of LDH was calculated by dividing the umoles of NADH oxidized per minute by the mg of protein in the incubation. For each tissue homogenate assayed, the reaction was performed in both the presence and absence of pyruvate. In all cases, the rate was negligible in the absence of pyruvate.

LDH Oviductal Flushings and Homogenates

Oviducts were collected from cycling mice (at any stage in the cycle) or pregnant and pseudopregnant mice on Days 1-5 after ovulation. For each reproductive state, oviducts were either flushed with buffer prior to homogenization or homogenized directly (as indicated in text). Individual oviducts were flushed with 100 ul of Kreb's Ringer Bicarbonate (KRB) after the pH was adjusted to 7.4 (6.95 g NaCl, 0.35 g KCl, 0.16 g KH_2P04, 0.14 g MgS04, 0.90 g glucose, 2.1 g $NaHC03$, and 0.19 g $CaCl_2$ per liter, pH 7.4). Each oviduct was flushed twice to remove the embryos or ova. Each flushing was evaluated microscopically for the presence of embryos or ova from pregnant or pseudopregnant mice respectively. Animals which did not have ova or embryos, as appropriate, were not used in these studies. On Days 4 and 5 of pregnancy or pseudopregnancy, all oviducts were not flushed because any embryos present

were expected to be in the uterus. All oviducts were placed on ice, and a wet weight of each oviduct was determined. The tissue was then placed in 1 ml of Kreb's Ringer bicarbonate buffer on ice and homogenized using a Brinkmann polytron at a setting of 3 for thirty seconds. For the LDH enzyme assay, 200 ul of homogenate from each oviduct were tested.

Uterine Preparation For LDH Assay

For each pregnant, pseudopregnant, or normally cycling animal, a total weight of uterine tissue was obtained after careful dissection from the oviducts. The tissue was then placed in 5 ml of Kreb's Ringer Bicarbonate buffer and homogenized with a Brinkmann polytron at a setting of 4 for two thirty second bursts, cooling on ice between bursts. All uterine homogenates were analysed as above except that only 100 ul of uterine homogenate were used in the assay.

Protein Assay

Protein from oviductal and uterine homogenates was evaluated by the method of Lowry et al., (1951) using bovine serum albumin (BSA) as the standard.

Statistics

Statistics were performed using analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) test. Data were considered to be significantly different at $p < 0.05$.

RESULTS

The specific activities of LDH in mice oviducts and uteri were determined for the various reproductive states between cycling and embryo implantation. When the procedure was used with fresh oviducal tissue, the absorbance of NADH at 340 nm was found to decrease linearly over time. When the assay was run using frozen tissue, the change in absorbance was minimal (data not shown). From this study, it was concluded that the rest of the research should be conducted with fresh tissue.

The oviducts were obtained from three reproductive states: cycling, pregnant, or pseudopregnant during the period from ovulation to implantation. The LDH specific activities of oviduct homogenates are shown in Table 1. On Day 2 the specific activity of the flushed pregnant mouse oviduct was statistically different ($p < 0.05$) from every other point on the graph. The specific activities of flushed pseudopregnant oviducts were not found to be different from the unflushed pseudopregnant oviducts at the same reproductive stage, so these values were combined for this graph. The Day 3 flushed pregnant oviducts were statistically different ($p < 0.05$) from the Day 1 and 2 flushed pregnant oviducts as well as Day 3 unflushed pregnant oviduct. In the mouse oviduct, the specific activity is the highest on Day 2 after ovulation for pregnant animals and the lowest on Day 4 after ovulation in both the pregnant and pseudopregnant oviducts. In

addition, there was no significant difference in the specific activity of oviducts from pseudopregnant mice receiving PMS and hCG only or PMSG, hCG and mating with vasectomised males (data not shown).

The specific activities of LDH in the mouse uterus are shown in Table 2. In pregnant mice, the specific activity of LDH in the uterus increased significantly from Day 0 to Day 2, then decreased from Day 2 to Day 4. Day 5 was not different from Days 0 or 4. The pseudopregnant animals followed a similar trend, except that the trend is shifted to the right by a day. The specific activity in the uterus of pseudopregnant mice was lowest on Days 0 and 1, increased on Days 2 and 3, then decreased on Day 4. Day 5 was not different from Days 0, 1, or 4. On Days 1 and 3, the specific activities of pregnant uteri were found to be statistically different ($p < 0.05$) from the specific activities found in the same day pseudopregnant uteri.

DISCUSSION

In the mouse oviduct and uterus, LDH was easily detected using individual oviductal or uterine homogenates. In all cases, the SA of unflushed pregnant oviducts were not significantly different from the SA of either flushed or not flushed pseudopregnant oviducts. With or without flushing, the specific activity of LDH in the oviduct did not increase in Day 1 pregnant animals (Table 1). This is in contrast to the findings of Varma et al., (1974) and Varma and Talwar (1975). They found that the specific activity increased in the rabbit oviduct 14 hours after mating. Our data also do not agree with another study, using the mouse oviduct, indicating that the specific activity of LDH increased 48 hours after mating in the isthmus of the oviduct (Nieder and Corder, 1983). We found an elevated LDH activity only after flushing the oviduct on Day 2. Whether this is due to difference in species or differences in the experimental design (natural mating versus hormone stimulated process) can not be determined from our data.

The specific activity of LDH in the mouse oviduct increased significantly on Days 2 and 3 of pregnancy only when the embryos were flushed from the oviduct. In Day 2 and 3 pseudopregnant animals, however, the specific activity did not increase when the ova were flushed from the oviducts. This suggests that the flushing process alone is not responsible for the increase in the specific activity of the enzyme. One possibility for this change in the enzyme activity which is detected is that an enzyme inhibitor, present only in pregnant animals, has been flushed from the oviduct. The inhibitor may be present in the pseuopregnant animal also, but it may not be in an active form. However, our experiments do not allow us to evaluate this possibility.

The inhibitor may originate from a number of sources. The embryo is one possible source of the inhibitor. Since the pseudopregnant animal does not contain a genetically distinct organism, no inhibitor would be present. The maternal system is another possible source of the inhibitor. The inhibitor may be inactive until the animal becomes pregnant, or until the maternal system receives some signal from the embryo. Another possible source of the inhibitor is from the male mouse. Since the pregnant animal has contact with the male, it is possible that the inhibitor originates in the sperm or semen. This possibility,

however, seems less likely since the significant increases are on Days 2 and 3 and not Day 1 of pregnancy. In addition, there were no significant differences in the LDH specific activity in the hormonally induced pseudopregnancy when compared to the females (pseudopregnant) housed with a vasectomised male.

The specific activity of LDH in the mouse uterus increased significantly, relative to normally cycling animals, in pregnant animals on Days 2 and 3, then decreased on Days 4 and 5. This agrees well with data obtained by Jelinek and Jelinkova (1977) in the rat uterus. Another study, by Holt and Rhe (1987), found that the maximum specific activity of LDH in ovarectomized rats, that were given injections of estradiol, occurred on Day 4 in the uterus. In the present study, the highest specific activity in mouse uterus was found on Days 2 and 3 of pseudopregnancy or pregnancy. Sakhuja et al., (1982) reported that the SA of Day 4 pregnant mice uteri was 1.262 activity units/mg protein. The article, however, did not define activity units, so a direct comparison with the present data could not be made. The increase seen in the specific activity of the uteri on Days 2 and 3 coincides with the period that the embryo spends in the oviduct. The specific activity on Day 4 or 5 had returned to a level which was not different from that of the cycling mouse (Day 0). In the pseudopregnant uteri, the specific activity decreased slightly on Day 1, then increased significantly on Days 2 and 3 relative to cycling values. When the pregnant uteri were compared with the pseudopregnant uteri, they were found to be significantly different on Days 1 and 3. However, the importance of this observation is not obvious. Since both pregnant and pseudopregnant uteri respond by increasing the LDH activity, this implies that the ovaries (the corpora lutea which are producing progesterone) are involved in this response. The embryos may have a role in shifting the time frame.

The specific activities of the oviducts, for cycling animals, were higher (approximately 1.5 fold) than the values of the uterine tissues. This trend was also observed by Patterson and Masters (1972) who reported higher LDH activities in rat oviducts than uteri. Since the specific activity of LDH increases in both oviducts and uteri after ovulation, the enzyme clearly is responsive to the reproductive state of the animal. In the oviduct these changes may regulate the pyruvate/lactate levels available for the developing embryo. In the uterus, these changes may reflect a change in carbohydrate utilization by the uterine tissue which is undergoing extensive remodeling to accommodate the new embryo. In both the uterus and the oviduct, the presence of the embryo appears to have a profound effect on the maternal system as judged by specific activity changes in the LDH. This is the first report suggesting a regulatory role for the embryo on maternal LDH activity. Further work is necessary to characterize the mechanism of the event.

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Table 1. Specific activity of LDH in oviductal homogenates.

Reproductive State: Day	Mean ± S.D. (N*) Not Flushed	Flushed
Cycling	0.383 ± 0.067^{c,d,e} (7)	
Pregnant		
Day-1	0.457 ± 0.021^{c,d,e} (3)	0.317 ± 0.081^{a,b,c} (3)
Day-2	0.440 ± 0.046^{d,e} (5)	0.594 ± 0.125^{f} (9)
Day-3	0.340 ± 0.056^{b,c,d} (3)	0 473 ± 0.070^{e} (4)
Day-4	0.243 ± 0.012^{a,b} (3)	N.D.
Day-5	0.295 ± 0.045^{a,b} (4)	N.D.
Pseudopregnant		
Day-1	0.382 ± 0.045^{c,d,e} (6)	N.D.
Day-2	0.417 ± 0.099^{c,d,e} (7)	N.D.*
Day-3	0.392 ± 0.078^{c,d,e} (6)	N.D.
Day-4	0.200 ± 0.020^{a} (3)	N.D.
Day-5	0.320 ± 0.063^{b,c} (3)	N.D.

N.D. Not significantly different from the same day flushed values, therefore values were pooled.

Values with the same letter are not statistically different ($p < 0.05$) by ANOVA followed by Fisher's LSD.

N*=Number of animals.

Table 2. Specific activity of LDH in uterine homogenates.

Reproductive State: Day	Mean ± S.D. (N*)
Cycling	0.247 ± 0.071[a,b]
Pregnant	
Day-1	0.310 ± 0.027[b,c] (3)
Day-2	0.363 ± 0.093[c,d] (3)
Day-3	0.328 ± 0.034[c] (5)
Day-4	0.200 ± 0.010[a] (3)
Day-5	0.230 ± 0.066[a,b] (3)
Pseudopregnant	
Day-1	0.190 ± 0.083[a] (5)
Day-2	0.407 ± 0.070[c,d] (3)
Day-3	0.417 ± 0.046[d] (3)
Day-4	0.210 ± 0.020[a] (3)
Day-5	0.220 ± 0.027[a,b] (3)

Values with the same letter are not statistically different ($p < 0.05$) by ANOVA followed by Fisher's LSD.
N*=Number of animals.