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Differential Activity of Matrix Metalloproteinases (MMPs) During Photoperiod Induced Uterine Regression and Recrudescence in Siberian hamsters (*Phodopus sungorus*)

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Abstract

Siberian hamsters adapt to seasonal changes by reducing their reproductive function during short days (SD). SD exposure reduces uterine mass and reproductive capacity, but underlying cellular mechanisms remain unknown. Because matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are important in uterine development, parturition, and postpartum remodeling, their expression in uterine tissue from Siberian hamsters undergoing photoperiod-mediated reproductive regression and recrudescence was investigated. Female hamsters were exposed to long day (LD, 16L:8D, controls) or SD (8L:16D) for 3–12 weeks (regression); a second group was exposed to SD or LD for 14 weeks and then transferred to LD for 0–8 weeks (recrudescence). Hamsters were euthanized, uteri collected, and homogenates analyzed by gelatin zymography or Western blotting for MMP and TIMP protein levels. Uterine weight decreased (67–75%) at SD weeks 12–14 and increased post-LD transfer (PT) reaching LD values by PT week 2. MMP-2, but not MMP-9 activity was reduced by SD week 12 or 14 but increased to LD levels at PT week 2. MMP-3 expression increased at SD week 9 compared to other SD and LD groups. MMP-14 and -13 protein levels decreased at SD week 3 but returned to LD levels by SD week 6. During recrudescence, MMP-3 (PT weeks 0–2), MMP-13 (PT week 4), and MMP-14 (PT weeks 2, 4) protein levels were higher than LD. TIMP-1 and 2 were present at low levels. Significant and differential variations in uterine MMP activity/expression during photoperiod-induced regression and recrudescence were observed. These changes likely reflect increases in tissue remodeling during both the adaptation to SD and the restoration of reproductive function.

Keywords

uterus; seasonal reproduction; protease; remodeling

INTRODUCTION

Seasonal limitation of reproductive function in Siberian hamsters (*Phodopus sungorus*) serves to maximize breeding success and is attained through measurement of changes in day length (Hoffman and Illnerova, 1986; Prendergast et al., 2002; Place et al., 2004). Long days (LD) maintain reproductive function, whereas, inhibitory short days (SD, 6–14 weeks) induce regression of uterine and ovarian function, decrease reproductive behavior, and reduce sex steroid hormone concentrations (Honrado et al., 1991; Edmonds, 1993; Schlatt, 1993; Place et al., 2004; Moffatt-Blue et al., 2006). In particular, photoperiod-induced changes in uterine

tissue are substantial; a significant reduction (70%), or regression in uterine mass occurs in Siberian hamsters after 12 weeks of SD exposure (Moffatt-Blue et al., 2006). Transfer from inhibitory SD to stimulatory LD restores endocrine function along the hypothalamic–pituitary–gonadal axis and induces reproductive recrudescence (return of gonad mass and function following quiescence), specifically inducing ovarian growth and remodeling (Ross et al., 2005; Salverson et al., 2007). Seasonal uterine regression and recrudescence both presumably involve tissue remodeling and the latter may also require new tissue growth. Although uterine recrudescence likely follows restoration of ovarian function, and changes in uterine mass in response to photoperiod have been reported in rodents (Honrado et al., 1991; Edmonds and Stetson, 1993; Schlatt et al., 1993), the mechanism of these changes in the uterus had not been investigated.

The mature uterus consists of two major compartments: the endometrium and the myometrium. The endometrial inner lining contains two predominant tissue types—luminal and glandular epithelium, and stroma. Uterine tissue undergoes cyclic synchronized tissue alterations in response to the ovarian hormones estrogen and progesterone during each sexual cycle and pregnancy. Progesterone is critical for implantation and successful gestation in hamsters (Harper et al., 1969; Zhang and Paria, 2006), and estradiol is necessary in other rodents, such as mice (Yoshinaga and Adams, 1966). During gestation in rats, uterine mass increases 13-fold to accommodate growing fetuses (Morton and Goldspink, 1986), and both apoptosis and proliferation play an important role in maintaining uterine changes (Zhang and Paria, 2006). Following parturition, uterine tissue involutes rapidly, regressing 25% within 3 days postpartum in rats, a decline mediated by protease activity (Shimada et al., 1985). Growth and development of the uterine epithelium involve synthesis and degradation of the extra-cellular matrix (ECM), a process partly regulated by matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) (Woessner, 1991; Alexander et al., 1996; Smith et al., 1999; Fata et al., 2000; Woessner and Nagase, 2000; Curry and Osteen, 2003). Indeed, changes in uterine mass as a result of hormonal alterations are often the result of changes in the total amount of uterine collagen, a primary target of many MMPs (Kao et al., 1969).

MMP activity is critical for regulating the interactions between cells and the ECM, including tissue remodeling, cell proliferation, differentiation, and cell death (Salmonsens, 1996; Massova et al., 1998; Hu et al., 2004; Kaitu'u et al., 2005; Page-McCaw et al., 2007). MMPs are Zn²⁺-dependent endopeptidases that cleave both ECM and nonmatrix proteins. These proteases are produced as latent zymogens and are activated by proteolysis by other MMP enzymes and can be regulated at the transcriptional, translational, and activity levels by hormones, cytokines, chemokines and oncogenes (Werb and Chin, 1998; Curry and Osteen, 2003; Braundmeier and Nowak, 2006). While MMPs are involved in the degradation of tissue, they are also critical for the release of growth factors that can initiate growth and development (Cheng et al., 2007). MMP activity influences the bioavailability of insulin-like growth factors (IGF) in the uterus (Coppock et al., 2004), and MMP cleavage of the ECM can influence IGF binding protein-1 expression in the uterine stromal fibroblasts (Strakova et al., 2003).

Specific MMP activity has been reported in pregnant and nonpregnant uterine tissue in rodents. Expression of MMPs-3, -7, -9, and -13 is noted in the uterus of nonpregnant rodents (Tsang et al., 1995; Rudolph-Owen et al., 1997; Nuttal and Kennedy, 1999), and MMPs -2, -3, -9 are expressed during gestation in mice (Das et al., 1997). MMP-14 (membrane type MMP-1) and MMP-2 are both increased during postpartum involution (Kengo et al., 2006), and a variety of MMPs and TIMPs are expressed during uterine development in neonatal mice (Hu et al., 2004). MMPs are also expressed during endometrial breakdown and repair during menstruation, however, these processes are not impaired with administration of general MMP inhibitors (Kaitu'u et al., 2005).

Although the role of MMPs and TIMPs in uterine matrix remodeling is well established, relatively little is known about their involvement in photoperiod-induced alterations in the uterus. We hypothesized that MMPs/TIMPs may play a crucial role in photoperiod-induced changes in the uterus of Siberian hamsters. To address this question, we investigated protein levels (MMPs-3, 13, and -14; TIMPs-1 and -2) and activity (MMPs-2, and -9) of MMPs and TIMPs in uterine tissue during regression (SD exposure for 3–12 weeks) and stimulated reproductive recrudescence (14 weeks of either SD and subsequent transfer to stimulating LD for 1–8 weeks). Animals exposed only to long day conditions that therefore lacked seasonal changes in uterus function served as control groups. We selected gelatinases, collagenases (secreted and membrane bound), stromelysin, and two of the four TIMPs for a first look at MMPs during seasonal changes in uterine tissue. Activity of MMP-2, and MMP-9 was measured by zymography whereas MMP-3, -13, -14, TIMP-1 and -2 were detected by Western blotting in uterine homogenates.

MATERIALS AND METHODS

Animals

Adult (>60 days of age) female Siberian hamsters were purchased from the breeding colony of Dr. Katherine Wynne-Edwards, Queen's University (Kingston, Ontario, Canada). Hamsters were housed individually in poly-propylene cages (28 cm × 7.5 cm × 13 cm) at 20±2°C, receiving food and water ad libitum. All experiments were conducted in our Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved facilities, in accordance with California State University, Long Beach (CSULB) and National Research Council (NRC) guidelines for use of laboratory animals; results from ovarian tissues harvested from these animals have been published elsewhere (Moffatt-Blue et al., 2006; Salverson et al., 2007), and subsets of these hamsters were used for the present study. Briefly, for the *regression* experiment, females were either assigned to 3, 6, 9, or 12 weeks of long day (LD, 16L: 8D, n = 20) or short day exposure (SD, 8L:16D, n = 40). Hamsters were weighed and their estrous stage was determined before tissue collection. All hamsters were anesthetized and euthanized while in diestrus II, as once normal cyclicity ceases with SD exposure, reproductive tissue assumes diestrus II characteristics, and this procedure allowed us to reduce variability among groups. Reproductive organs were harvested and stored as described previously (Moffatt-Blue et al., 2006).

For the *recrudescence* experiment hamsters were exposed to either LD (n = 18) or SD (n = 50) for 14 weeks (Salverson et al., 2007). Subsequently, SD exposed hamsters were transferred to LD for 0, 1, 2, 4, or 8 weeks (n = 10/group) and euthanized for reproductive tissue collection as described above. Controls were maintained in LD and tissue collected on weeks 0, 2 and 8 (n= 6/group). Uterine tissue from both regression and recrudescence experiments was weighed, flash frozen in liquid nitrogen and stored at -80°C until processed for protein extraction.

Protein Extraction and Quantification

Frozen uterine tissues were homogenized (1:5) in 0.1M Tris-HCl (pH 7.6) buffer containing 5mM CaCl₂, 150mM NaCl, 0.05% Brij35, 0.02% NaN₃, 1% Triton X-100 and protease inhibitor cocktail (Sigma Chemical Co., St. Louis, MO), and centrifuged for 30 min at 12,500 rpm at 4°C (Gu et al., 2005). Total protein in the supernatant was determined by the Bradford method (Bio-Rad, Hercules, CA) and was expressed as total protein (mg/uterus weight).

Gelatin Zymography

Gelatin zymography was done using pre-cast zymography gels according to manufacturer's directions (Bio-Rad). An aliquot containing 40 µg total protein/lane was applied on the gel. Electrophoresis was conducted at constant voltage (100 V) for 90 min. Gels were then washed

in two changes (30 min each) of renaturation buffer (2.5% Triton X-100) and subsequently incubated overnight at 37°C in zymogram development buffer (50mMTris, 200mMNaCl, 5mM CaCl_2 and 0.02% Brij pH 7.5) (Bio-Rad). To verify the observed bands as MMPs, 5mMEDTA, GM6001 (1 μM), MMP-1 inhibitor-1 (10 μM) or serine protease inhibitor cocktail were included in the development buffer as controls. Gels were then stained in 0.5% Coomassie blue R250, destained until bands were visible, and photographed. Relative gray scale band density was then determined using NIH image software as directed by the online manual (V1.61) for 1D gels to measure area and mean pixel value.

Immunoblotting

Aliquots of uterus homogenates (empirically determined 20 μg total protein) were separated on 10% Tris-HCl pre-cast gels (Bio-Rad) as per manufacturer's direction, and electro-transferred on to nitrocellulose membranes. Membranes were blocked in blocking solution (Bio-Rad) and immunoblotted using primary antibodies (Chemicon/Millipore, Temecula, CA) against MMP-14 (MT-MMP-1, MAB3317), MMP-3 (MAB3369), MMP-13 (MAB3321), TIMP-1 (AB8112), and TIMP-2 (MAB3310). In addition, as a protein loading control, β -actin antibody (SC4778, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was also used. Membranes were incubated overnight with the primary antibodies (1:2,000), washed and incubated with appropriate horseradish peroxidase secondary antibodies (1:3,000), and developed by Amplified Opti-4CN detection kit (Bio-Rad) as per the manufacturer's protocol or by chemiluminescence. Positive controls for MMPs and their tissue inhibitors (Chemicon International) were simultaneously run and immunoblotted to ascertain the predicted band location. Relative band density was determined using NIH image software as directed by the online manual (V1.61).

Statistical Analysis

Results are presented as mean $\pm$ SEM and analyzed by one-way ANOVA with Newman-Keuls post hoc tests using Prism 4 software (GraphPad Software, Inc., San Diego, CA). $P \leq 0.05$ was considered statistically significant.

RESULTS

For the LD controls in both the regression and recrudescence experiments, there were no significant differences in either the uterus weight, total protein, band densities in zymograms or Western blots, therefore to simplify presentation of results, data from the respective LD groups were pooled to serve as a single control group for each experiment.

Total Uterine Protein and Uterus Mass

Total protein concentrations or uterine weight showed no significant differences in LD control groups either during regression or recrudescence. However, exposure to 3–12 weeks of SD photoperiod decreased uterine weight (30–67%) and total protein concentration (33–66%) as compared to LD control (Fig. 1A,B; $P < 0.05$). Similarly, in the recrudescence experiment, the mass of paired uterine horns decreased (75%) after 14 weeks of SD exposure (Post transfer (PT) week 0), but then increased steadily upon transfer to LD from PT weeks 1–4 ($P \leq 0.05$), reaching LD control levels at PT week 2 (Fig. 1C). As expected, total protein concentration (mg/uterus weight) showed a similar pattern in the recrudescing uterus with levels returning to LD values at PT week 4 (Fig. 1D).

MMP Zymography

Gelatin zymography showed three major (80, 72, 60 kDa) and one faint minor band (88 kDa) in both regressing and recrudescing uterine homogenates (Fig. 2A, B). The 88 and 80 kDa

bands, although of lower density, are consistent with the pro- and active forms of MMP-9. The higher density 70 and 60 kDa bands correspond to pro- and active forms of MMP-2. In the regressing uterus, MMP-9 activity was low and remained unchanged (data not shown; $P > 0.05$), however, MMP-2 activity (60 kDa) showed a small (16%) but significant decrease after 12 weeks of SD exposure (Fig. 2A; $P < 0.05$). In recrudescing uteri, no significant changes were noted in the low MMP-9 activity (data not shown; $P < 0.05$) however, MMP-2 activity (60 kDa) was significantly lower (40%) after 14 weeks of SD exposure (PT week 0), and then increased steadily and significantly at PT weeks 2, 4, and 8 by 29%, 45%, and 38% compared to PT week 0 (Fig. 2B; $P < 0.05$). Although increasing, MMP-2 activity at PT weeks 0–1 ($P < 0.05$) remained lower than the LD control, and reached LD levels by PT week 2 (Fig. 2B).

To ensure that above bands represent MMP activity and not other proteases, control zymograms were developed in the presence of MMP-1 inhibitor (10 μ M), serine protease inhibitor cocktail, EDTA (5 mM), or MMP inhibitor GM6001 (1 μ M) (Fig. 2C). MMP-1 inhibitor reduced the intensity of all major bands, the serine protease inhibitor had no effect, and no bands were visible either in the presence of EDTA or GM6001 (Fig. 2C).

Western Blot Analysis of MMP-14, MMP-3, MMP-13, and TIMP-1, 2

In the regressing uterus, levels of MMP-3 (55 kDa, major band) at SD week 9 were 25–58% higher as compared to all other groups (Fig. 3A). Protein levels of MMP-13 (60 kDa) and MMP-14 (52 kDa) in the regressing uterus were lower at SD week 3 by 45% and 58%, respectively but no differences were noted at SD weeks 6, 9, or 12 as compared to LD control (Fig. 3B,C). The protein loading control β -actin showed no changes during regression (Fig. 3D). In the recrudescence experiment, MMP-3 protein levels were 35–39% higher at PT weeks 0, 1, and 2 as compared to the LD control (Fig. 3E). Levels of collagenase MMP-13 protein were significantly higher at PT week 4 (38%) as compared to the LD control group (Fig. 3F). Although levels of MMP-14 protein at PT week 0 (14 weeks SD) were not significantly different than the LD control group, MMP-14 levels at PT week 2 were significantly higher than PT weeks 0 (52%), 1 (39%) and the LD control group (35%) (Fig. 3G). MMP-14 levels at PT week 4, but not at PT week 8, were significantly higher (42%) than PT week 0 (Fig. 3G). No changes were observed in β -actin during recrudescence (Fig. 3H). TIMP-1 (30 kDa) and TIMP-2 (18–20 kDa) proteins were detected at very low levels both in regressing and recrudescing uterus, and were therefore not quantifiable (data not shown).

DISCUSSION

The results presented here show alterations in the expression of MMPs during photoperiod induced regression and recrudescence in the uterus of Siberian hamsters. Changes in photoperiod induced uterine tissue remodeling is evidenced by changes observed in uterine mass following exposure to SD for 12–14 weeks, and exposure of regressed animals to LD. Our results show for the first time that these photoperiod-induced changes in uterine remodeling are, at least in part, concomitant with differential and significant variations in MMP activity.

Twelve to 14 weeks of SD exposure induced significant declines in uterine weight and total protein (Fig. 1A–C), with both parameters returning to LD control levels within 2–4 weeks post-LD transfer (Fig. 1B,C). These data are consistent with the cessation of reproductive activity observed in Siberian hamsters during SD induced regression and the return of reproductive and endocrine function with recrudescence (Schlatt et al., 1993; Moffatt-Blue et al., 2006; Salverson et al., 2007). Decreases in uterine mass are associated with a loss of collagen (Kao et al., 1969; Shimizu et al., 1995), whereas uterine growth during pregnancy is associated with reduction in protein degradation without increased rates of protein synthesis (Morton and Goldspink, 1986). In the current study, reduction in uterus mass during SD

induced regression appears to be correlated with a decline in total protein. Similarly, the restoration of uterine mass following 4, and particularly 8, weeks of LD exposure is also associated with parallel increases in total protein (Fig. 1).

MMPs and their tissue inhibitors are crucial for tissue remodeling in the developing, pregnant, postpartum, and cycling uterus (Curry and Osteen, 2003; Page-McCaw et al., 2007). However, to our knowledge the role of the MMP/TIMP system during photoperiod induced uterine regression and recrudescence has not been examined previously. In the current study, gelatinases (MMPs-2 and 9), collagenases (MMPs-13 and -14), stromelysin (MMP-3), and TIMP-1 and 2 were found to be expressed to various degrees in the uterus from Siberian hamsters exposed to either 12–14 weeks of SD (regression) or upon transfer to LD (recrudescence).

Photoperiod induced uterine regression is a slow process that occurs over 12–14 weeks of SD exposure. This timeframe is distinct from the swift and dynamic uterine involution that occurs postpartum in rodents. Indeed, in rats, the uterus returns to its pre-pregnancy state rapidly, and the involution is predominantly a result of extracellular matrix degradation (Kengo et al., 2006). Postpartum regression is associated with increases in the mRNA and activity of MMP-14 (MT-MMP-1) and MMP-2 within 18–36 hr of parturition, with increased expression peaking prior to Day 5 postpartum (Kengo et al., 2006). In the present study, activity of gelatinases MMP-2 and 9 during SD induced regression did not significantly change for up to 9 weeks and then only MMP-2 activity showed a small (16%) but significant decrease at SD week 12 (Fig. 2A), a time point where uterine weight is decreased by 65% as compared to LD controls (Fig. 1A). These data suggest that gelatinases, although abundant in uterine tissue, may not be the major players in the chronic decline in uterus mass during regression. A different protein expression pattern was noted for MMPs-13 and -14 following SD exposure. Initially, these collagenases reacted to SD exposure with reduced expression ($P < 0.05$; SD week 3) but progressively increased to LD control levels at SDweeks 6, 9, or 12. This expression pattern may reflect a return to normal uterine function, or it may be that the same MMPs, necessary for the normal uterine cycle, are upregulated in a different capacity to aid with regression. MMP-13 exhibits collagenase activity at high levels during the rapid phase of postpartum uterine involution in rats (Weeks et al., 1976), and it may be that, following an initial SD adaptation period, this collagenase is also up-regulated in the extended uterine regression observed in seasonal breeders. MMP-14 (MT-MMP-1) is present in stromal and epithelial cells of mouse endometrium (Hu et al., 2004), cycling uterus in humans (Määttä et al., 2000), and, according to data from the present study, may be associated with photo-induced uterine regression, again after an initial reduction in expression following SD exposure.

In contrast to the other MMPs and TIMPs examined, MMP-3 protein expression showed a clear pattern of increase during SD exposure, peaking at SD week 9 as compared to all other groups (Fig. 3A). In mice, MMP-3 (stromelysin) is expressed at low levels in the uterus (Rudolph-Owen et al., 1997), and is specifically noted in stromal cells in cycling rats (Nuttall and Kennedy, 1999). The nuclear localization of MMP-3 suggests that it may play a role in apoptosis (Si-Tayeb et al., 2006) and may have a direct role in seasonal uterine regression. It is interesting that peak MMP-3 protein expression occurs just prior to significant declines in total uterine protein content (Fig. 1A and Fig 3A), this further suggests that this protease may have a prominent role in seasonal uterine regression. Stromelysin is upregulated in TIMP-1 null mice, suggesting that TIMP-1 may be important in regulating its activity (Nothnick, 2001). In the present study we observed low levels of TIMPs -1 and -2; this low expression may also contribute to higher levels of MMP-3 in hamster uterus. The low levels of TIMPs noted in the present study are, however, congruent with the low expression of TIMPs-1, -2 and -3 reported in proliferating human endometrium (Määttä et al., 2000). Again, the rapid timeframe of postpartum involution is quite distinct from the longer process (12–14 weeks) of

photoperiod induced uterine atrophy in the present study, where only MMP-3 was significantly upregulated (week 9) with SD regression. Additionally, we observed modest changes in MMP-2, -13 and -14, therefore suggesting that MMPs are not likely the key mediators of uterine atrophy in response to SD.

Transfer of photo-inhibited hamsters (14 weeks in SD) to long days in the present study was concomitant with progressive increases in uterine weight and total protein, and changes in specific MMP proteins during this stimulated recrudescence. The activity of MMP-2 peaked at PT week 4 (significantly higher than PT week 0 and LD control) in the midst of recrudescence (Fig. 2B). MMP-2 is expressed in stromal cells in humans and cats during the uterine cycle and gestation, respectively (Rogers et al., 1994; Freitas et al., 1999; Walter and Schönkypl, 2006), and may also play a role in stimulated uterine recrudescence. Although we saw no changes in the activity of MMP-9 in the current study, this gelatinase has a wide distribution in the cycling and hormonally primed uterus, with mRNA expression localized to the glandular and stromal cells of the endometrium, macrophages and other immune cells, and vascular tissue (Freitas et al., 1999; Skinner et al., 1999; Vincent et al., 1999). It may be that the diverse roles played by MMP-9 in uterine tissue prevent any large-scale changes in expression levels during photo-period-stimulated recrudescence. In general, protein expression of collagenases (MMP-13, -14) showed progressive increases and returned to LD levels essentially in parallel with changes in uterine weight. These proteases may be involved with the tissue remodeling that must occur to restore uterine function. MMP-3 expression was higher than LD controls early in recrudescence, however was reduced to LD levels by week 4 (Fig. 3), suggesting that this protease has a distinct function during recrudescence not apparent in normal LD reproductive activity. As with regression, TIMP-1 and TIMP-2 expression was low in the recrudescing uterus, again signifying that other mechanisms may also regulate MMP activity during seasonal change.

MMPs are present as proenzymes and are activated as needed by proteolytic cleavage, growth factors, specific inhibitors, and a variety of other factors (Curry and Osteen, 2003 for review). Previous studies from our laboratory have shown that plasma concentrations of estradiol decreases significantly at SD weeks 6, 9, and 12 as compared to LD controls (Moffatt-Blue et al., 2006) and are restored to LD levels by PT week 8, coinciding with the return of ovarian function (Salverson et al., 2007). Although estrogens may play a significant role in endometrial growth and uterine remodeling (Zhang et al., 2007), the effect of estradiol on MMP expression and activity is not fully understood. Estrogens may be involved in activating MMP gene transcription via the induced binding of AP-1 elements found in MMP gene promoters (Benbow and Brinkerhoff, 1997), or via induction of growth factors or inflammatory cytokines (Osteen et al., 2002). However it is not known if this is the case during seasonal changes in uterine function. Other reproductive hormones, including progesterone, have a suppressive effect on some uterine MMPs (Osteen et al., 2000); however, no changes in plasma progesterone were noted in Siberian hamsters following 3–12 weeks of SD exposure (Moffatt-Blue et al., 2006). In the present study, uterine tissue was harvested when hamsters were in the diestrus phase, with reduced estradiol and potentially increased progesterone concentrations. Thus it is likely that the relative levels of these ovarian steroids may also play a regulatory role in MMP activity, especially during the progressive return of ovarian function during recrudescence of reproductive function.

CONCLUSIONS

Results from the present study clearly show alterations in the expression of several MMPs, although not in TIMPs, during photoperiod induced changes in uterine function. However, changes in the activity of MMPs do not directly mirror significant changes observed in uterine weight during regression or recrudescence. Unlike pregnancy, parturition, or menstruation,

where uterine tissue experiences rapid and dynamic tissue remodeling, repair, and growth, photoperiod induced changes in uterus are slow and gradual (12–14 weeks for regression, 8 weeks for recrudescence) and modest changes in MMPs reported here are consistent with this view. In summary, data from the present study show that alterations in photoperiod changes total protein and uterine mass in Siberian hamsters. This is the first study to show the expression of MMPs/TIMPs in uteri from photoinhibited and photostimulated hamsters. Significant changes in the expression of MMP-2, MMP-3 and MMP-13 and MMP-14 were observed during photoperiod induced regression and recrudescence. Expression patterns of these proteins, although distinct, are consistent with some role in both uterine atrophy and restoration of uterus function, notably with MMP-3 peaking above LD levels during regression and MMPs-3, -13 and -14 upregulated above LD levels during recrudescence. Further investigations are necessary to explore the cellular events and specific roles of the MMP/TIMP system during the photoperiod-mediated seasonal loss and regain of reproductive function.

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Fig. 1.

A Paired uterine horn mass and **(B)** total extracted uterine protein concentrations (mg/uterus) in Siberian hamsters exposed to short days (8L: 16D) ($n = 10/\text{group}$) or LD (controls) ($n = 20$ per group) for 3, 6, 9, or 12 weeks. **C:** Paired uterine horn mass and **(D)** total extracted uterine protein concentrations (mg/uterus) from Siberian hamsters exposed to either SD ($n = 10$ per group) or LD (controls, $n = 18$) for 14 weeks then SD exposed animals were subsequently transferred to LD for 0, 1, 2, 4, or 8 weeks. Columns with different letters differ significantly ($P < 0.05$).

Fig. 2.

A: Gelatin zymography of MMP-2 and MMP-9 in uterine homogenates from Siberian hamsters ($n = 10/\text{group}$) exposed to short days (8L: 16D) or LD (control) for 3, 6, 9, or 12 weeks. Columns with different letters differ significantly ($P < 0.05$). Active MMP-9 (80–82 kDa); proMMP-2 (72 kDa) and active MMP-2 (60 kDa) bands are labeled. **B:** Gelatin zymography of MMP-2 and MMP-9 in uterine homogenates from Siberian hamsters ($n = 10/\text{group}$) exposed to either SD or LD (controls) for 14 weeks then SD exposed animals were subsequently transferred to LD for 0, 1, 2, 4, or 8 weeks. Columns with different letters differ significantly ($P < 0.05$). **C:** Zymograms were also developed in the presence of: no inhibitors, MMP-1 inhibitor, serine protease inhibitor, 5 mM EDTA or GM6001 as described under methods.

Fig. 3.

Representative Western blots and quantified means of Western analysis of MMP-3, MMP-13, and MMP-14 protein in uterine homogenates from Siberian hamsters ($n = 10/\text{group}$) exposed to (A–C) either SD or LD (controls) for 14 weeks and (D–F) either SD or LD (controls) for 14 weeks then SD exposed animals were subsequently transferred to LD for 0, 1, 2, 4, or 8 weeks. Uterus homogenates ($20 \mu\text{g}/\text{lane}$) were separated on 10% SDS–PAGE gels and immuno-blotted with specific antibodies as described in Materials and Methods Section. Columns with different letters differ significantly ($P < 0.05$).