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Mast cell involvement in human cervical ripening

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ABSTRACT

Objective: Cervical ripening resembles an inflammatory process in many aspects, involving invasion of inflammatory cells, collagen breakdown and remodelling of the extracellular matrix. Mast cells produce a variety of inflammatory agents and are attributed a functional role in cervical ripening. The aim of this study was to examine if cervical mast cells are increased in number and stimulated during pregnancy.
Study design: Cervical biopsies were obtained with a biopsy needle prior to surgical termination of pregnancy in the first trimester, surgery for first-trimester miscarriage, elective caesarean section, and benign gynaecological surgery in non-pregnant women. After fixation, semithin sections were prepared and stained with toluidine blue. The number of mast cells was counted under a light microscope and their secretory activity was scored (0.5–4) according to specified criteria and further visualised with electron microscopy. For pairwise comparison between groups Fisher's nonparametric permutation test was used.
Results: The number of mast cells was increased from 3.4 ± 1.65 mast cells per 10 visual fields in non-pregnant women to 7.70 ± 0.35 per 10 visual fields in first trimester control women ($p < 0.05$). The highest number of mast cells was observed at term with 10.8 ± 2.1 per 10 visual fields, a number that was significantly higher than in first trimester control women ($p < 0.05$). At term mast cell activity scores were 3.39 ± 0.37 compared with 2.69 ± 0.27 in control first trimester women and 2.21 ± 0.86 in women with missed miscarriage ($p < 0.05$). The percentage of mast cells with activity score 4 was significantly higher at term compared with in the first trimester. Free mast cell granules were predominantly observed in areas with disorganized collagen fibres.
Conclusion: The findings confirm that an increased influx of mast cells to the cervix occurs during pregnancy. The stimulated mast cell secretory activity in conditions associated with cervical tissue remodelling, such as term pregnancy and symptomatic miscarriage, provides further evidence that mast cells play a physiological role in cervical ripening.

Introduction

Mast cells derive from bone marrow and are widely distributed and chemotactically recruited in tissues throughout the body [1]. They are heterogeneous, and their functional properties depend on the local microenvironment in which they mature [2]. Mast cells are known to be involved in a diversity of biological processes, such as allergic reactions, tissue remodelling, wound repair and inflammation [3,4]. Morphologically, mast cells are characterized by numerous electron-dense cytoplasmic granules, containing a variety of biological mediators such as histamine, cytokines, proteases, and heparin, which are released from the mast cells in response to specific stimulation. The inflammatory mediators may in turn contribute to remodelling of the extracellular matrix, directly or indirectly by interaction with other inflammatory cells [4].

Cervical ripening in term pregnancy is characterized by invasion of inflammatory cells, tissue oedema and reorganization of the extracellular matrix [5], a process believed to be initiated by the shift in sex steroid levels occurring toward term [6]. This hormonal shift may also influence the migration of inflammatory cells to the uterus and the recruitment of mast cells, their maturation and degranulation have been demonstrated to be regulated by oestradiol and progesterone [7–10]. The potential of mast cells to release uterotonic mediators makes them powerful regulators of myometrial contractile activity. Thus, mast cell degranulation has been shown to initiate contractions in isolated human myometrium [11,12].

Previous studies indicated that there are more mast cells in the pregnant uterine cervix than in the non-pregnant cervix [13,14]. In

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addition, cervical mast cells appeared to be activated during spontaneous miscarriage as well as after induction of cervical ripening by vaginally administered misoprostol or isosorbide mononitrate [13–16].

The aim of the present study was to perform a systematic quantification of mast cells in the cervix of non-pregnant women and women in early and term pregnancy. We also estimated mast cell activation according to specified morphological criteria, and light-microscopic observations were followed up with electron microscopy.

Materials and methods

Study group 1

Healthy primigravid women treated vaginally with either 200 μg misoprostol or 40 mg isosorbide mononitrate to promote cervical ripening prior to surgical termination of pregnancy in the first trimester, as previously described in detail [13]. Primigravid women not given pre-surgical treatment served as controls. Before cervical dilatation, tissue biopsies were obtained from the central anterior cervical lip with a Tru-Cut® biopsy needle (Allegiance, Healthcare Corporation, McGaw Park, IL, USA). After fixation in 0.1 M Na-Cacodylate-buffered 3% glutaraldehyde, the middle portion of each tissue strip was dissected, washed in 0.1 M Na-Cacodylate-buffer containing 4% sucrose, post-fixed with 1% osmium tetroxide, dehydrated in graded series of ethanol and propylene oxide and embedded in epoxy resin (AGAR 100 RESIN®, Agar Scientific LTD, Stansted, Essex, England) [13].

Study group 2

Healthy nulliparous women in the first trimester, presenting with either symptomatic or missed (no bleeding or pain) miscarriage. Cervical biopsies were obtained prior to evacuation. Primigravid, non-pretreated women served as controls. The specimens were treated as described above (see study group 1).

Study group 3

Healthy women (gestational length > 37 weeks, no prior vaginal delivery, uncomplicated singleton pregnancy, without symptoms of labour and cervical Bishop score < 6) who were scheduled for elective caesarean section. Tissue biopsies were taken transvaginally from the anterior cervical lip under spinal analgesia before caesarean section. The specimens were snap-frozen in liquid nitrogen and the middle portion of each strip was dissected and prepared for microscopy as described.

Study group 4

Non-pregnant healthy women having regular cycles and being in the follicular phase of the cycle (cycle day 2–11) who were scheduled for benign gynaecological surgery. The biopsies were obtained under spinal analgesia before surgery was performed. The specimens were fixed and treated as described.

The number of women included in each study group and their demographics and characteristics are presented in Table 1.

For microscopic examination, semithin (1 μm) sections (three-six from each cervical specimen, obtained at different levels) were

Table 1
Variables in each study group.

Study group	Non-pregnant (n = 5)	Term pregnant (n = 5)	First trimester controls (n = 4)	Symptomatic miscarriage (n = 5)	Missed miscarriage (n = 4)	Misoprostol in first trimester (n = 4)	IMN in first trimester (n = 6)
Age, years	36.6 (22-47)	30.0 (26-33)	22.2 (20-24)	29.0 (23-35)	30.8 (25-36)	23.0 (20-25)	23.3 (20-26)
Gest. age, weeks		38.5 (37.5-40.0)	8.0 (7-9)	10.2 (9-12)	10.0 (9-11)	8.3 (8-9)	8.2 (7-10)
Number of MCs/10 vfs	3.4 (1.65)/ 3.4 (1.3-5.5)	10.8 (2.1)/ 9.6 (9.1-14.0)	7.7 (0.35)/ 7.8 (7.2-8.0)	10.6 (1.9)/ 10.2 (8.0-12.9)	3.03 (2.24)/ 3.1 (0.4-5.5)	10.0 (1.99)/ 9.65 (8.0-12.6)	5.1 (2.95)/ 4.6 (2.0-10.2)
Activity score	2.33 (0.31)/ 2.41 (1.89-2.71)	3.29 (0.37)/ 3.20(2.80-3.70)	2.69 (0.27)/ 2.78 (2.31-2.88)	2.88 (0.20)/ 2.82 (2.70-3.14)	2.21 (0.86)/ 2.48 (1.00-2.90)	2.90 (0.34)/ 2.96 (2.48-3.20)	2.74 (0.40)/ 2.67 (2.34-3.45)
% activity score 4	15.6 (5.4)/ 12.0 (11.0-22.0)	61.6 (25.2)/ 54.0 (31.0-90.0)	21.8 (4.5)/ 21.0 (18.0-27.0)	28.8 (11.3)/ 28.0 (12.0-43.0)	20.8 (18.9)/ 19.0 (0.0-45.0)	25.8 (13.0)/ 24.0 (13.0-42.0)	27.1 (17.6)/ 21.7 (13.0-61)

Gest. = gestational; MCs = mast cells; Mean (SD)/Median (min-max); n = number; vfs = visual fields.

Table 2
Pairwise comparison between groups.

Study Groups	Nonpregnant vs Term pregnant	Nonpregnant vs First trimester controls	Nonpregnant vs Symptomatic miscarriage	Nonpregnant vs Missed miscarriage	Nonpregnant vs Misoprostol in first trimester	Nonpregnant vs IMN in first trimester	Term pregnant vs First trimester controls	Term pregnant vs Symptomatic miscarriage	Term pregnant vs Missed miscarriage
Number of MCs/10 vfs	0.008 *	0.016 *	0.008 *	0.75	0.008 *	0.30	0.024 *	0.87	0.008 *
Activity score	0.008 *	0.10	0.024 *	0.83	0.040 *	0.093	0.032 *	0.063	0.024 *
% activity score 4	0.008 *	0.12	0.056	0.63	0.17	0.15	0.016 *	0.024 *	0.040 *

IMN = isosorbide mononitrate; MCs = mast cells; n = number; * = $p < 0.05$; vfs = visual fields.

stained with toluidine blue. Microscopy and photography were performed with a Nikon EFD-3 microscope connected to a Nikon digital camera. Mast cells were assessed at 400 x magnification, covering the whole area of each section, including 50–78 visual fields. The number of mast cells was counted and expressed as number of cells per 10 high power visual fields.

The activity of the mast cells was scored, including the following parameters: cell size (small, middle-sized, big); cell contour (round, irregular, cytolemmal elongations); blue-staining intensity of granules (strong blue, moderate blue, purplish); location of cells (vicinity of intact or distorted collagen); nuclear chromatin (compact or dispersed); prominent nucleolus; granule swelling (moderate, marked); intracellular distribution of granules (compact, dispersed); distribution of granules in neighbouring stroma. These parameters were subjectively evaluated, yielding a score between 1 and 4 for each single mast cell. Small, round mast cells with compact blue granules were scored at 1. Middle-sized mast cells with moderate blue and moderately swollen granules were scored at 2. In addition, small and middle-sized mast cells that exhibited at least two other signs interpreted as expressions of stimulated activity (dispersed chromatin, prominent nucleolus, dispersed intracellular distribution of granules) were upgraded by 0.5 to activity score of 1.5 and 2.5, respectively. Large mast cells with irregular contours, cytolemmal elongations and swollen, purplish granules were scored at 3. Cells that also exhibited free granules in the neighbouring stroma as an expression of active secretion were scored at 4. The mean score for each cervical specimen was calculated from the mean scores from all sections of that specimen. In addition, the percentage of mast cells given activity score 4 was calculated.

Counting and scoring of mast cells were performed on two occasions by one investigator who was blinded to the origin of the specimen. Repeated measurements were obtained without knowledge of the previous values. The intraobserver variability was calculated from 30 randomly selected specimens and expressed as the percentage variability and standard error of the mean according to accepted guidelines [17].

Ultrathin sections (60–90 nm) from women in early and term pregnancies were prepared for electron microscopy. The sections were retrieved on 150 mesh formvar-coated copper grids, contrasted with uranyl acetate/lead citrate, and examined with a Philips CM electron microscope as previously described [13,14].

Statistics

For pairwise comparison between groups Fisher's nonparametric permutation test was used. A p-value < 0.05 was considered statistically significant.

Ethical approval

The study was approved by the Regional Ethics Committees at University of Gothenburg, Sweden (S099-02) and at Lund University, Sweden (2015/857). The study was carried out in accordance with The Code of Ethics of the Declaration of Helsinki, and written consent was obtained from all participating women.

Results

In pregnant women, except in those suffering from missed miscarriage and women treated with isosorbide mononitrate, the number of mast cells was significantly higher than in non-pregnant women (Tables 1 and 2). Moreover, the number of mast cells was significantly higher in women at term and in women with symptomatic or missed miscarriage compared with control women in the first trimester (Table 2). The number of mast cells per 10 visual fields were in the same range (10.0–10.8) in term pregnant women, in women with symptomatic miscarriage and in women treated with misoprostol (Table 1).

In all groups of women mast cells were predominantly located in areas with disorganized collagenous tissue, exhibiting signs of tissue oedema as indicated by an increased distance between single fibroblasts (Fig. 1A). Generally, mast cells observed in the vicinity of intact collagen bundles or adjacent to smooth muscle bundles were rather small and fairly regularly contoured in all groups of women, albeit most prominently in non-pregnant women (Fig. 1B–D). The secretory granules in these cells were relatively small, compact and bluish giving them a low activity score. On the contrary, bigger mast cells with irregular contours or cytolemmal elongations were most frequently encountered in tissue areas with disorganized and disrupted collagen fibrils (Fig. 1B, E—H). These cells mostly demonstrated swollen, purplish or faint blue granules and/or scattered accumulations of secreted granules in the neighbouring stroma resulting in a high activity score.

The highest activity scores were found in term pregnant women and in first trimester women having either symptomatic miscarriage or being treated with misoprostol (Table 1). In these groups the activity score was significantly higher than in non-pregnant women (Table 2). In addition, the activity score was higher in term pregnant women than in first trimester control women. The maximal score of 4 was significantly more frequent at term than in all the other groups (Table 2). Although the number of mast cells was significantly lower in non-pregnant women, the percentage of mast cells with an activity score of 4 did not differ from that in first trimester pregnant women (Table 2).

As judged from the measurements of 30 randomly selected specimens the intraobserver variability was $7.3 \pm 1.12\%$ for the mast cell counting and $5.7 \pm 0.89\%$ for the activity scoring.

Electron microscopy demonstrated mast cells with varying expressions of secretory activity in both early and term pregnancy. Mast cells exhibiting elongations and granules of varying density and size were seen in all specimens. The mast cell granules tended to be denser and more homogeneous and more densely packed within the cell in areas with rather intact collagen (Fig. 2A, B). Free granules were predominantly observed in stroma where the collagen fibres were disorganized (Fig. 2C, D). In some mast cells, irregularity and varying density of the granules were prominent (Fig. 2D, F, G). The granules were observed throughout the cytoplasm (Fig. 2D, E, H).

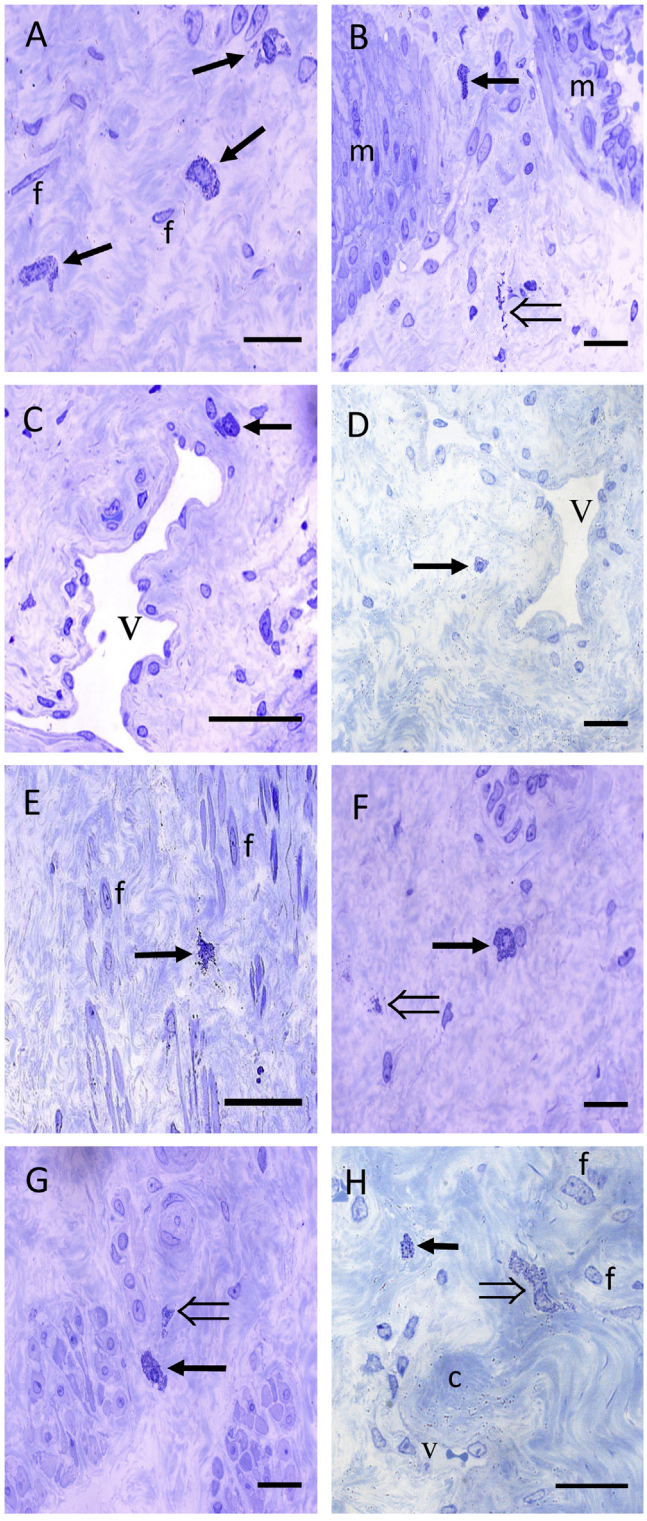

Fig. 1. Toluidine-blue stained sections of cervical tissue from pregnant and non-pregnant women (scale bar = 50 μm). A. Gestational week 9, pre-surgical treatment with isosorbide mononitrate. Three mast cells (arrows) with loosely packed, slightly swollen, bluish-purplish granules (activity scores 2–2.5) located in partly disintegrated, oedematous tissue with fibroblasts (f) separated apart. B. Term pregnancy, gestational week 40. One mast cell (arrow) with bluish, fairly compact granules, visible nucleolus and slightly irregular contour in rather compactly organized connective tissue between muscle bundles (m) (activity score 2.5). Free granules (open arrow) are observed in the disorganized collagenous tissue (activity score 4). C. Symptomatic miscarriage gestational week 10. One small mast cell (arrow) close to a vessel (v) in intact perivascular collagenous tissue; bluish, tightly packed granules (activity score 1). D. Non-pregnant, cycle day 8. One small mast cell (arrow) close to vessel (v). The mast cell granules are purplish (activity score 2). E. Symptomatic miscarriage gestational week 10. One mast cell (arrow) with irregular contour, cytoplasmic elongations containing granules. Secretory granules are seen in the adjacent matrix (activity score 4). f = fibroblast. F. Gestational week 9, presurgical treatment with misoprostol. One mast cell (arrow) with visible nucleolus, bluish-purplish loosely packed granules and slightly irregular contour (activity score 2.5) and one mast cell (open arrow) showing secretory activity, surrounded by free granules in the tissue stroma (activity score 4). G. Control, gestational week 9. Two mast cells with swollen, purplish granules and irregular contours (activity scores 2.5 (arrow) and 3 (open arrow), respectively. H. Non-pregnant, cycle day 9. Two mast cells, one relatively small with more bluish granules (arrow) (activity score 2), and one with irregular contour and swollen purplish granules (open arrow) (activity score 3). The collagen (c) is intact. f = fibroblast; v = vessel.

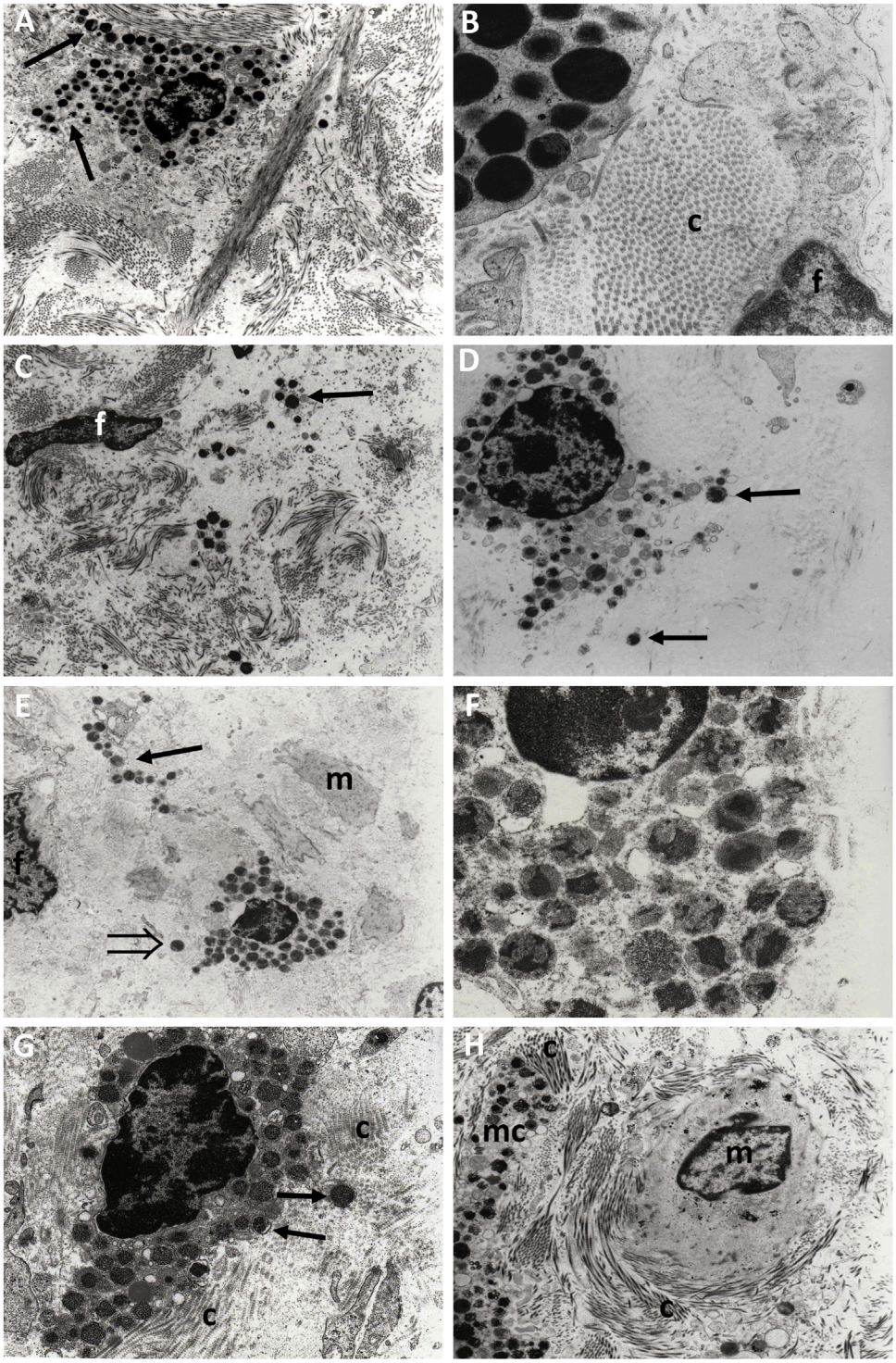

Fig. 2. Electron micrographs of cervical tissue from pregnant women. A. Gestational week 42. The stroma is partly disorganized. Mast cell with cytoplasmic elongations (arrows) and granules of varying density and size (x 5,750). B. Symptomatic miscarriage, gestational week 10. Section of a mast cell close to transversely sectioned collagen (c). The granules are of fairly homogeneous density. f = fibroblast (x 41,000). C. Gestational week 42. Free secreted MC granules (arrows) are seen in a disordered matrix indicating collagenolysis. f = fibroblast (x 7,250). D. Control, gestational week 8. An area with loose stroma and a mast cell with a prominent nucleolus, irregular contour and cytoplasmic projections, granules of varying density, size and internal structure, free secreted granules in the stroma (arrows) (x 14,500). E. Symptomatic miscarriage, gestational week 11. Partially disorganized stroma. Mast cell with prominent cytoplasmic elongation containing granules of varying size and density (arrow). Secreted granule indicated (open arrow). f = fibroblast; m = smooth muscle (x 7,250). F. Micrograph demonstrating secretory granules of different internal morphology indicating the heterogeneity of the mast cell population (symptomatic miscarriage, gestational week 10) (x 41,000). G. Gestational week 38. Partly disrupted collagen bundles (c). Mast cell exhibiting secretory granules of various sizes exhibiting granular contents of low density. Granules under exocytosis indicated (arrows) (x 22,000). H. Missed miscarriage, gestational week 11. Projection of a mast cell (mc) surrounded by relatively intact collagen bundles (c) in the vicinity of a smooth muscle cell (m), secretory granules of varying size, density and internal structure (x 14,500).

Comments

This study confirms our previous hypothesis that the number of cervical mast cells is increased in pregnant women compared non-pregnant women [13,14]. In addition, it shows that mast cells increase in number as gestation progresses. Similar observations have been previously made in women undergoing spontaneous early miscarriage [18]. The observed increased invasion and activation of mast cells in tissue areas being under obvious remodelling emphasizes their involvement in the regulation of the extracellular matrix. In this context, it is interesting to note that the highest individual number of mast cells as well as the highest mast cell activity scoring were registered in women at term and in women having symptomatic miscarriage.

We have previously reported that cervical collagenous tissue in women with missed miscarriage appeared to be even more disorganized than in women with ongoing spontaneous miscarriage [13]. Therefore, it was unexpected to find that the number of mast cells in the cervices of women with missed miscarriage was lower than in other first trimester groups. However, it must be emphasized that the number of women in this group is small and that the miscarriage process might have proceeded differently among the women. Considering the suggestion that mast cells are involved in the regulation of uterine contractility [6], it is tempting to speculate that limited recruitment of mast cells may impair not only cervical ripening but also the establishment of uterine contractions to promote the abortive process in women with missed miscarriage.

In a previous study, we observed that the disorganization of the cervical collagenous tissue was more prominent after pretreatment with either misoprostol or isosorbide mononitrate before termination of pregnancy in the first trimester [14]. However, as assessed from subtle cytoplasmic and nuclear findings, the reactive phenomena were slightly more pronounced after misoprostol. We also observed that misoprostol was superior to isosorbide mononitrate to induce cervical ripening though the clinical response to isosorbide mononitrate varied [19]. In accordance with this, we noticed in the present study a wide range both in the number of mast cells and mast cell activity in the isosorbide mononitrate group of women whereas the mast cell response to misoprostol was fairly consistent. Nevertheless, very high activity scores were found in some specimens from women treated with either isosorbide mononitrate or misoprostol (Table 1). Therefore, it appears reasonable to assume that both isosorbide mononitrate and misoprostol can exert a direct effect on mast cells [20,21].

Although the number of mast cells in cervical biopsies from non-pregnant women was lower than in pregnant women, the activity scores and the proportion of cells with maximal activity were in the range found in the control first-trimester cervices. Since mast cells have the potential to express steroid receptors [7–9] it seems reasonable to believe that mast cells also are involved in cellular events that regulate cervical tissue remodelling during the menstrual cycle [22]. A similar theory was proposed, based on light microscopy and electron microscopy studies, demonstrating cyclical changes in mast cell morphology [23].

Ultrastructural observations have supported the concept of mast cell heterogeneity and presented theories for the influence of the microenvironment on the maturation and differentiation of mast cells [24–26]. Storage and release of the mast cell granular material appear to occur in a discriminating and sequential manner by selective mechanisms depending on the activating agents involved. In the present study, electron microscopy, in accordance with the light microscopical findings, demonstrated mast cell heterogeneity with substantial variation in the size, density and structure of the secretory granule contents, probably reflecting an adaptation of the mast cells to an ongoing tissue remodelling. Thus, mast cell granules were denser and more densely packed within the cell in rather intact collagenous tissue and more freely distributed in disorganized tissue. However, since mast cells with both low and high activity score were diagnosed in all groups of women, electron microscopy could not discriminate mast cells with features specifically related to gestational conditions.

Toluidine blue staining is an established technique for visualizing mast cells. The size and swelling of the granules and increased metachromacy are phenomena attributed to differentiation and activation of mast cells and may reflect the functional adaptation of the cells [2,27]. Based on these observations as well as our initial findings at a preliminary screening of all included cervical tissue slides, we selected the parameters that were subsequently applied to score mast cell activity. In light of the fact that the intraobserver variability in the repeated scoring procedures was reasonable, the used scoring method appears adequate and reliable.

Taken together, the results show that the number of cervical mast cells is increased during pregnancy and indicate that mast cells are actively involved in cervical ripening.

Disclosure of interests

The authors have no competing interests to disclose.

Contribution

All authors have contributed significantly and are responsible for the content of this manuscript.

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