ORIGINAL ARTICLE

Optical properties, physiologic parameters and tissue composition of the human uterine cervix as a function of hormonal status

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Abstract The influence of sex hormones on the human uterine cervix is likely to be important in the process of cervical ripening. Frequency domain near-infrared spectroscopy (FD-NIRS) was used to investigate non-invasively the changes in the optical properties that reflect physiologic parameters and tissue composition of the uterine cervix in the different phases of the menstrual cycle. Twenty premenopausal and nine postmenopausal women were examined. Optical properties of the uterine cervix were measured, and physiological parameters [concentration of water, oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb), total hemoglobin (tHb), oxygen saturation (StO_2), water, and scattering power] were calculated. Analysis of variance (ANOVA) was used to test for statistical significance. The optical properties of the anterior cervical lip did not differ from those of the posterior lip. HHb was significantly lower in cervices during menstrual bleeding than during the follicular, luteal, or postmenopausal phases. The ratio of O_2Hb to HHb was highly significantly increased by a factor of 2 when cervices during the menstrual bleeding were compared with those during the follicular, luteal, or postmenopausal phases. The scattering power was significantly lower during menstrual bleeding than during the follicular or postmenopausal phases. We demonstrated that withdrawal of sex hormones during menstrual bleeding is associated with a significant decrease in HHb and scattering power, with stable values of O_2Hb, tHb, StO_2, and H_2O compared with the values during the follicular, luteal or postmenopausal phases of the menstrual cycle. Cervical softening during menstrual bleeding seems to be different from cervical softening for labor.

Keywords Uterine cervix · Cervical ripening · Frequency domain near-infrared spectroscopy (FD-NIRS) · Optical properties of the human uterine cervix · Hormonal status

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Introduction

Preterm labor is responsible for 70% of perinatal deaths and nearly half of long-term neurological morbidity [1], and it is probably one of the most challenging problems in modern obstetrics and gynecology. One of the keys to treating preterm labor and preventing severe neonatal complications could be the early identification of women at risk. Evaluation of cervical ripening to predict preterm labor is commonly performed by digital examination or sonography.

Preterm cervical ripening may be induced by local infection, cervical incompetence or uterine contractions. Undoubtedly, cervical ripening is associated with many biochemical and physiological changes of the cervical tissue. Hormone-regulated biochemical changes occur

during pregnancy, ultimately leading to cervical ripening and birth [2]. We recently demonstrated that prostaglandin-induced cervical ripening results in a significant change in optical properties (i.e., the absorption and scattering of light), a significant decrease in total hemoglobin (tHb), oxyhemoglobin (O_2Hb), deoxyhemoglobin (HHb), and a highly significant increase in water content (H_2O) [3]. So far, the influence of sex hormones on the optical properties of the human uterine cervix has not been investigated, but it is likely to be important in the process of cervical ripening [4].

Near-infrared spectroscopy (NIRS) is used to characterize the optical properties of thick tissues in vivo in a non-invasive way [5]. Optical properties can be quantitatively defined in terms of the absorption and reduced scattering coefficients (μ_a and μ'_s) of light. These parameters are sensitive to tissue concentrations of light-absorbing molecules and light scattering structures. The primary tissue contributors to light absorption are O_2Hb, HHb, H_2O), lipids, cytochromes and melanin [6]. Light scatterers are the structural elements of tissue, such as cell components and fibrous materials in the extracellular matrix (e.g., collagen/elastin). Frequency domain NIRS (FD-NIRS) is a technique capable of quantifying absolute tissue μ_a and μ'_s values. Absolute concentrations of tissue O_2Hb, HHb, tHb, oxygen-saturation (StO_2) and H_2O are derived from the μ_a of the tissue [7, 8]. We used FD-NIRS to examine in vivo the optical properties of human cervical tissues in various menstrual phases. Postmenopausal woman with low levels of estrogen and progesterone were compared with premenopausal woman during the three phases of the menstrual cycle, namely menstruation, the follicular phase, with high levels of estrogen, and the luteal phase, with high levels of estrogen and progesterone.

The aim of this study was to investigate the optical properties and tissue composition of the human uterine cervix during the above-mentioned menstrual phases to elucidate the effects of the sex steroid hormones on cervical ripening in non-pregnant women.

Patients, materials and methods

A total of 29 women scheduled for hysterectomy were included in the study after we had obtained their written informed consent. Four woman were menstruating (i.e., days 1–4 of the menstrual cycle), seven woman were in the follicular phase (i.e., day 5–14 of the menstrual cycle), nine woman were in the luteal phase (i.e., days 16–28 of the menstrual cycle), and nine woman were postmenopausal.

In the operating theater during anesthesia, immediately prior to surgery, a routine gynecological examination was performed by an experienced gynecologist. The vaginal probe was sterilized according to hospital standards for clinical instruments. At the end of the routine clinical checkup, while the gynecological speculum was still in situ, the uterine cervix was cleaned to remove mucous and detritus in order to allow optimal light penetration and signal detection. The vaginal probe of the FD-NIRS instrument was inserted into the vagina and placed on the vaginal portion of the uterine cervix with gentle pressure, ensuring good probe-to-cervix contact but without altering perfusion. Measurements were taken once on each quadrant of the cervix. The data acquisition time was approximately 30 s per measurement; thus, the overall examination time for the study was no more than 2 min per patient, leading to negligible additional discomfort for the patient. The probe was withdrawn from the vagina. The data acquisition technique is standardized, and has been performed by the same physician for all patients, ensuring high precision.
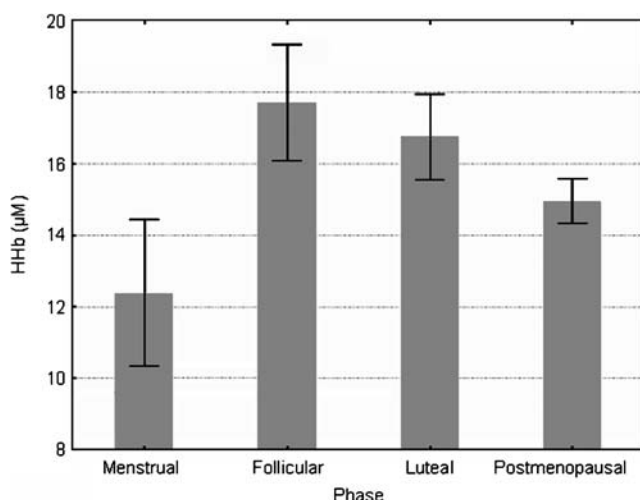
The FD-NIRS instrument used at the Department of Obstetrics and Gynecology, University Hospital of Zurich, is a modified Imagent (ISS, Champaign, IL, USA). It is equipped with seven near-infrared diode lasers (emitting at 690 nm, 730 nm, 750 nm, 808 nm, 870 nm, 920 nm and 970 nm) and one avalanche photodiode light detector. The instrument includes a pair of frequency synthesizers (PTS 500 frequency synthesizer, Programmed Test Sources Inc., Littleton, USA) capable of switching frequencies within less than 1 ms. One frequency synthesizer modulates the intensity of the laser diodes with frequencies between 130 MHz and 490 MHz, while the other modulates the detector at a frequency which is 5 kHz different from the source modulation, thus demodulating the light frequency at the detector. The instrument is equipped with a complete software package (Boxy, ISS) for data acquisition and two calibration blocks with known optical properties. Once data acquisition has been initiated, the frequency dependence of the phase and amplitude of the photon density wave is measured in steps of 10 MHz and fit to analytically derived model functions in order to calculate μ_a and μ'_s parameters for each wavelength. In addition, the wavelength dependence of absorption is automatically used to calculate O_2Hb, HHb, tHb, StO_2 and H_2O. The wavelength-dependent decrease of scattering (scatter power) is calculated by an exponential fit. The system is fully automated.

For our study we utilized the previously described probe, specifically designed for measurements of the human uterine cervix [3]. The vaginal probe was slightly bent, allowing good visualization during placement on the cervix. Eight multimode silica fibers (400 μm diameter, 3 M FT 400 EMT, Thorlabs Inc., USA) were coupled to the laser source to transmit the light to the tip of the probe. One glass fiber bundle (3 mm in diameter) conducted the light from the tip of the probe to the detector. The source-detector separation was 10 mm. Condom-like latex-covers were used to prevent transfection from patient to patient.
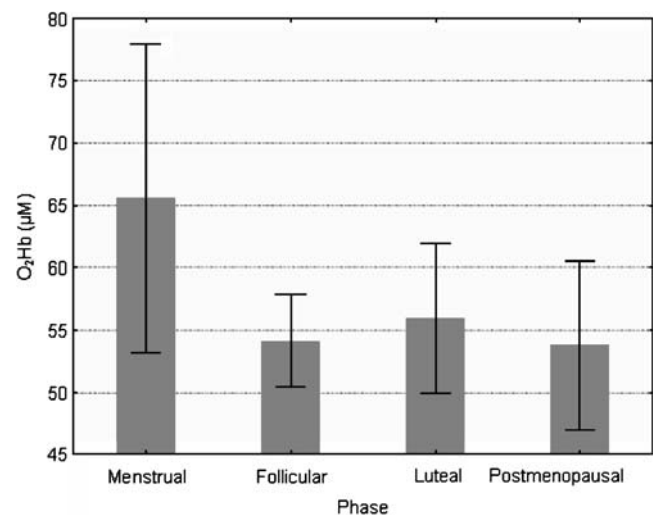
The software package STATISTICA 7.0 (Statsoft Inc., Tulsa, USA.) was used for statistical analysis. All statistical procedures were applied to untransformed data. The Kolmogorov–Smirnov test was used to check data for normal distribution. None of the response variables differed significantly from a normal distribution (all $P > 0.2$). One-way, two tailed, analysis of variance (ANOVA) was used to calculate statistical significance of differences between optical properties of the uterine cervices grouped into the four menstrual stages (m=menstruation, f=follicular, l= luteal phases, p=postmenopause). P levels below 0.05 were considered as statistically significant, and those below 0.005 as highly significant.

Results

The optical properties of the anterior cervical lip did not differ from those of the posterior lip (data not shown). Figure 1 shows HHb as a function of the four menstrual stages. It was significantly lower in cervices during menstrual bleeding than during the follicular or the luteal phase ($P = 0.016$, $P = 0.038$). A substantial, but not significant, increased level of O_2Hb was measured in cervices during the menstrual cycle, as shown in Fig. 2. Similarly, tHb was highest during menstrual bleeding and lowest in postmenopausal cervices (Fig. 3). However, the differences were not significant. The ratio O_2Hb/HHb was highly significantly increased by a factor of 2 when cervices during menstrual bleeding were compared with those during the follicular, luteal or postmenopausal phases. Pair-wise comparisons demonstrated that the menstrual
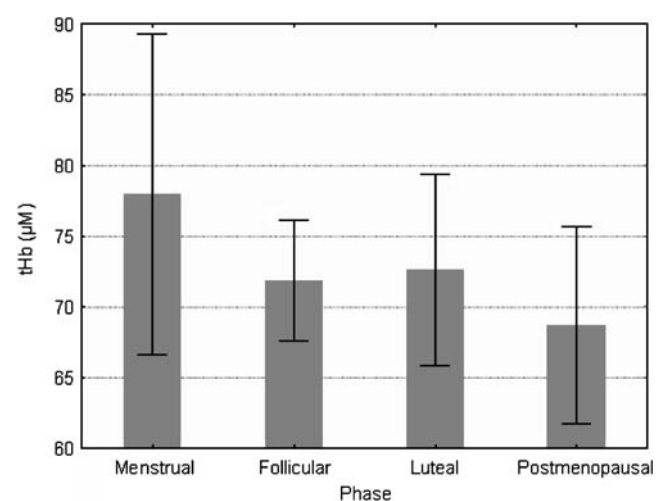

Fig. 2 Oxyhemoglobin (O_2Hb) is shown as a function of the four menstrual stages. A substantial, but not significant, increased level of O_2Hb was measured in cervices during the menstrual cycle. *Error bars* represent the standard deviation

phase differed significantly from all other phases ($P = 0.004$, $P = 0.003$, $P = 0.005$ for follicular, luteal and post-menopausal phases, respectively). Likewise, oxygen saturation (StO_2) was markedly increased during menstrual bleeding (Fig. 4). However, the differences when StO_2 values during menstruation were compared with those during the other physiological phases were not significant. No differences in tissue water content were observed when cervices were grouped into the four menstrual phases (Fig. 5).

The scattering power (SP; Fig. 6) as a function of the four menstrual phases was significantly lower during menstrual bleeding than during the follicular or postmenopausal

Fig. 1 Deoxyhemoglobin (HHb) is shown as a function of the four menstrual stages. Deoxygenated hemoglobin was significantly lower in cervices during menstrual bleeding than during the follicular or luteal phases, or during the postmenopause phase. *Error bars* represent the standard deviation

Fig. 3 Total hemoglobin (tHb) is shown as a function of the four menstrual stages. tHb was highest during menstrual bleeding and lowest in postmenopausal cervices. *Error bars* represent the standard deviation
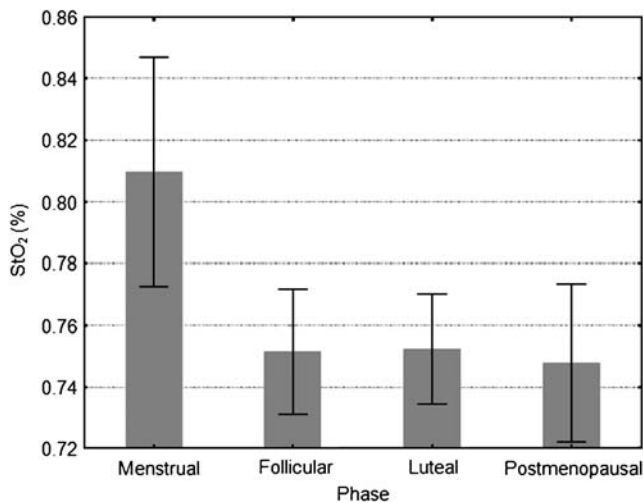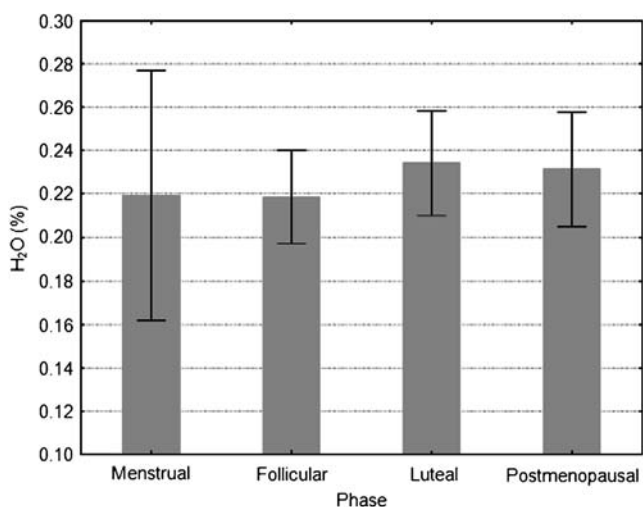
Fig. 4 Oxygen saturation (StO_2) is shown as a function of the four menstrual stages. StO_2 was markedly increased during menstrual bleeding. *Error bars* represent the standard deviation

phases, but not when compared with the luteal phase ($P = 0.043$; $P = 0.039$; $P = 0.15$).

Discussion

Preterm labor is a major obstetric problem, with a potentially adverse fetal outcome. Early identification of women at risk is one the most important aims in the daily routine of every obstetrician. Women at risk are identified on the basis of their history, their microbiological and serological test results, and on the evaluation of their uterine cervices. Bimanual examination and transvaginal sonography of the uterine cervix provide information on

Fig. 5 Tissue water content (H_2O) in percent is shown as a function of the four menstrual stages. H_2O was stable throughout the cycle. *Error bars* represent the standard deviation
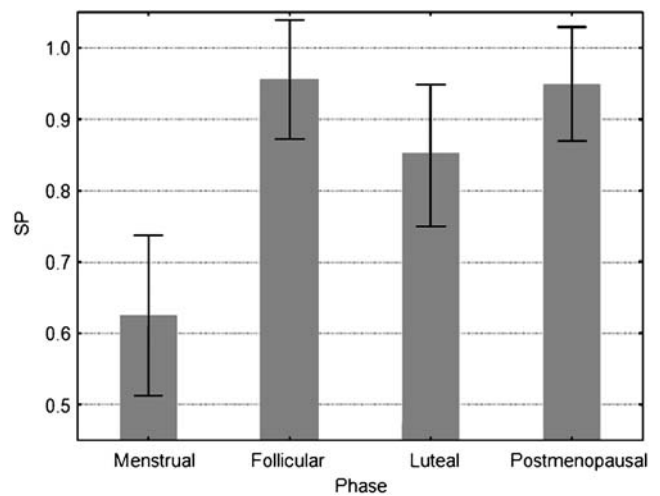
Fig. 6 The scatter power (SP) is shown as a function of the four menstrual phases. SP was significantly lower during menstrual bleeding than during the follicular or postmenopausal phases. *Error bars* represent the standard deviation

morphology that developed before. The assessment of physiologic profiles predicting preterm delivery is difficult and, so far, very unsatisfactory. Tocography and electromyography can be utilized to monitor contractions of the uterine muscle [9]. The development of new techniques that measure physiologic parameters of the uterine cervix with the potential to predict preterm delivery is of the utmost clinical importance.

In a complex research project we are currently investigating various influences affecting the physiology of the uterine cervix. Among these influences, the effects of hormones on the supply of blood, oxygen and water in the cervix is an important focus [4]. We recently demonstrated that FD-NIRS technology is highly sensitive and accurate in detecting changes in cervical physiology [3, 10]. This is in good agreement with our study, where the comparison of data of the anterior and posterior cervical lips as an internal quality control did not show any differences. In addition, in previous work, we demonstrated that prostaglandin-induced cervical ripening results in a significant change of optical properties, a significant decrease in tHb, O_2Hb, and HHb, and a highly significant increase in water content [3]. The influence of sex hormones on the optical properties of the uterine cervix and its physiology in non-pregnant individuals is the focus of the study described here.

As shown in Fig. 1, HHb is significantly lower in cervices during menstrual bleeding than during other menstrual phases. O_2Hb, tHb, StO_2, and H_2O showed slight changes throughout the cycle. Interestingly, however, the ratio of O_2Hb to HHb was highly significant lower during the menstrual bleeding than during any other phases evaluated. Proliferative processes during the follicular

phase, i.e., estrogen-dominated phases, and secretory processes during the luteal phase, i.e., the progesterone-dominated phases, seem to metabolize substantially more oxygen than during the menstrual phase. In addition, menstrual bleeding is characterized by a strong increase of blood flow into the uterus and its cervix. The blood, however, does not stay in the uterine and cervical tissues but leaves the uterus and is known as menstrual bleeding. Thus, the increased blood influx into the uterine tissue is counterbalanced, in part, by the menstrual bleeding, which is well represented by the tHb that increased only slightly during the menstrual bleeding (Fig. 3).

The human uterine cervix is a complex tissue. The cervix is composed of an extracellular matrix and cellular components. The extracellular matrix consists predominantly of collagen, elastin and proteoglycans. The cell components consist of smooth muscle cells, fibroblasts, epithelial cells, and blood vessels [11]. The scattering of light is a boundary phenomenon. Boundaries in biologic matters are cell membranes, organelles, or extracellular matrix fibers (i.e., collagen and elastin). As photons propagate in tissues and hit boundaries, the change in refractive index alters the photons' angles of direction, the so-called scattering of light. The scatter power depends on the size distribution of the intra- and extracellular scatterers, which change during apoptosis and extracellular matrix remodeling. In our measurements we observed a significant decrease in scattering power during menstruation. This may be explained by shrinkage of the cell components of the uterine cervix after programmed cell death, so-called apoptosis [12, 13]. The concept of apoptosis with reduced oxygen consumption by the cells during menstrual bleeding is also supported by the highly significant increase in the ratio of O_2Hb to HHb during menstrual bleeding. On the other hand, oxygen saturation remained almost stable during menstrual bleeding. Although it can be assumed that inflammatory processes resulted in increased oxygen consumption by the tissue, this seems to be counterbalanced, in part, by the reduced oxygen requirement of the tissues undergoing apoptosis. The decrease in the scattering power may also be explained by changes in collagen ultrastructure. In naturally cycling mice, circulating estradiol was positively correlated with stromal cell proliferation and collagen type-I content, while it was negatively correlated with loss of collagen type-IV surrounding glandular epithelium and apoptosis in luminal, glandular, and stromal compartments [14]. This may explain why, during the follicular and luteal phases in our study, the scattering power remained at a high level and dropped by one-third during menstrual bleeding. While the high estrogen and progesterone levels during the follicular and luteal phases inhibit collagenase activity [15, 16], the decrease in the sex steroid hormones during menstrual

bleeding results in increased collagenase activity, with increased collagen fragmentation leading to reduced light scattering and a drop in scattering power (Fig. 6). Similarly, when a mifepristone-induced progesterone withdrawal was induced in the rat cervix, there was a decrease in collagen organization and an increase in collagen fragmentation, with a significant decrease in fibril length and diameter, although fibril bundling remained unaffected [17, 18]. This, again, fits well with our data, with those reported by other groups [19, 20], and, most importantly, with the clinical appearance. During the menstrual phase, the cervix becomes clinically softer; the cervical canal opens for the menstrual bleeding, similarly to the cervical ripening for labor. Interestingly, the clinical softening of the uterine cervix during menstruation is not based on an increase in water content (Fig. 5). This phenomenon has also been described in a non-pregnant cow model, where differences in texture of the cervix could not be explained by differences in water content [20]. In pregnant humans, in turn, cervical softening was associated with a dramatic increase in water content [3], which is consistent with the findings in the cow model during pregnancy and labor [21]. Apparently, cervical softening during menstrual bleeding has a different physiology and biochemistry than cervical softening for labor. While cervical softening during menstrual bleeding seems to be based mainly on the withdrawal of sex steroids, leading to a disinhibition of metalloproteinases and disintegration of collagen, cervical ripening for labor seems to be based on inflammatory processes, with hyperemia and edema.

It is surprising that there was no difference between the data of the postmenopausal women and those of the premenopausal women. Apparently, postmenopausal atrophy of the cervical components does not affect their optical properties and the physiologic parameters derived from them.

In this study we demonstrated that withdrawal of sex hormones during menstrual bleeding is associated with a significant decrease in HHb and scattering power, with stable values of O_2Hb, tHb, StO_2, and H_2O compared with the values during the follicular, luteal or postmenopausal phases of the menstrual cycle. Cervical softening during menstrual bleeding seems to be different from cervical softening for labor.

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