
CONFERENCE REPORT

Prediction of term and preterm parturition and treatment monitoring by measurement of cervical cross-linked collagen using light-induced fluorescence

HOLGER MAUL^{1,2}, GEORGE SAADE¹ AND ROBERT E. GARFIELD¹

From the ¹Division of Reproductive Sciences, The University of Texas Medical Branch Galveston, TX, USA, and ²Department of Obstetrics and Gynecology, University of Heidelberg, Heidelberg, Germany

Acta Obstet Gynecol Scand 2005; 84: 534–536. © Acta Obstet Gynecol Scand 84 2005

Submitted 22 September 2004

Accepted 29 November 2004

One of the keys to treating preterm labor is the early detection of changes indicating the onset of parturition.

Recently, we have developed a non-invasive method for the objective evaluation of the status of the cervix, where changes in collagen content of the cervix can be detected using an optical system and light-induced autofluorescence (LIF). This system measures the collagen fluorescence in the cervix as an indirect estimate of collagen concentration. Studies of pregnant women during the past few years support the use of this technique.

Cervical function and assessment

The cervix is composed of smooth muscle (ca. 10%) and a large component of connective tissue (90%) consisting of collagen, elastin, and macromolecular components, which make up the extracellular matrix (1–3). Many biochemical and functional changes occur in cervical connective tissue at the end of pregnancy (4–7). This process, termed cervical ripening, results in softening, dilatation, and effacement of the cervix. Ripening is required for appropriate progress of labor and delivery of the fetus.

Fluorescence spectroscopy of collagen

Fluorescence spectroscopy is a widely utilized research tool in the biosciences, primarily because of the amount of information that it can reveal in terms of molecular and physical states (8–14). We have used this methodology recently to evaluate the cervix during gestation.

The collascope and measurement of cervical ripening

Collagen gives characteristic fluorescence whose maximum is around 390 nm. The intrinsic fluorophore is believed to be pyridinoline, which is considered one of the major crosslinks within the primary structure of collagen fibrils (10, 15, 16). In our initial investigations, measurements were obtained from the serosal surface of the medium band of the cervix of rats *in vivo*. The results showed a decrease in fluorescence intensity decrease in the later gestational days and at parturition corresponding to the decrease in collagen. We also found a drop in collagen fluorescence intensity in rats treated with the anti-progesterone compound RU 38.486 and which delivered prematurely.

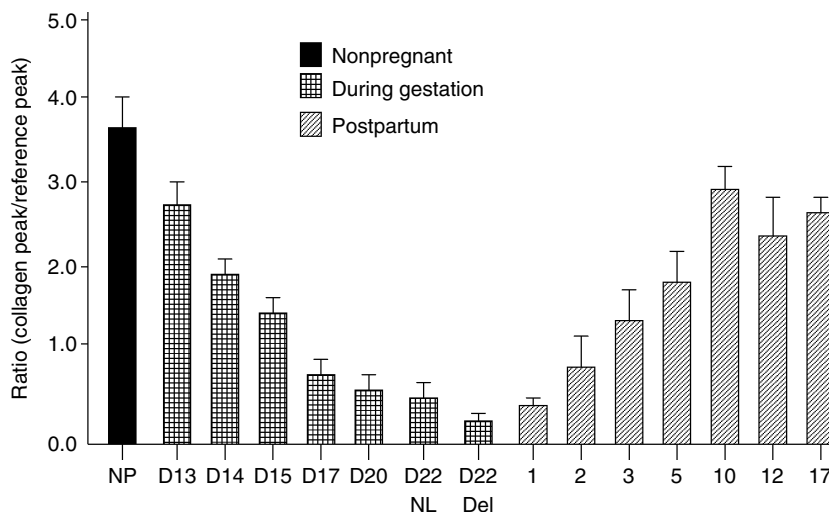


Fig. 1. Overview of rat cervical collagen fluorescence intensity changes: average values of ratio of collagen peak to reference peak obtained in non-pregnant, various times during pregnancy, during delivery and at various times postpartum in rats. Note the progressive decline in fluorescence with lowest values obtained on day 22 (DL, during labor; NL, non-labor).

Further studies of rats

With the collascope, we were able to measure the fluorescence signal from the cervix in anaesthetized rats (17). The advantage to this technique is that one can follow cervical changes longitudinally in the same animal under a variety of conditions and treatments (Fig. 1).

In the postpartum period, the fluorescence gradually increased from the low value observed during delivery (Fig. 1). These results demonstrate the progressive decline in fluorescence during pregnancy to reach low values during delivery; these findings correlate well with cervical resistance as measured by the slope of stress-strain curves and a decline in cervical collagen content in electron micrographs of ripened versus unripe cervix. In addition, we examined rats at various times prior to and during preterm labor induced with the anti-progesterone onapristone. This study showed that ripening occurred with anti-progesterone treatment and

that R5020, a progestin agonist, prevented ripening and preterm birth.

We conclude from these studies that the collascope can be used as a non-invasive tool to measure changes in cervical collagen content of the cervix under a variety of conditions. Results of these measurements correspond with known physiological changes in the cervix during pregnancy.

Studies of humans

We have also initiated human studies with the Collascope (17). Non-pregnant, pregnant, and postpartum human volunteers were recruited for the study. The first step was to establish a longitudinal distribution profile according to the weeks of gestation and postpartum. The cervical external os was gently wiped with rayon-tipped proctoscopic swabs prior to measurements being made. The measuring site was selected at the 12 o'clock position. Fluorescence decreased

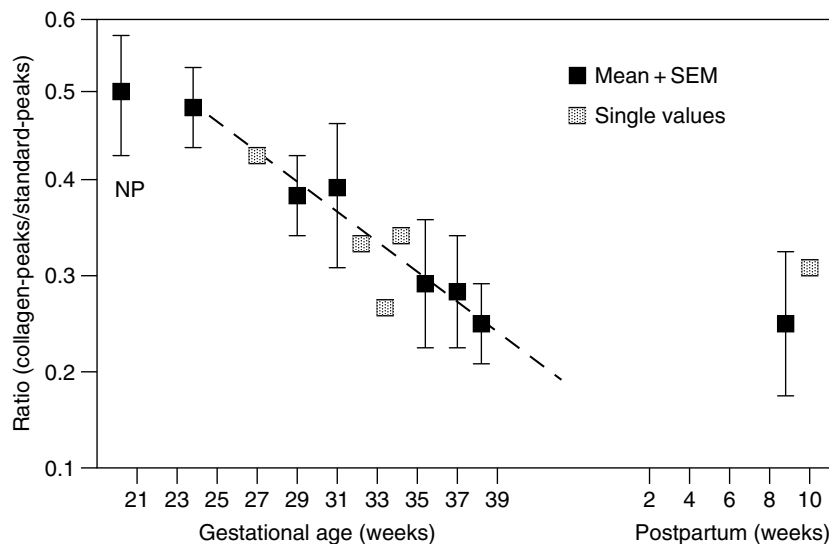


Fig. 2. Cervical fluorescence – pregnant women, during pregnancy, and postpartum: average and single patient data on fluorescent spectra obtained from 40 non-pregnant and pregnant patients at various times of gestation and postpartum.

progressively during the final 15 weeks of pregnancy (Fig. 2). So far, several hundred patients (including nonpregnant patients) have been recruited. Several of the subjects have been measured two or three times during their pregnancy and postpartum. The results show a gradual decrease of the fluorescence as pregnancy approaches term followed by a slow recovery during the postpartum period (17–19). Recent studies demonstrated that the collascope could be used for the monitoring of the effect of induction agents on cervical ripening (20).

In view of the important role of collagen fibers and their turnover in the process of cervical function during pregnancy, the light-induced autofluorescence of cervical collagen could be a useful tool for evaluating cervical status and monitoring treatment strategies.

References

- Danforth DN. The morphology of the human cervix. *Clin Obstet Gynecol* 1983; 26: 7–13.
- Woessner JF, Brewer TH. Formation and breakdown of collagen and elastin in the human uterus during pregnancy and postpartum involution. *Biochem* 1963; J89: 75–82.
- Leppert PC, Keller S, Cerreta J, Mandl I. Conclusive evidence of elastin in the uterine cervix. *Am J Obstet Gynecol* 1982; 142: 179–82.
- Danforth DN, Vies A, Breen M, Weinstein HG, Buckingham JC, Manalo P. The effect of pregnancy and labor on the human cervix. changes in collagen, glycoprotein and glycosaminoglycans. *Am J Obstet Gynecol* 1986; 120: 641–51.
- Liggins GC. Cervical ripening as an inflammatory reaction. In: Ellwood DA, Anderson ABM, eds. *Cervix in Pregnancy and Labour: Clinical and Biochemical Investigation*. New York: Churchill Livingstone, 1981: 1–9.
- Leppert PC. Anatomy and physiology of cervical ripening. *Clin Obstet Gynecol*, 1995; 38: 267–79.
- Ekman G, Almström H, Granström L, Malmström A, Norman M, Woessner JF Jr. Connective Tissue in Human Cervical Ripening. In: Leppert PC, Woessner JF, eds. *The Extracellular Matrix of the Uterus, Cervix and Fetal Membranes: Synthesis Degradation and Hormonal Regulation*. New York: Perinatology Press, 1991: 87–96.
- Udenfriend S. *Fluorescence Assay in Biology and Medicine*, Vol. I New York: Academic Press, 1962.
- Cantor CR, Schimmel PR. *Biophysical Chemistry*. New York: W.H. Freeman, 1980.
- Lakowicz JR. *Principles of Fluorescence Spectroscopy*, 3rd Print. New York: Plenum Press, 1986.
- Ramanujam N, Mitchell MF, Mahadevan A, Thomsen S, Silva E, Richards-Kortum R. Fluorescence spectroscopy: a diagnostic tool for cervical interepithelial neoplasia (CIN). *Gynecol Oncol* 1994; 52: 31–8.
- Lam S, Hung JYC, Kennedy SM, Leriche JC, Vedal S, Nelems B et al. Detection of dysplasia and carcinoma in situ by ratio fluorometry. *Am Rev Respir Dis* 1992; 146: 1458–61.
- Cothren RM, Richards-Kortum RR, Sivak MV, Fitzmaurice M, Rava RP, Boyce GA et al. Gastrointestinal tissue diagnosis by laser induced fluorescence spectroscopy at endoscopy. *Gastrontest Endosc* 1990; 36: 105–11.
- Schomaker KT, Frisoli JK, Compton CC, Flotte TJ, Richter JM, Nishioka NS et al. Ultraviolet laser-induced fluorescence of colonic tissue: Basic biology and diagnostic potential. *Lasers Surg Med* 1992; 12: 63–78.
- Glassman W, Byam-Smith M, Garfield RE. Changes in rat cervical collagen during gestation and following antiprogestone treatment as measured in vivo with light induced autofluorescence. *Am J Obstet Gynecol* 1995; 173: 1550–6.
- Fujimoto D. Isolation and characterization of a fluorescent material in bovine achilles tendon collagen. *Biochem Biophysics Res Commun* 1977; 76: 1124–9.
- Glassman WS, Liao Q-P, Shi S-Q, Goodrum L, Olson G, Martin E et al. Fluorescence Probe for Cervical Examination during various Reproductive States. *Proc SPIE – Adv Fluorescence Sensing Technol III* 1997; 2980: 286–92.
- Olson G, Goodrum L, Martin E, Saade G, Garfield RE. Noninvasive measurement of cervical collagen content in women approaching delivery. *Am J Obstet Gynecol* 1998; 178: S91.
- Maul H, Olson G, Fittkow CT, Saade GR, Garfield RE. Cervical light-induced fluorescence in humans decreases throughout gestation and before delivery: Preliminary observations. *Am J Obstet Gynecol* 2003; 188: 537–41.
- Fittkow CT, Maul H, Olson G, Martin E, MacKay L, Saade G et al. Light-induced fluorescence of the human cervix decreases after prostaglandin application for induction of labor at term. *Eur J Obstet Gynecol Reprod Biol* (in press).

Address for correspondence:

Robert E. Garfield
 Division of Reproductive Sciences
 Department of Obstetrics and Gynecology
 The University of Texas Medical Branch
 Galveston, TX 77555-1062
 USA
 e-mail: rgarfiel@utmb.edu