FSH Biosensor to Detect Postpartum Ovarian Recrudescence

H. W. Taylor[1], S. E. Shidler[2], B. L. Lasley[2], L. Ngalamou[3] and F. E. Taylor[4]
[1]School of Engineering, Fairfield University, Fairfield, CT, USA
[2]Institute of Toxicology and Environmental Health, University of California, Davis, CA, USA
[3] Department of Electrical and Computer Engineering, University of the West Indies, Trinidad and Tobago
[4]Department of Electrical Engineering, University of Washington, Seattle, WA, USA

Abstract—**Preliminary evidence of FSH suppression as a marker of postpartum ovarian recrudescence is presented. Falling FSH concentration in response to ovarian follicular estrogenic secretory activity apparently signals the reestablishment menstrual cycles. We propose to investigate further this phenomenon with the development of a portable FSH biosensor. The FSH biosensor design is based on SPR and biological thin-film technologies and is incorporated as a key element of the fertility information appliance. It is possible that the device could be a useful natural family planning tool, especially during the return to fertility after childbirth.**

Keywords—**amenorrhea, anovulation, biosensors, breast-feeding, fertility information appliance, follicle stimulating hormone, FSH, menopause, natural family planning, NFP, ovarian recrudescence, ovulation detection, pregnancy, postpartum infertility, reproductive ecology, SPR, surface plasmon resonance**

I. Introduction

Today, there exist several key methods of natural family planning (NFP), including the Symto-Thermal Method [1], the Ovulation Method [2] and the Creighton Model [3]. However, women may experience long intervals of fertility interruption, up to two or three years, resulting from pregnancy followed by extensive breastfeeding and lactation [4]. The external symptom of returning fertility and ovarian recrudescence (cervical mucorrhea) may be ambiguous and confusing during the transition.

The purpose of the current work is the development of a biosensor system capable of detecting trace levels of hormone in aqueous media (urine or saliva). The biosensor could be based on the latest developments in sensor technology, with potential candidates including micro-electromechanical systems (MEMS) cantilever micro-thermopile devices [5]. However, we chose surface plasmon resonance (SPR) devices [6] because they are inexpensive and already commercially available.

Beyond their potential usefulness for natural family planning, hormonal biosensor systems could have a wide range of important applications, including the monitoring of estrogen analogs and mimetics in the environment. Our focus here is on the application of biosensors in the study of human reproductive ecology. We are particularly motivated by the potential development of a fast and accurate method of recording hormonal data in the field, including remote villages in Africa and elsewhere in the developing world.

A biosensor system is designed to detect small quantities of specific hormones through affinity interactions with specific antibodies bound to the sensor surface. On-board processors amplify the resulting signals to be logged directly to a personal digital assistant or PDA. A portable biosensor system could be considerably more economical than current methods which involve collecting and storing biological samples to be shipped to the laboratory for assay [7].

Of particular interest, is the apparent loss of negative feedback inhibition of follicle stimulating hormone (FSH) during lactatonal amenorrhea. This may be similar to the elevated FSH in females after menopause. Interestingly, a search of the internet now produces several devices on the market which are designed to test for sustained FSH elevation to diagnose the advent of menopause [8].

Without the use of any form of birth control, the pregnancy rate before the first menses after childbirth is about six percent [1]. The ultimate aim of this project is to develop a biosensor-based early warning system to avoid or delay pregnancy, if so desired.

II. Methodology

Study Participants

Women recruited to participate in our studies chose one of two groups: full breast-feeding or partial breast-feeding. Recruitment took place through the US membership of the Couple to Couple League (CCL); and in Cameroon, through the Family Life Office of the Diocese of Kumbo with the permission of the local traditional ruler, the Fon Nso. The fact that CCL has a network of more than 1,000 teaching couples in the US and thirty in Cameroon providing instruction in natural family planning and ecological breast-feeding made it an ideal organization through which to recruit breast-feeding participants for this study.

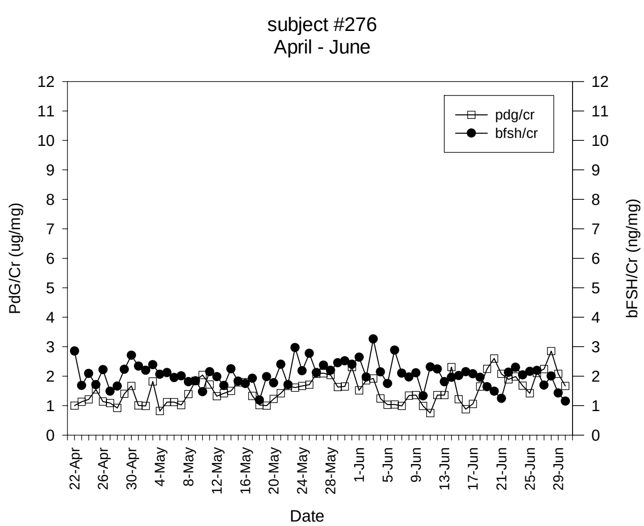
FSH Studies

First, we participated in the field testing of a new technology using EIA (enzyme immunoassay) for ovarian hormone metabolites such as pregnanediol-3-glucuronide (PdG) in dry urine samples [9]. Our study of the duration of the postpartum anovulatory interval was reported at last year's EMBS meeting [7]. Since it is now possible to assay for the beta subunit of FSH in urine [10], we then also recruited three of the study participants to collect liquid samples in addition to urine samples dried on filter paper. We were interested in learning whether postpartum FSH concentration would remain relatively low during postpartum amenorrhea with a rise in FSH prior to ovarian recrudescence signaling imminent fertility, as had been suggested by some [11]. Or, on the other hand, would we find elevated FSH because of the absence of negative feedback inhibition by estradiol in accordance with the observations of others [12]. Also, FSH has been shown to be a useful marker of early pregnancy [13] and effective in identifying the day of ovulation [14].

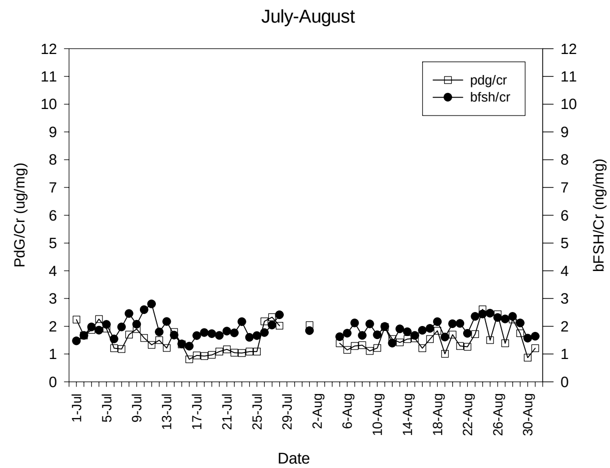
III. Results

For each of our three FSH-study participants, we found the beta-FSH/creatinine (cr) ratio relatively elevated for an extended time during depressed ovarian function. However, concurrent with the onset of steroidogenesis (as evidenced by an increased PdG/cr ratio), we found an immediate decline in FSH; presumably due to negative feedback at the hypothalamus and pituitary (see the three panels of Fig. 1). These data support the hypothesis that the ovaries are rendered unresponsive to FSH during “deep” lactational amenorrhea [15]. Furthermore, the recovery of FSH responsiveness at the ovarian follicle enables the reproductive functioning of the hypothalamic-pituitary-ovarian axis, including ovulation and luteinization. Such refractoriness continues well beyond pregnancy-induced ovarian suppression [16]. For this subject, ovarian recrudescence apparently occurred in the 94th week postpartum, whereas the 56th week was the median found for the study population as a whole.

<u>*Panel A*</u>



<u>*Panel B*</u>



<u>*Panel C*</u>

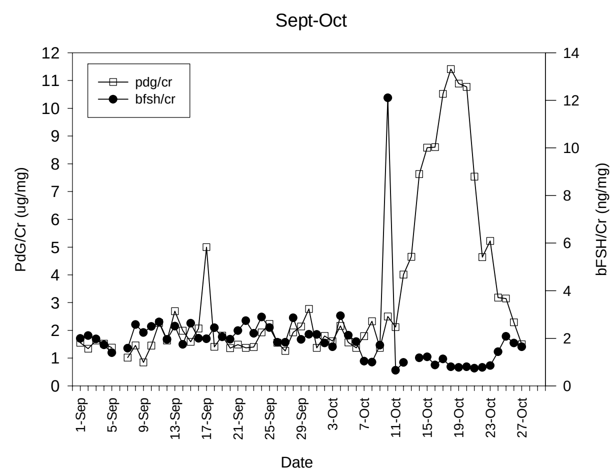


Fig. 1. The last six consecutive months of postpartum amenorrhea for one subject is seen in *Panels A*, *B* and *C* above, followed by ovarian recrudescence indicated by a spike in urinary FSH, followed by as sustained rise in urinary PdG. Concentrations in each case are indexed to creatinine (cr) to control for urinary dilution

SENSOR DESIGN

We chose SPR technology for the biosensor because it is selective to surface binding, sensitive to small changes, portable and has proven to be feasible in similar applications [6].

To aid in the discussion why we chose a given SPR sensor type, a brief overview of the sensor theory will be helpful [17]. A light source is typically used to excite SPR. The light source is designed to have a spectrum of energies. The incident photons are made incident on a thin gold layer. A quantum of energy is transferred from the photons to electrons that are free to oscillate on a gold surface. The electrons that oscillate generate a surface plasma wave (SPW). The SPW is bound to the gold surface. The energy of the SPW is at a maximum at the sensor surface. It exponentially decays in the normal direction to the sensor surface and extends only a few hundred nanometers. This makes the sensor ideal for surface detection.

During SPR, the light reflected off the gold layer. A dip in intensity will be observed where the energy was transferred to generate the SPW. If the refractive index on the sensor surface changes, the propagation constant of the SPW changes. This results in a new location of the dip in the reflected light spectra. This change in the position in the dip is used to sense biological binding.

There are two common ways of generating a spectrum of photon energies. One technique is wavelength interrogation, using a range of wavelengths at a fixed angle. The range of wavelengths is achieved using a polychromatic or white light source. Another practice is angular interrogation. As the name suggests, it uses a range of incident angle while a monochromatic light source is used.

SPR Angular Interrogation

Using angular interrogation, the tangential wave vector of the incoming light varies as the incident angle of the light changes. There are different ways to vary the angle of the incident light, such as using a stepper motor to sweep the monochromatic light source through a range of angles. A more robust technique has been devised with collaborations with Texas Instruments. This produced the Spreeta™ [18] SPR sensor. Figure 2 shows this sensor with a LED in the bottom left corner. This monochromatic light source is reflected off the "Surface Plasmon Layer" sensing surface. It can be seen how the incident angle varies across the sensor surface. The trigonometric relationship shows that each angle of incidence has a different angle tangential component that may excite the SPW.

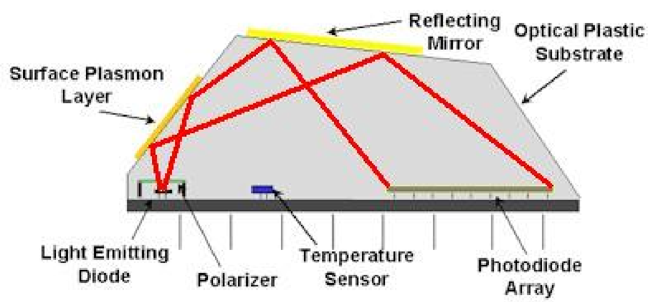


Fig. 2. The Spreeta™ SPR sensor has the ability to vary the incident angles (angular interrogation). The LED is used at a fixed wavelength.

The reflected light is detected at the photodiode array. During SPR, a dip in the intensity of the reflected light is noticed at a specific pixel. When binding on the sensor surface occurs, the position of the dip will change. This variation is correlated to specific binding levels of the analyte.

Angular interrogation is not as sensitive as wavelength interrogation, but the Spreeta™ still is sensitive enough for our application. Furthermore, it is by far the smallest, cheapest, and most robust sensor commercially available.

FSH Antibody Immobilization on Gold Film

Various antibodies to the FSH peptide are now commercially available. One of these will be immobilized on the Spreeta™ sensing surface. The sensing surface includes a thin layer (50 µm) of gold. Therefore the FSH can be immobilized using a gold binding protein layer [6;19].

Fertility Information Appliance

The electronics design was developed and reported previously [20] and shown below in Figure 3 with a FSH sensor in addition to the luteinizing hormone (LH) sensor. Briefly, NFP methods are combined using neuro-fuzzy algorithms as a means of monitoring and predicting days of fertility. A wakeup circuit alerts the user for pending measurements and a TCP/IP stack module allows information transfer in the form of email (SMTP protocol) or file (FTP protocol) using a mobile phone or handheld PDA. Further enhancements of the system could enable remote querying and data transfer.

Recent progress in the miniaturization and integration of biosensors and micro-fluidics [21;22] give the promise of the development of truly portable, integrated biosensors suitable for field use.

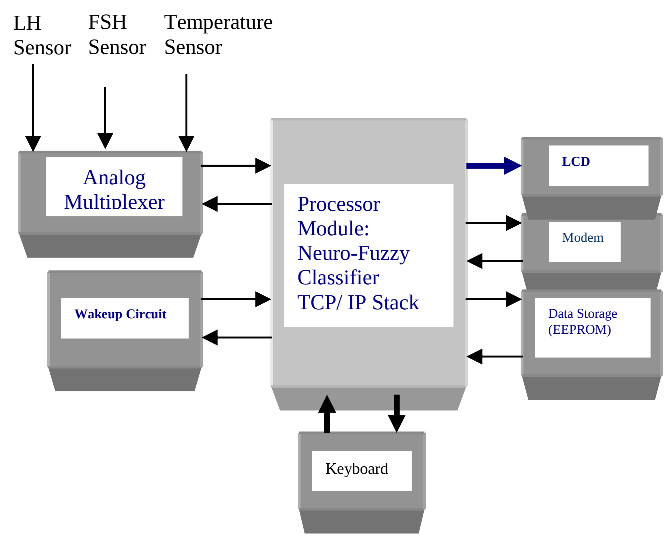


Fig. 3. Block diagram of the Fertility Information Appliance. A wakeup circuit alerts the user of pending temperature or hormonal input and the keyboard allows manual entry of her fertility symptoms. The neuro-fuzzy classifier uses NFP rules to detect and predict the fertility state.

V. Conclusion

Our preliminary studies of FSH suppression and the return of fertility after childbirth suggest that the noticed drop in FSH concentration could become a useful marker of ovarian recrudescence. The convergence of portable SPR sensor and immobilization technologies makes it possible to develop a portable FSH biosensor for use in human reproductive ecology field work. Depending on the results of the planned field studies, full clinical trials may be indicated to determine the efficacy of a fertility appliance monitor as an aid to natural family planning during the transition from postpartum amenorrhea to regular menstrual cycles.

Acknowledgment

The authors would like to acknowledge the three women who volunteered to collect, freeze and ship urine samples for many months in addition to their daily dry urine samples and continuous recording of breastfeeding data.

The authors are grateful for the generous support of the National Institute of General Medical Sciences through grant number S06-GM08066 and also would like to acknowledge the generous support of the West Virginia NSF EPSCoR Mini-Grant Program.

References

[1] Kippley, J. F. and Kippley, S. K., *The Art of Natural Family Planning*, 4th ed. Cincinnati: Couple to Couple League International, Inc., 1997, pp. 1-511.

[2] Billings, J. J., *The Ovulation Method: Natural Family Planning*, 5th ed. Liturgical Press, 1992.

[3] Hilgers, T. W., *Medical Applications of Natural Family Planning: A Contemporary Approach to Women's Health Care* Pope Paul VI Inst Pr, 1991.

[4] Kippley, S. K., *Breastfeeding and Natural Child Spacing: How Ecological Breastfeeding Spaces Babies*, Fourth ed. Cincinnati, OH: The Couple to Couple League International Inc., 1999, pp. 1-212.

[5] Wu, G., Ji, H., Hansen, K., Thundat, T., Datar, R., Cote, R., Hagan, M. F., Chakraborty, A. K., and Majumdar, A., "Origin of nanomechanical cantilever motion generated from biomolecular interactions," *Proceedings of the National Academy of Sciences*, vol. 98, no. 4, pp. 1560-1564, Feb.2001.

[6] Woodbury, R. G., Wendin, C., Clendenning, J., Melendez, J., Elkind, J., Bartholomew, D., Brown, S., and Furlong, C. E., "Construction of biosensors using a gold-binding polypeptide and a miniature integrated surface plasmon resonance sensor," *Biosens.Bioelectron.*, vol. 13, no. 10, pp. 1117-1126, Nov.1998.

[7] Taylor, H. W., Shideler, S. E., Samuels, S. J., and Lasley, B. L., "Survival-time analysis of the postpartum anovulatory interval as measured by rise in urinary pregnanediol-3-glucuronide in lactating women," *Proc.25th Annual International Conference of the IEEE EMBS*, vol. 4 pp. 2995-2997, 2003.

[8] http://www.menocheck.com/ . 2004.

[9] Shideler, S. E., Munro, C. J., Johl, H. K., Taylor, H. W., and Lasley, B. L., "Urine and fecal sample collection on filter paper for ovarian hormone evaluations," *Am.J.Primatology*, vol. 37 pp. 305-315, 1995.

[10] Qiu, Q., Kuo, A., Todd, H., Dias, J. A., Gould, J. E., Overstreet, J. W., and Lasley, B. L., "Enzyme immunoassay method for total urinary follicle-stimulating hormone (FSH) beta subunit and its application for measurement of total urinary FSH," *Fertil.Steril.*, vol. 69, no. 2, pp. 278-285, Feb.1998.

[11] Hanson, F. W., Powell, J. E., and Trelford, J. D., "Postpartum FSH levels. Report of 2 cases," *Obstet.Gynecol.*, vol. 35, no. 3, pp. 462-464, Mar.1970.

[12] Glasier, A., McNeilly, A. S., and Howie, P. W., "Fertility after childbirth: changes in serum gonadotrophin levels in bottle and breast feeding women," *Clin.Endocrinol.(Oxf.)*, vol. 19, no. 4, pp. 493-501, Oct.1983.

[13] Qiu, Q., Overstreet, J. W., Todd, H., Nakajima, S. T., Stewart, D. R., and Lasley, B. L., "Total urinary follicle stimulating hormone as a biomarker for detection of early pregnancy and periimplantation spontaneous abortion," *Environ.Health Perspect.*, vol. 105, no. 8, pp. 862-866, Aug.1997.

[14] Li, H., Chen, J., Overstreet, J. W., Nakajima, S. T., and Lasley, B. L., "Urinary follicle-stimulating hormone peak as a biomarker for estimating the day of ovulation," *Fertil.Steril.*, vol. 77, no. 5, pp. 961-966, May2002.

[15] Taylor, B., Taylor, D., Clark, D., and Duran, A., "Ovaries are non-responsive to follicle stimulating hormone (FSH) before the postpartum return of ovarian cyclicity," *The Fed.of Am.Soc.for Exp.Biol.J.- Abstracts Part I*, vol. 13, no. 4, 1999.

[16] Taylor, H. W., Vázquez-Geffroy, M., Samuels, S. J., and Taylor, D. M., "Continuously recorded suckling behaviour and its effect on lactational amenorrhoea," *J.Biosocial Science*, vol. 31 pp. 289-310, 1999.

[17] Homola, J., "Present and future of surface plasmon resonance biosensors," *Anal.Bioanal.Chem.*, vol. 377, no. 3, pp. 528-539, Oct.2003.

[18] Sommers, D. R., Stubbs, D. D., and Hunt, W. D., "Investigation of SPR technology using Texas Instruments' Spreeta™ sensor," *Sensors, 2002.Proceedings of IEEE*, vol. 2 pp. 1244-1249, 2002.

[19] Brown, S., "Metal-recognition by repeating polypeptides," *Nat.Biotechnol.*, vol. 15, no. 3, pp. 269-272, Mar.1997.

[20] Ngalamou, L. and Rose, D., "Fertility information appliance," *Proc.of the 15 th IEEE Symposium on Computer-Based Medical Systems*, pp. 335-338, 2002.

[21] Hodge, A., Newcomb, R., Zaghloul, M., and Tigli, O., "System architecture for multi-technology testbench-on-a-chip," *IEEE International Symposium on Circuits and Systems*, vol. 2 pp. 736-739, 2002.

[22] Romani, A., Campi, F., Ronconi, S., Tartagni, M., Medoro, G., and Manaresi, N., "A system-on-a-programmable-chip for real-time control of massively parallel arrays of biosensors and actuators," *Proc.of the 3rd IEEE International Workshop on System-on-Chipfor Real-Time Applications*, pp. 236-241, 2003.