The Use of Urinary Hormonal Assessments in Human Studies

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OVERVIEW

Unlike most other health functions, reproduction is discontinuous in its expression, and even in healthy individuals, reproductive function can be perturbed by changes in the daily routine or adverse environmental factors. Most importantly to health providers and epidemiologists, reproductive function is composed of a series of related events which, in the female, are for the most part concealed. Ovulation, fertilization and implantation occur in the absence of any overt event which would be obvious to the woman and can only be confirmed by clinic or laboratory analyses with multiple sampling. Even menstrual calendars from daily diaries and basal body temperature charts, which are often considered to be reliable indicators of ovarian function, are blunt tools in tracking early pregnancy losses due to variations in menstrual cycle lengths. Even the absence of ovulation or the presence of an early fetal loss (EFL) may not be identified by the most complete log of daily physical and emotional occurrences.

For clinic and out-patient evaluations, the relationship of hormonal dynamics are used to assess the integrity of the hypothalamo-pituitary-gonadal (HPO) axis. Changes in the production of pituitary glycoproteins and ovarian steroid hormones are intimately related to ovarian and the menstrual function. The resulting changes in the concentration of these hormones in blood provide an accurate assessment of ovarian function. Similarly, the production of trophoblastic chorionic gonadotropin following implantation and the resultant acceleration of ovarian steroid hormone production can be monitored by changes in blood hormone levels. The dynamic nature of these hormonal events often require that serial blood sampling and measurement be made over several days. Unfortunately, the collection of daily blood samples leads to apprehension and noncompliance in all but the most motivated subjects. It is also largely not efficient or feasible in non-clinic-based populations, such as in occupational settings.

The combined need for special equipment and trained personnel, together with a low compliance for serial venipuncture thus eliminates blood sampling as a viable approach to evaluate reproductive events in population-based, epidemiologic studies. Even if only a few blood samples could be considered sufficient to be representative of a woman's reproductive status, it is not practical to obtain even a few individual blood samples from non-clinic subjects. Since multiple biological samples are required to assess female reproduction, other biological samples, such as urine and saliva samples, have begun to be employed in population-based studies.

Over the past fifteen years, many studies have been published using urine to monitor reproductive function. Since urine is not homeostatically controlled and, in fact, is altered by the kidney in order to maintain serum osmolarity, the concentration of solutes in urine is a function of the concentration or dilution of urine produced. A

relatively constant amount of solute produced over a given interval of time may be found in widely different concentrations in urine due to variation of urine volume. Thus, the earliest studies relied on the collection of total urine volume during a 24-hour time period in order to calculate the total solute excreted. From a logistic aspect of sample handling alone, this requirement to perform daily 24-hour urine collection made such studies quite impractical. The recognition that creatinine could be used to "index" hormone metabolite excretion in small samples of urine to adjust for urine concentration was an important factor in the current popularity of urinary hormone assays. The recognition that urine samples tend to be more consistently concentrated at the first voiding of the day further reduced the variations in urine concentration when collected on a daily basis.

Nowhere is urinary monitoring used more frequently than in facilities that maintain non-domestic animals.[1] Compared to humans, there are many additional problems associated with the collection of even a single sample from a non-tractable animal that must be captured and restrained for any type of examination. The collection of serial blood samples from non-domestic and non-laboratory animals simply cannot be obtained; modern zoos now recognize that most species can be evaluated with relatively simple assays for gonadal or placental steroid hormone metabolites in urine. While the ubiquity of sex steroids across the animal kingdom supports the broad application of this approach, differences in metabolism and routes of excretion even between closely related species are variables that require consideration. Two of the more notable caveats are the difference in progesterone metabolites in higher primates[2] and the difference in both estrogen and progesterone excretion in different rhinoceros species.[3]

Despite differences in hormone metabolism, more than thirty different species of non-domestic animals have been monitored and their ovarian cycles characterized by urinary steroid metabolites using variations of estrogen conjugate and pregnandiol-3-glucuronide assays. Most of these studies have involved captive animals, but field studies have been reported in which urine was collected from the ground from elephants,[4] feral horses,[5] and buffalo.[6] In a few laboratory studies in which paired blood and urine samples have been collected and analyzed, the ability of urine measurements to approximate blood values is quite promising. In at least two cases[7,8] the urinary estrogen metabolite profiles are more informative than those provided by serum measurements, suggesting that in some cases urinary monitoring is superior to serum measurements, even when blood samples are available. Two reports of human studies suggest that, despite its limitations, urinary monitoring has decided advantages over serum evaluations in terms of obtaining critical information which had not been collected with blood samples.[9,10]

HUMAN STUDIES

The recognition that measurable quantities of intact human chorionic gonadotropin (hCG) is stable in frozen urine samples, suggested that serum assays for intact hCG could be directly adapted to urine samples. While most of the circulating hCG is metabolized to unrecognizable fragments in urine, the concentrating effect of the kidney allows the small portion of intact hCG which does pass into the urine to be detected at about the same time that it is detected in blood. Steroid hormones are not metabolized to a great degree and, when conjugated in the liver, become water soluble and cleared through the urine. Conjugated steroid metabolites are found in urine at

concentrations several-fold higher than the concentration of their parent circulating steroid. These relatively high concentrations of hormones, or their metabolites in the absence of large proteins, permits a wide range of assays to be applied to urine which cannot be applied to blood.

Because of the relatively high concentration of the hormone metabolites and low concentration of large protein molecules in urine, unprocessed urine can often be analyzed directly or following a dilution with water. These direct assays save time and are optimal, particularly in large epidemiological studies. This quality of urine has led to increasingly simpler assays for both laboratory and non-laboratory uses; for example, economical and practical over-the-counter kits for hCG, pregnanediol-3-glucuronide, and luteinizing hormone are available. Laboratory assays are now being adapted for large, population-based studies in which tens or hundreds of thousands of samples need to be analyzed in a relatively short time period. We may even anticipate home assays which will obviate the need for sample transfer and storage altogether.

While the collection of urine samples has the advantage of being a self-collectable and non-invasive technique, and could provide theoretically unlimited collection intervals with a proven high compliance rate, this strategy is not without limitation. After more than twenty years of applying urinary assays to both clinic and non-clinic-based populations, some reservation remains regarding the reliability of such evaluations. Due to these reservations, urinary monitoring has not replaced blood collection in the clinic, where blood samples are relatively easily obtained. The reservation of many clinicians in employing urinary measurements stems primarily from the paucity of convincing studies, demonstrating a constant relationship of urinary and circulating serum hormone profiles. In most attempts to demonstrate such relationship, urinary data have been compared to serum measurements rather than analyzed for their own merit. Evaluation of urinary hormone profiles as independent measures are needed to appreciate fully their value. Such studies are just now appearing, and the next year or two will likely witness the beginning of urinary assays applied in clinical settings.

Despite the reservations of clinicians, the use of urinary assays for non-clinic-based populations in monitoring reproductive function has gained recognition and popularity. In the past ten or twelve years more reliable and more efficient techniques have been developed and applied to increasingly larger and more complex studies.[11,12] Owing to the number of changes which have taken place in assay development and the kinds of populations to which different assays have been applied, there is currently a wide range of methods being applied and many different results being cited. The purpose of this report is to review both the methods and the outcomes of some of the more prominent studies which have employed urinary monitoring to assess reproductive function in women.

METHODS AND RESULTS

Early Fetal Loss

Radioimmuno-assays (RIA) for the detection of hCG in urine have been refined in terms of sensitivity and specificity over the last ten years, and a limited number of prospective studies based on hCG have been carried out. While the structure of hCG is distinct from that of pituitary luteinizing hormone (LH), these two hormones have a large degree of structural and bioactive similarities. Few of the early radioimmunoassays for hCG were sufficiently specific to eliminate concerns related to cross-reactivity with LH when they were used to detect very early pregnancy losses. Even today with

exquisitely specific assays, some concern exists regarding cross-reactions with non-trophoblastic LH/CG-like molecules.[13,14]

The earliest studies which employed urinary hCG assessments to detect early fetal loss applied relatively less sensitive and specific assays than are available today. Thus, there were even greater concerns relating to their validity. In addition, confirmation of the day of ovulation was not available through urinary assays, and normal menstrual cycles could only be assumed. Since these early studies were performed prior to any knowledge that hCG could also be produced by the pituitary in normal, non-pregnant women, no attempt was made to control for detecting hCG from non-trophoblastic sources. All of these issues allowed questions to be raised regarding the specificity of the early assays for early pregnancy. Equally important, each study involved different populations as well as variations in the sample collection protocol, which also contributed to the different outcomes (*i.e.,* different proportions of pregnancies ending in EFL). The progression of assays as they were applied to clinic and non-clinic populations of women are listed in TABLE 1.

Miller *et al.*[15] prospectively measured the incidence of post-implantation pregnancy loss in 623 cycles of 197 women, using urinary radioimmunoassays (RIA) for hCG in early morning urine samples, collected on alternate days from day 21 of the menstrual cycle until menses. Of 152 detected conceptions, 64 (43%) ended in early loss (< 20 weeks), of which 50 (78%) were subclinical losses, using an hCG sensitivity level of 1 ng/ml. A mean concentration level of urinary β-hCG higher than 2 ng/ml on two consecutive occasions, or 5 ng/ml on one occasion was chosen empirically as a criterion for eliminating false positive trophoblastic activity and pregnancy. An overall early fetal loss (EFL) rate of 33% (78% of 43%) was reported (TABLE 1).

Edmonds *et al.*[16] also measured hCG to study early embryonic mortality in women. Early morning urine samples were obtained on alternate days in the luteal phase of the cycle between day 21 and menses and were assayed for hCG using RIA. With an assay sensitivity of less than 1 ng/ml, and a discriminator based on a group of 18 sterilized women with normal cycles, a concentration of greater than 4.2 ng hCG/ml on at least one day was found to provide a statistically conservative cut off, above which an embryo was believed to be present. An apparent sub-clinical spontaneous abortion rate of 62% was reported in 198 menstrual cycles (TABLE 1).

In both the Miller *et al.*[15] and the Edmonds *et al.*[16] studies, the collection of urine on alternate days meant that short-lived rises in hCG (> 48 h) or low rises (< 4.2 ng hCG/ml) occurring in some of the women would be missed, thereby decreasing the sensitivity. Furthermore, in both studies the specificity of the hCG assay in regard to the interference of LH and the contribution of non-trophoblastic hCG was not clarified.

By the mid-1980s the specificity of hCG assays had improved, and Ellish *et al.*[17] studied early pregnancy in 20 healthy women and a total of 92 urine samples to investigate the feasibility of detecting early pregnancy and EFL. In an attempt to improve compliance, urine was collected as monthly pooled 3-day samples (days 24 through 26 if the woman usually had a 28-day cycle, and days 26 through 28 if she had a 30-day cycle) for up to six months. A 100% specific hCG RIA with a sensitivity of 0.5 ng/ml hCG concentrations were indexed by creatinine to adjust for the dilution of urine, and a discriminator of 0.2 ng/mg Cr was used for determining conceptions. Of the 92 urine samples, 25 had positive hCG values, of which 13 were collected from eight clinically confirmed pregnant women. A total of 44% (11/25) EFLs and one unknown outcome occurred in this study population (TABLE 1).

This study relied heavily on the assumptions that trophoblastic hCG would be secreted during specific three-day intervals prior to the end of each menstrual cycle, that the length of each menstrual cycle could be accurately predicted, and that

TABLE 1. Early Fetal Loss Detection Methods by Employing Urinary hCG Levels

Author	Date	Study Population	Assay Method	Results
Miller *et al.*[15]	1980	Normal population (n = 197; 623 cycles; no controls)	RIA Sensitivity = 1 ng/ml	50 (33%) of 152 conceptions ended in EFL
Edmonds *et al.*[16]	1982	Normal population (n = 82; 198 cycles; 18 sterile controls)	RIA Sensitivity = 1 ng/ml	61 (62%) of 118 conceptions ended in EFL
Ellish *et al.*[17]	1986	Normal population (n = 20; 92 cycles; no controls)	RIA Sensitivity = 0.01 ng/ml	11 (44%) of 25 conceptions ended in EFL
Sweeney *et al.*[18]	1988	Normal population (n = 88; 306 cycles; no controls)	Five monoclonal Ab kits Sensitivity = 2–4 ng/ml	6 (18%) of 36 conceptions ended in EFL
Wilcox *et al.*[11]	1988	Normal population (n = 221; 707 cycles; 31 sterile controls)	IRMA Sensitivity = 0.01 ng/ml	43 (22%) of 198 conceptions ended in EFL
Taylor *et al.*[19]	1992	Normal clinical (AI) population (n = 92; 224 cycles, no controls)	IEMA IRMA Sensitivity = 0.05 ng/ml	6 (16%) of 38 conceptions ended in EFL; 84% sensitivity achieved with IEMA

concentrations of hCG would not be diluted below detection limits by pooling samples. All of these assumptions would tend to lead to an underdetection of EFLs.

The most sensitive and specific hCG assay applied to an early pregnancy study reported to date is that used by Wilcox *et al.*[11] Using a method which extracted 4 milliliters of urine and an immunoradiometric assay (IRMA) for hCG, urines were analyzed in over 700 menstrual cycles from 221 women to study the risk of EFL; daily early-morning urine samples, collected up to six months from 221 healthy women and 31 healthy sterilized controls, were studied. The investigators found that among 198 (out of 707) cycles with increased hCG levels (>0.025 ng/ml on three consecutive days), 22% (43 of 198 pregnancies) ended in subclinical pregnancy loss and a further 9% (19 of 198) terminated in clinically detected fetal losses. The lowest hCG concentration detectable by the IRMA used was 0.01 ng/ml (TABLE 1).

According to the investigators, their strategy for detecting early pregnancy certainly missed some losses. Only those pregnancies in which intact hCG was produced and reached the woman's urine were detected. Also any early pregnancy that failed to produce enough hCG to meet the criterion could not be distinguished from the background levels of hCG observed in women who had undergone sterilization.

Sweeney *et al.*[18] used five commercial monoclonal antibody kits to monitor early pregnancy and to detect EFL in a group of 88 normal women (306 cycles), who were trying to become pregnant. The reported sensitivity of the kits ranged between 2–4 ng hCG/ml and were used for the measurement of hCG in urine, collected daily and frozen, beginning seven days postovulation. A serum RIA was performed on the 16th postovulatory day, if no bleeding had started, to confirm the pregnancy. All of the urine samples of participants who had a confirmed pregnancy were then monitored for hCG levels with the commercial kits. Overall 32 women became pregnant, with six pregnancies (18%) resulting in EFL. On the average the kits were able to detect a pregnancy 14 days postconception, and therefore any losses prior to this would not be included in the findings. Although the study showed that these commercial kits can be and are efficient and cost-effective, it also showed that their lack of sensitivity compared to radioimmunoassays will present the researcher with an underestimation of EFLs, especially those that occur prior to day 14 postconception.

The most recent report relating to early pregnancy monitoring represents an attempt to maintain the specificity of the IRMA but to reduce laboratory costs by "screening" samples with a highly sensitive immunoenzymometric assay prior to analysis with the more specific immunoradiometric assay. This strategy was developed to be able to analyze the very large sample set which theoretically would be generated in population-based studies in the future. In order to develop an economical, nonradiometric immunoenzymometric assay (IEMA) for the detection of urinary hCG, Taylor *et al.*[19] investigated daily early morning urine samples ($n = 224$ cycles) of 92 healthy volunteer women undergoing artificial insemination (AI) because of their partner's infertility. Both the sensitivity and the specificity of IEMA were compared to IRMA. The IRMA had a mean assay sensitivity of 0.051 ng/mL and the positive-negative discriminator for the IRMA was set at 0.25 ng/mg Cr for any 3 out of 4 consecutive days within the 15-day assay window (10 days prior to and 5 days following onset of vaginal bleeding). The IEMA had a mean assay sensitivity of 0.054 ng/mL and an estimated screening specificity of 84%. Using both assays combined a EFL rate of 16% (6 out of 38 conceptions) was detected (TABLE 1). The differences of outcomes compared to other studies may be related to the lower fecundibility of the AI patient population, which may have included women with unrecognized infertility problems. The authors concluded that with usage of IEMA nearly 80% of screened menstrual cycles can be eliminated without the need for further testing by IRMA.

Ovarian Function

Just as early fetal losses cannot be confirmed by physical signs or symptoms, neither can the events of ovulation and luteal function. While menstrual calendars have been used to indicate that menstrual regularity is strong evidence that ovulation occurs, we now have evidence to demonstrate that not all intermenstrual intervals are ovulatory.[9] Measurement of hormone patterns associated with ovarian function, are the only accurate indicators of ovarian function and these dynamics cannot be evaluated with anything less than serial sampling. Thus, studies which are investigating ovarian function require the same approach as those directed towards early fetal loss, and urinary monitoring has provided the solution.

To study urinary estrogen and pregnanediol metabolites, and therefore to increase inference about ovarian function, Stanczyk *et al.*[20] developed and validated direct urinary steroid glucuronide RIA assays for estrone glucuronide ($E_1$3G), estradiol-3-glucuronide ($E_2$3G), estradiol-17-glucuronide ($E_2$17G), estriol-3-glucuronide ($E_3$3G), estriol-16-glucuronide ($E_3$16G), and pregnanediol-3-glucuronide (PdG). Daily 24-hour urine samples were collected in two batches, a night collection and a day collection, from 7 healthy women volunteers (aged 24–40 years) for one menstrual cycle. The urinary assay results were correlated to daily serum estradiol (E_2), progesterone (P), and lutenizing hormone (LH) levels. Urinary $E_2$17G levels correlated best with serum E_2 levels and seemed to be the most suitable hormone to predict ovulation (TABLE 2). These authors suggested that the marked difference between preovulatory and postovulatory PdG levels could show this hormone to be indicative of ovulation, especially using a urinary dipstick method. While there is still some controversy with regard to the best urinary estrogen metabolite to measure, this study provided the first data from paired measures of blood and urinary profiles and encouraged future developments in urinary monitoring of ovarian function.

Branch *et al.*[21] studied the effect of conception on urinary concentration levels of $E_1$3G, PdG, and LH/hCG using RIA and developed an additional algorithm to measure ovarian function using the $E_1$3G/PdG ratio. Daily early morning urine samples were collected for up to 5 months from 6 women who did not conceive and were compared to 5 women who did conceive. Results showed that the ratio of $E_1$3G to PdG and the LH levels during the follicular phase of the cycle did not differ in the two groups of women. But $E_1$3G and PdG levels increased significantly after 7 and 12 days, respectively, following the LH peak in conceptive cycles when LH/hCG levels were also significantly elevated. HCG levels were 3 times the mean basal level 11 to 15 days after the LH peak. The rapid and sustained rise in urinary steroid conjugates in association with the urinary hCG rise provided the first demonstration that the response of the ovary to peri-implantational hCG could be used to confirm pregnancy using urine samples (TABLE 2).

In order to study urinary hormone levels at the time of ovulation and implantation, Lasley *et al.*[22] extended Branch's *et al.*[21] observations by comparing conceptive and nonconceptive ovarian cycles. Early morning urine samples were collected on a daily basis from healthy women who were receiving AI because of their partner's infertility. A total of 23 conception cycles and 11 nonconception cycles, as well as nine cycles that ended in either spontaneous abortion or medical termination of pregnancy were investigated. Urinary hormone profiles for estrone-3-sulfate ($E_1$3S), $E_1$3G, and PdG were analyzed by direct immunoassay and for LH/hCG by RIA. The estrogen conjugate peak coincided with the LH/hCG peak, and the authors suggest that ovulation and fertilization occurs 1 day after these peaks. Implantantation was assumed to take place on days 7 or 8 after the estrogen peak, which was consistent with

TABLE 2. Ovarian Function Detection Methods Using Urine Samples

Author	Date	Study Population	Assay Method	Results
Stanczyk *et al.*[20]	1980	Normal population (n = 7)	RIA (Estrogen Conjugates; PdG)	PdG & $E_2$17G best to predict ovulation
Branch *et al.*[21]	1980	Normal population (n = 11)	RIA (LH/hCG; E3G; PdG)	E3G, PdG & LH/hCG all increased significantly between day 7–12 after conception
Lasley *et al.*[22]	1985	Normal clinical (AI) population (n = 23; 11 controls)	RIA (hCG/LH) dir. IA (E-Conjugates, PdG)	E-Conjugates precede hCG rise & are early indicator of implantation
Shideler *et al.*[9]	1989	Perimenopausal population (n = 5)	Direct IA (E_1-Conj; PdG)	Sustained & dramatic estrogen release; higher LH levels
Baird *et al.*[12]	1991	Normal population (I: n = 28, 87 cycles; II: n = 188, 283 cycles; III: n = 17, 60 cycles)	RIA (E_1-Conj; PdG LH)	E_1G/PdG ratio algorithm provides estimate of ovulation day; 25%–30% cycles had non-detectable LH peaks
Munro *et al.*[24]	1991	Normal population (n = 10)	RIA EIA (E_1-Conj; PdG)	Both RIA and EIA showed similar E_1-Conj & PdG profiles
Clough *et al.*[25]	1992	Normal clinical (AI) population (n = 16; 25 cycles)	ELISA (LH)	LH peaks in frozen/thawed urine samples highly correlated to serum LH peaks (91.7%)

the rise of LH/hCG between days 10 and 11 after the estrone conjugate peak. In most cases the excretion of estrone conjugates in women who aborted tended to diverge from successful pregnancies by day 16 after the estrone conjugate peak (TABLE 2), again suggesting that urinary steroid hormone metabolite patterns can be used to confirm EFLs detected by hCG.

Shideler *et al.*[9] studied ovarian-pituitary hormone interactions during the perimenopause by analyzing daily urine and opportunistic blood samples from five healthy women (aged 42–47 years) for three or four consecutive cycles. Urinary estrone conjugates ($E_1$3S and $E_1$3G) and PdG levels were measured using a direct immunoassay technique. RIA measurements of serum LH, FSH, E_1, E_2, and P were also performed. A dramatic and sustained release of estrogen during prolonged intermenstrual intervals was observed in women who exhibited irregular menstrual cycles (TABLE 2). These data remain today as the only demonstration that the highly variable gonadotropin and steroid hormone production rates are a consequence of alternating periods of ovarian failure followed by an ovarian response to elevated gonadotropins during the transition to the menopause. This report demonstrates the advantage of urinary assays to clarify events which occur over prolonged time periods.

DeVane and co-workers[23] presented a preliminary report that demonstrated the utility of urinary assays to monitor ovarian events in premenarcheal girls. In this study, daily samples for up to two years were collected from girls as young as 10 and 11 years old. Increasing estrogen levels were observed to occur more than one year prior to menarche. Estrogen-only cycles in the absence of ovulation were associated with menarche and the first six to ten months of episodic vaginal bleeding. Ovulatory cycles, as confirmed by PdG elevations, did not occur until six to twelve months following menarche.

Munro *et al.*[24] studied the relationship of serum estrogen (E_2) and progesterone (P) concentrations to the excretion profiles of their major urinary metabolites, E_1-Conjugates (E_1-3-G plus E_1-3-S) and PdG, as measured by enzymeimmunoassay (EIA) and radioimmunoassay (RIA). Paired daily blood and urine samples were collected from 10 healthy women (aged 23–40 years) for one complete menstrual cycle and evaluated by EIA and RIA, and the effect of sample dilution on hormone concentrations was determined. Profiles of urinary E_1-Conj and PdG concentrations were similar when determined by RIA and EIA (TABLE 2). Comparison of the paired hormone values in the late follicular phase indicates that estrogen metabolites reach the urine 12–24 hours after free estrogen appears in the blood. The correspondence of serum P and urinary PdG was also parallel with a 24–48 hour lag. The authors conclude that because of simplicity, flexibility, and economy, EIA of urinary steroid metabolites is an optimal method to detect steroid conjugates for determination of ovulation. This report is one of the few that correlates blood and urine profiles with regard to ovarian function. The more important contribution of this report, however, was to demonstrate that non-radioactive methods could be applied to urine samples and reduce costs without incurring a loss of accuracy or reliability.

Using urinary ovarian hormone data, Baird *et al.*[12] studied early-morning urine samples in three groups of healthy women in order to develop and validate a method of estimating day of ovulation. Direct radioimmunoassay (RIA) was employed to assay urinary LH, E_1G, and PdG levels for a total 430 cycles from 233 women. The new algorithm for estimating the day of ovulation based on the ratio of E_1G to PdG required that ratio values for day 4 and day 5 of the five-day sequence be no more than 40 per cent of the day 1 ratio value. With the 0.4 descent criterion, a day of luteal transition could be identified in 88% of the cycles (TABLE 2). This report addressed the importance of an accurate identification of the day of ovulation in order to assess events occurring in intermenstrual intervals and the problem associated with measure-

ment of intact LH to detect the midcycle LH surge; in this investigation 25–30 per cent of the cycles assayed had undetectable LH peaks. Therefore, the algorithm permits the day of ovulation to be estimated with a high degree of accuracy using only estrogen and progesterone metabolite measurements.

In order to develop and validate an assay for urinary lutenizing hormone (LH) that can be used for frozen-thawed urine, Clough *et al.*[25] studied daily early morning urine samples from 16 healthy women, undergoing AI because of their partner's infertility. A total of 25 complete menstrual cycles were assayed using an enzyme-linked immunosorbent assay (ELISA) for urinary LH component evaluation (LH and LH-α), and matched daily blood and urine samples were compared for urinary LH patterns and levels. The simultaneous measurement of LH together with its α subunit not only detected more of the midcycle surge components (since free α subunit would also be measured), but it obviated concerns relating to the dissociation of the LH subunits in frozen:thawed urine samples. This approach is suggested to detect greater than 90% of the midcycle surges when compared to the midcycle LH peak in ovulatory cycles. The urinary LH peak was highly correlated to the midcycle serum LH peak, therefore implicating practical applicability for self-collected frozen urine samples in determining the day of ovulation (TABLE 2).

DISCUSSION

The past twenty years have witnessed many societal and technological changes of importance to women's reproductive health. Women have increasingly joined the paid workforce, so that today approximately 70% of pre-menopausal women work outside the home. Often women in the workplace have placed themselves in occupations which may expose them to chemical, physical or emotional stressors which may adversely affect their reproductive systems. In addition, increased development and dissemination of chemical and physical agents in the environment provide additional opportunities for exposure of women to reproductive toxicants.

Along with these societal and exposure-related changes have occurred technological advances in our abilities to measure reproductive effects that are not readily observed by the woman or her health care provider, such as early fetal loss, occurrence or absence of ovulation, and alterations in metabolism or excretion of reproductive hormones. These advances have included greater acceptability of sample collection (*i.e.,* daily urine instead of blood samples) and improved sensitivity and specificity, increased efficiency, and reduced costs of assays through use of non-radiometric techniques.

Societal changes have increased our interest in monitoring and evaluating women's reproductive function—and concomitant technological changes have improved our ability to do so. From a public health perspective, given the potential for women's increased exposure to occupational and environmental agents which may have adverse reproductive effects, there is increased research interest in identifying such toxicants and effects, so that exposure can be appropriately reduced and adverse effects prevented. However, prior to 1980 such detection involved either less sensitive measures, such as menstrual calendars[26–29] or invasive procedures (*i.e.,* frequent blood samples) which tend to be less acceptable and result in reduced participation or participation by only highly select and motivated (*e.g.,* clinic) populations. Indeed some of the variation in the literature in the detected prevalence of outcomes such as early fetal loss may be due to the nature of the populations studied as well as to differences in the assay methods.

The development of sensitive and specific urinary assays has provided a greatly

improved, less invasive, more acceptable and efficient, and less expensive alternative that now makes assessment of female reproductive function available for large-scale, population-based studies. The availability of assays, based on daily urine sample collection, for hCG provide the opportunity for detection of early fetal loss, with confirmation using multiple biomarkers to confirm ovulation. The availability of urinary assays for LH, PdG, and estrogen conjugates permits evaluation of the occurrence and timing of ovulation for assessment of subfertility, as well as potentially in the future for alterations in production and metabolism of reproductive hormones and alterations in ovarian function in women approaching menopause. Future studies may well show that detection of reproductive dysfunction may be a more sensitive indicator for determination of occupational and environmental exposures standards, which have previously largely been established based on acute (usually respiratory or dermatologic) effects.

CONCLUSION

Over the last ten years, there has been much progress in monitoring ovarian function and especially early pregnancy loss by means of urinary assays. The development of more sensitive and economical assays have lead to lower cost and more consistent EFL detection. Normal frequencies of EFLs have not been established yet, probably because of the homogeneity within most of the study populations; the rules by which these methods are to be applied are still being formulated. The need for multiple biomarkers to distinguish between the hCG released by the trophoblast in pregnant women and the hCG secreted by the pituitary gland in nonpregnant women, seems clear to be able to accurately predict early pregnancy failures. Nonetheless, urinary assays have contributed greatly to the emerging field of investigating gynecologic and reproductive health problems in women and are likely to gain popularity in the future.

The field of monitoring ovarian function is just now beginning to be explored. The transition from radioimmunoassays to less expensive enzyme-based assays permits the evaluation of the large numbers of samples which would be required for large population-based studies. It seems likely that reproductive epidemiology will soon expand its horizons beyond early fetal loss and begin to address questions relating to ovarian function such as ovulatory failure and menstrual function.

While these techniques are not yet accepted as methods for clinical evaluations in most settings, this will change as more information is collected. The advantages of self-collection for indefinite periods of time make this strategy superior to blood collections and serum analyses.

SUMMARY

The collection and analysis of urine samples provides a practical method for monitoring female reproductive events in non-laboratory and non-clinic populations. Collection of biologic samples permits objective assessment of reproductive health endpoints in epidemiologic studies and for epidemiologic research purposes can provide validation of information provided by the subjects, especially outcomes which are usually concealed and thus unknown to the participant. Urine sampling has several advantages over the collection of blood samples, such as simplicity, non-invasiveness, and cost efficiency. Several studies have shown that endocrine information similar to

that obtained in blood samples can be obtained from assays of daily urine samples. The measurement of human chorionic gonadotropin in daily and selected urine samples has been incorporated into several recent epidemiologic studies focusing on early fetal loss, and ovarian and pituitary hormone metabolites have been measured in daily urine samples to evaluate ovarian function in studies focusing on women's reproductive health. As the strategy of urinary monitoring becomes more accepted as a legitimate research tool, laboratory methods are being modified to improve performance, reduce costs and adapted to sophisticated algorithms using multiple hormonal measurements to identify a number of end-points.

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