62 77-033 CRL

Principal Investigator DR B. SEATON Trainee investigator(if any) NONE

Application No 77-002 (REVISED) Supporting Agency(if Non-CRL)

Title of study HORMONAL CORRELATES OF PREGNANCY AND LACTATIONAL AMENORRHEA.

77-033

Project status:
( ) New Study
(✓) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following(If Not Applicable write NA):

2. Source of Population:
   a) Ill subjects Yes (No)
   b) Non-ill subjects (Yes) No
   c) Minors or persons under guardianship (Yes) No
   Does the study involve:
   a) Physical risks to the subjects Yes (No)
   b) Social risks Yes (No)
   c) Psychological risks to subjects Yes (No)
   d) Discomfort to subjects (Yes) No
   e) Invasion of Privacy Yes (No)
   f) Disclosure of information possibly damaging to subject or others Yes (No)
3. Does the study involve:
   a) Use of records (hospital, medical, death, birth or other) Yes (No)
   b) Use of fetal tissue or abortus Yes (No)
   c) Use of organs or body fluids (Yes) No
4. Are subjects clearly informed about:
   a) Nature and purposes of study (Yes) No
   b) Procedures to be followed including alternatives used (Yes) No
   c) Physical risks (Yes) No
   d) Sensitive questions (Yes) No
   e) Benefits to be derived (Yes) No
   f) Right to refuse to participate or to withdraw from study (Yes) No
   g) Confidential handling of data (Yes) No
5. Will signed consent form be required:
   a) From subjects (Yes) No
   b) From parent or guardian (if subjects are minors (Yes) No
6. Will precautions be taken to protect anonymity of subjects: (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview(all other requirements will be submitted with individual studies.
   ✓ Protocol (Required)
   ✓ Abstract summary (Required)
   ✓ Statement given or read to subjects - nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (REQUIRED)
   ✓ Informed consent form for subjects
   ✓ Informed consent form for parent or guardian (included with subject consent form)
   ✓ Procedure for maintaining confidentiality
   ✓ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Board for review.

It is to obtain approval of the Review Board on Use of Human Volunteers for any changes involving the rights and welfare of subjects before making such change.

Dr. Seaton
Principal Investigator

Trainee

Please return 2 copies of entire protocol to Chairman, Review Board on Use of Human Subjects.

Attachment 1a

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

The Board will not consider any application which does not include an abstract. The abstract should summarize the purpose of the study, the methods and procedures to be used, by addressing each of the following items. If an item is not applicable, please note accordingly:

1. Describe the requirements for a subject population and explain the rationale for using in this population special groups such as children, or groups whose ability to give voluntary informed consent may be in question.

2. Describe and assess any potential risks - physical, psychological, social, legal or other - and assess the likelihood and seriousness of such risks. If methods of research create potential risks, describe other methods, if any, that were considered and why they will not be used.

3. Describe procedures for protecting against or minimizing potential risks and assessment of their likely effectiveness.

4. Include a description of the methods for safeguarding confidentiality or protecting anonymity.

5. When there are potential risks to the subject, or the privacy of the individual may be involved, the investigator is required to obtain a signed informed consent statement from the subject. For minors, informed consent must be obtained from the authorized legal guardian or parent of the subject. Describe consent procedures to be followed including how and where informed consent will be obtained.

   (a) If signed consent will not be obtained, explain why this requirement should be waived and provide an alternative procedure.

   (b) If information is to be withheld from a subject, justify this course of action.

6. If study involves an interview, describe where and in what context the interview will take place. State approximate length of time required for the interview.

7. Assess the potential benefits to be gained by the individual subject as well as the benefits which may accrue to society in general as a result of the proposed work. Indicate how the benefits outweigh the risks.

8. State if the activity requires the use of records (hospital, medical, birth, death or other), organs, tissues, body fluids, the fetus or the abortus.

The statement to the subject should include information specified in items 2,3,4,5,7 as well as indicating the approximate time required for participation in the study.

Received 18/11/77

77-033

SECTION I - RESEARCH PROTOCOL

1) Title: Hormonal correlates of pregnancy and lactational amenorrhea

2) Principal Investigator: Dr. Brian Seaton

3) Starting Date: As soon as necessary equipment procured.

4) Completion Date: One year after starting date.

5) Total Direct Cost: $ 92,480

6) Abstract Summary:

Despite the fact that there is a substantial and growing body of evidence to sugges that "oestrous-like" cyclic phenomena are not completed abolished during pregnancy, this remains a largely ignored area of endocrinology. Clarification of the situation is of considerable importance since the existence of such periodic phenomena at a time when, in women, estrogens and progestogens are substantially elevated above the levels in normal cycling women raises many important questions about our present understanding of the hormonal interaction involved. The purpose of this project is to make a careful study of the endocrinology of pregnancy under condition designed to detect any periodic phenomena in order that the nature and significance of such fluctuation may be evaluated, with particular reference to their implications for improved methods for the control and management of pregnancy.

7) Reviews:

a) Research Involving Human Subjects _____

b) Research Committee _____

c) Director _____

d) BMRC _____

e) Controller/Administrator _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: The long-term objective of this project is a better understanding of the role of steroidal hormones in regulating the reproductive process in general and pregnancy in particular. It is expected that such information will be of value in the development of improved procedures for pregnancy management and reproduction control.

2. Background: It is a commonly held view that pregnancy suppresses the periodic phenomena associated with the estrous cycle; for example Heap, Perry and Challis (1) state that "pregnancy is defined as the period of fertilization to parturition and the emphasis is on the role of hormones in maintaining gestation and in the arrest of the estrous or menstrual cycles" and Ryan (2) states that "pregnancy .... begins for the mother only when .... ovarian cyclicity is replaced by pregnancy-specific endocrine mechanisms". However, whilst it is certainly true that the overt signs of normal menstrual cycles (e.g. menstruation in the human) cease during pregnancy there is a growing body of evidence that some form of periodic phenomena, resembling the estrous cycle but which do not result in full estrous or menstrual effects, occur throughout pregnancy in a wide range of mammals. Some of this evidence is quite unequivocable; for example, Swezy and Evans (3) showed that rats continue to ovulate at regular intervals whilst pregnant, Richards (4) showed that hampsters continue to show periodic changes in activity patterns whilst pregnant, D'Souza (personal communication) has found evidence of periodic changes in the vaginal cytology of pregnant tree-shrews and cats, Seaton (5) has found evidence of regular fluctuations in estrogen excretion in a pregnant gorilla and similar fluctuations can be seen in the date of Bielert, Czaja, Scheffer, Robinson and Goy(6) and of Bosu, Johansson and Gemzell (7) obtained from the rhesus monkey. On the other hand, the data of Reyes, Winter, Faiman and Hobson (8)

from the chimpanzee and of Raeside and Liptrays (9) from the horse are equivocable in this respect.

There has been, of course, an enormous amount of work done on hormone levels during pregnancy in the human, not only on estrogen levels in plasma (e.g. 10-15), urine (e.g. 16-21) and other fluids (e.g. 22-23) but also on progestogens (10, 15, 24-25) and other hormones (e.g. 10, 13, 15, 25-31). It is therefore pertinent to enquire whether there have been any previous reports of periodic phenomena occurring during pregnancy in women, and if not, why not?

Since women, in common with other primates, have a poorly defined behavioural estrus at the time of ovulation and intercourse may occur at any stage of the cycle, behavioural patterns are of no value; ovarian studies (e.g. by laparoscopes) are excluded for normal subjects on ethical grounds, hence the only remaining areas available for study are (i) occurrence of menstruation (ii) hormonal patterns and (iii) vaginal cytology (which reflects hormonal activity).

Although missed menstruation is the classical symptom of pregnancy in humans, there have been many cases of "large-for-dates" babies which have been ascribed to incorrect dating of conception due to the occurrence of one or (rarely) more post-conceptual menstrual periods; the so-called "short-periods"; However, the occurrence of these "short-periods" has not received properly controlled scientific study and is still a subject of controversy (32). Similarly, it is a commonly held view (33) that women are more likely to have sponstaneous abortions at the times when they would have had their next menstrual

period (had they not been pregnant), particularly in the first trimester. Again, this is a poorly studied, and therefore controversial area. Both these phenomena imply that aspects of the menstrual cycle do persist, at least for a while, post-conception in women.

The existing data on hormonal patterns(particularly periodic phenomena) focus exclusively on diurnal rhythms. The concensus of opinion (e.g. 34-36) is that circadian fluctuations in estrogens do occur during pregnancy though there is at least one report (37) which claims that they do not. Whatever the position with regard to diurnal variations, the period of such rhythms (1 day) is so much shorter than the rhythms of interest in this study (20-30 days) that their main relevance is as a confounding variable. This point will be covered in the "Methods of Procedure" section. All the other longitudinal studies on hormone levels which have been cited fail to give a conclusive answer as to whether low-frequency periodic fluctuations occur during pregnancy and it is clearly pertinent to enquire why this should be so. The studies so far reviewed all suffer from one or more of the following features which tend to obliterate any signs of such periodic fluctuations:-

1) The data are not obtained serially from a selected cohort of women;

2) The data from individual subjects are averaged before being evaluated for longitudinal trends;

3) The inter-sample interval is too long (i.e. over 1 week) and/or the period studied in one individual is too short (less than 3 months);

4) Because of the large increase in estrogen production during pregnancy, estrogen levels are plotted on logarithmic scales. It can readily be verified that, on a logarithmic scale covering 3 decades, a 50% difference in absolute hormone concentration (i.e. $\Delta \log \approx 0.3$) is attenuated to 10% (of full scale) linear difference on the graph.

However, when these factors are borne in mind it is possible to discern the presence of regular, periodic fluctuations in those papers (e.g. 12, 38) where the data are sufficiently detailed. These data, though, cannot be regarded as conclusive evidence for the existence of periodic phenomena during pregnancy. Thus, in order to establish whether or not regular fluctuation in hormonal levels are a normal feature of human pregnancy a properly designed study (avoiding the problems listed previously) needs to be undertaken.

It should be noted that, whilst the dogma that pregnancy abolishes the menstrual cycle is based on the observation that menstruation ceases during pregnancy in women, there is absolutely no justification for assuming that the absence of menstruation means the absence of any form of sub-menstrual cycle. Indeed, the occurrence of periodic fluctuations in hormone levels has been observed in young girls who are several years pre-menarche (39).

It should also be noted that the resemblance of periodic fluctuations in hormone levels during pregnancy to normal menstrual cycles does not necessarily mean that they are of maternal origin. It is well-established that the majority of pregnancy estrogens are of feto-placental origin (40) and Faiman, Reyes and Winter (41) have produced evidence that female chimpanzee neonates exhibit cyclic fluctuations in estrogen excretion during the first few months of life. Some evidence that gorilla neonates

also exhibit cyclic fluctuations in estrogen excretion has also been obtained (Seaton, unpublished data). Thus, the possibility that the feto-placental unit (and the sex of the foetus) plays an active role in the occurrence of pregnancy cycles must be considered seriously.

It is now becoming accepted that the occurrence of regular estrous/menstrual cycles in non-pregnant females (human or other mammals) is an artifact of civilisation or domestication and that, in fact, for the majority (if not all) of species in the wild the normal conditions of the female are either (i) immature, (ii) pregnant or (iii) in lactational or seasonal anestrous/amenorrhea. This raises the interesting possibility that the complex feed-back mechanisms which result in the estrous/menstrual cycle were, in fact, evolved to serve a different purpose. Papanicolaou (42), in a study on vaginal cytology in menstruating and pregnant women, observes that "the two post-ovulatory stages of the normal cycle, the proliferative and the growth-secretory state, may be considered as a short imitation of the more extensive and fundamental processes occurring during gestation" and "as a whole, the smear of advanced pregnancy resembles the pre-menstrual smear". It would therefore seem that, from a physiological point of view, the estrous/menstrual cycle should be regarded as a "faulty" pregnancy cycle, rather than pregnancy being an "interruption" of the "normal" estrous/menstrual cycle.

Studies on estrous/menstrual cycles and pregnancy in domesticated species or women in developed countries have undoubtedly contributed much to our understanding of reproductive endocrinology. By constrast there have been few studies on women in the underdeveloped countries (e.g. 43, 44) and there is undoubtedly considerable value in studying the endocrinology of a group of women in whom pregnancy spacing still occurs largely by "natural" means (e.g. lactational amenorrhea) rather than by voluntary abstinence from intercourse or by active contraception.

Quite apart from the fundamental knowledge on the endocrinology of reproduction which may be expected to accrue from such a study, the data obtained would provide essential baseline information (which does not, at present, exist) for evaluating the impact (at the endocrionological level) of health-care and fertility control programs in such populations.

3. Rationale: The dogma that pregnancy abolishes cyclic activity has meant that there have been no longitudinal studies on hormone levels in pregnant women which have been specifically designed to investigate the possibility that cyclic phenomena (other than diurnal rhythms) might occur. In those studies on patterns of hormone levels during pregnancy which have been undertaken, data have been obtained either as individual values for large numbers of subjects or as longitudinal studies but with a sampling frequency not greater than one per week. In either case, the data collection effectively masks any periodicity which might be present.

Since to establish that hormone levels do show periodic fluctuations throughout pregnancy would have far-reaching implications in terms of our understanding of the control mechanisms involved and their significance it is now imperative to re-examine the endocrinology of pregnancy in order to clarify the position.

It should be noted that, whatever emerges from this study in terms of the role of periodic phenomena during pregnancy valuable baseline data (which at present are not available) will be obtained on hormone levels during pregnancy in Bangladeshi women. These will be of some importance in other projects on pregnancy care or family planning.

B. SPECIFIC AIMS

The specific aims of this project are to collect data on the patterns of hormone excretion in pregnant or lactating Bangladeshi women and in neonates in order to:-

1) Substantiate and clarify the phenomenon of sub-extrous cycles.

2) Obtain baseline data on hormone levels in these populations.

C. METHODS OF PROCEDURE

Since it is anticipated that periodic phenomena during pregnancy, if they really occur, will be observed in the majority of women the number of subjects for this project need not be large. Indeed, the large number of samples that will be required from each subject dictates that the number of subjects should be kept to a minimum.

Two groups of women will be studied:-

Group 1 will consist of women of middle to high socio-economic status selected from patients who attend privately for ante-natal care at the Dept. of Obstetrics and Gynaecology, IPGM, Dacca. It is expected that these women will be comparable to those in developed countries;

Group 2 will consist of women of low socio-economic status selected from the village of Nandepara on the outskirts of Dacca.

Volunteers will be taken into the project as early as possible during pregnancy (in general, this will be from about 2-3 months post-conception) and will be studied until 2-3 months post-partum. Each subject will therefore participate in the project for about 9 months. A minimum of 5 volunteers will be needed in each group so, allowing for a drop-out rate of 50%, each group will consist initially of 10 subjects, (this

is close to the maximum which can be accomodated.) In addition 6 normal, non-pregnancy women from each socio-economic group will be recruited and similarly studies to serve as controls. However, whilst the pregnant subjects will be studied for nine consecutive months, the controls will be studied for only two consecutive months.

Prior to entry into the project each potential subject will first be informed (see appendix 1a) of the purpose of the project, the nature of their involvement in it, the risk (albeit minimal) involved, the benefits they may expect to receive and the fact that they may withdraw at any time without prejudice to their normal entitlement to medical care.

After being given an opportunity to ask questions a signed consent will be obtained. The potential subject will then be given a thorough medical examination including a vaginal examination and the withdrawal of 5-10ml blood by venapuncture. This withdrawal of blood is a normal procedure in antenatal care but, in addition to the routine tests for clinical purposes, the remaining blood will be retained for further examination for research purposes. Potential subjects with any of the following characteristics will be excluded from the study:-

1) Multi-gravid women with a previous history of difficult pregnancies;

2) Primi-gravid women in whom the initial examination reveals reasons for anticipating pregnancy difficulties (e.g. small pelvic arch);

3) Women whose general health or nutritional status are appreciably lower than the norm for their socio-economic group;

4) Women with acute illness at the time of the initial examination;

5) Women whose participation in this project might be detrimental to their physical or mental well-being.

In all of the above criteria the opinion of the examining physician (Prof. T.A. Chowdhury of Dr. S. Bhatia) will be decisive.

6) Also excluded will be:-

(a) Women outside the age-range 18-35 at the time of potential entry.

(b) Women with more than 4 previous pregnancies.

(c) Women who have used oral contraceptives or IUDs in the 6 months prior to estimated date of conception.

Collection of samples

1) A blood sample (5-10 ml) will be collected by venapuncture at the time of the initial examination. This sample will be collected whether or not part of it is required for routine clinical assays.

2) If, at any time during the study period, it is necessary to take additional blood samples for clinical purposes, the volume collected will be approximately 5ml in excess of that required for the routine tests in order to permit additional assays to be performed for research purposes (this will not apply, of course, if, in the opinion of the physician, withdrawal of the additional blood would be detrimental to the patient's well-being). These additional samples will not be collected in the absence of clinical necessity.

3) Subjects will be required to collect daily early morning (first voided) specimens of urine. A female urine receptacle will be supplied to each subject for this purpose. The subject will then transfer a portion of the urine to a specimen tube (35 ml capacity, any excess urine being discarded) which contains 0.01 gm thiomersal ($C_2H_5.Hg.S.C_6H_4.COONa$, a powerful bacterocide) as a preservative. Later the same morning the urine sample will be collected by a field assistant, brought to the laboratory, divided into aliquots and deep frozen. Urine from both male and female neonates (blood will not be required from neonates) will be similarly collected except that a pediatric urine collector will be used.

4) Once a week a sample of vaginal epithelial cells will be taken (either by the field assistant or, if appropriate, by the subject herself) during the field assistant's visit and immediately smeared on to a clean microscope slide and fixed with "Dry fix" (a mixture of polythylene glycol, ethanol and acetic acid) to both preserve and protect the smear.

5) At the same time as the vaginal smear is taken the field assistant will record a weekly report about the general health of the subject (see attachment 2).

6) Shortly (i.e. approximately 4-6 weeks) prior to parturition each subject will again receive a thorough medical examination as is common in ante-natal care. If a blood sample is thought to be necessary for clinical purposes, at that time, an additional 5-10ml blood will be taken (subject to the approval of the examining physician) for research investigations.

7) The date and time of birth, the weight and sex of the baby and any other clinical features of the birth will be recorded.

8) Urine collections will continue for 2 months post-partum but vaginal smears will not be collected during the post-partum period of bleeding.

9) Early morning urine samples will be collected daily from the baby as far as is possible during the post-partum period that maternal urine samples are collected.

Medical Care for the Subject

There are no scientific requirements for medical intervention within the context of this project; indeed, the need for medical intervention implies a confounding variable which, in other circumstances, might be resolved by dropping the subject from the study. Nevertheless, the nature of this project is such that there would be a clear moral obligation on the CRL to provide the best medical care at its disposal in the event of a volunteer subject encountering difficulties with her pregnancy. For this reason, provision has been made in the budget for hospitalization and additional assays although these are not an integral part of the research itself.

Analysis of urine samples

The following apply to both maternal and neonatal urine samples. Samples will be assayed for creatinine concentration by the alkaline pricrate method (45, 46).

Steroid conjugates will be hydrolysed enzymatically using a B-glucuronidase and/or a sulphatase (47-49).

Hydrolysed samples will be assayed for estrodiol, estriol, testosterone and progesterone by optimised radio-immunoassays (5, 50) using highly specific antisera (from Miles-Yeda Ltd.) which obviate theneed for prior chromatographic isolation of

the steroids of interest. In view of the large number of samples involved in this project, only 3 samples per week will be assayed initially, the remaining samples will be kept in reserve to cover losses (spillages, etc) and to permit a daily study throughout critical periods as appropriate. It should be noted that experience in previous studies of this kind have shown that it is more convenient for the subjects to collect daily samples (even though not all are to be used) because they get into a routine which is less troublesome than having to remember on which days samples should be collected.

One sample per week from each subject will be assayed by a gas-liquid-chromatography profiling technique (e.g. 51,52) which enables more than twenty different steroids (androgens, progestogens, corticosteroids, but excluding estrogens) in pregnancy urine to be identified and quantified. These samples will normally correspond with the weekly vaginal smear. Randomly selected samples (selected using tables of random numbers) will also be assayed for total estrogens by the Kober/Ittricht fluorimetric method (16, 53-56). this is the method which is in routine use in hospital laboratories all over the world (e.g. 57-59) and is included to provide a comparison of the data obtained in this study with the data obtained elsewhere.

Evaluation of vaginal smears

Smears of vaginal epithelial cells will be stained according to the method of Papanicolaou (42, 60) under the supervision of Dr. M. islam, a histopathologist in the IPH. Instruction in the interpretation of the cell distribution patterns during pregnancy will be provided by a visiting consultant from the UK, Dr. F. D'Souza, who has had extensive experience in this field. Subsequently, randomly selected smears (approximately 10 per month) will be sent to Dr. D'Souza (who will not be informed of the interpretation made here) for her opinion in order to ensure consistency in the interpretation of the smears. In addition, any smears which appear ambiguous or abnormal will be sent to Dr. D'Souza for her opinion.

Analysis of blood samples

Blood samples will be divided into two portions, one heparinised the other non-heparinised and assayed as follows:-

Heparinised blood

(a) hematocrit,
(b) hemoglobin, by the cyano-methemoglobin method (61),
(c) vitamin C, by the 2, 4-DNP method (61),
(d) red blood cell folate by RIA (62),
(e) plasma carotene and vitamin A (61).

Non-heparinised blood

(a) total serum protein by the biuret method (61),
(b) serum zinc by atomic absorption spectrophotometry (63).

Any remaining plasma or serum will be stored at -40°C in case any additional assays are subsequently found to be desirable. The purpose of these assays is to obtain a crude estimate of the nutritional status of the mother (64) to assist in the interpretation of the endocrinological data. As such, these assays are not a crucial part of this study.

Evaluation of the data

As set out above, the following data will be obtained:-

1) High frequency (≥3/week) determinations of urinary estrone estriol testosterone and progesterone;

2) weekly observations of >20 other urinary hormones;

3) weekly observations on vaginal cytology;

4) weekly onservations of maternal weight and blood pressure, together with a record of the occurrence of other relevant events e.g. vaginal blood loss, fetal movement, maternal illness;

5) date and time of birth, the birthweight (or as soon as possible thereafter) and sex of the child, a note of any complications in the delivery;

6) blood levels of various nutritional parameters at the time of entry into the study and at subsequent clinical examinations.

All urinary hormone levels will be corrected for variations in urine volume by relating them to urinary creatinine levels.

Graphs of hormone levels, maternal weight and blood pressure throughout pregnancy will be drawn for each individual subject and the occurrences of relevant events (e.g. parturition, abortion, maternal illness) indicated.

Correlations between the levels of different hormone will be determined

(a) by construction regression lines and calculating the correlation co-efficients;

(b) by observing the constancy (or otherwise) of hormone ratios.

The appearance of the vaginal smears will be "quantified" on an arbitrary scale (0-4) by inspection since the interpretation of smears depends on a complex evaluation of the number, relative distribution and stage of development of the different types of cells. The vaginal smear "index" obtained in this way will similarly be correlated with the hormonal data.

The hormonal and vaginal cytological data will be evaluated for the occurrence of periodic phenomena by the autocorrelation technique. At its simpliest, the autocorrelation technique consists of visually examining the data for repeating patterns.

perhaps assisted by tracing the data on to an overlay which is then moved sideways (over the original data) to find other positions of correspondence. At its most sophisticated, the autocorrelation technique consists of a computer-based fast-Fourier transform of the data (65, 66) to locate fundamental frequencies in the data (this is essentially a computer stimulation of the simple visual technique) and may include a compensation for data trends (e.g. the steady rise in the level of estrogens during pregnancy). Autocorrelation techniques give both the frequency and the significance of any periodicity which exists. The data will also be evaluated, by the usual statistical proceudres (e.g. t-tests), for significant differences between

(a) data obtained from women in Group 1 as compared with women in Group 2;

(b) data obtained from women who give birth to girls as compared to those who give birth to boys.

Confounding variables

The major confounding variables in this project are

(i) diurnal variation in hormone excretion rates (34-36) and

(ii) day-to-day and hour-to-hour variations in urine volume (which affects the measured hormone concentration).

The taking of daily blood samples (even it if were ethnically and culturally acceptable - which it is not) instead of urine does not solve these problems since there are considerable fluctuations (some as short as 15 min) in the plasma concentrations of some hormones (67). Urine has the dual advantage over blood that it is easier to collect and represents the integration (i,e, the average) of high frequency hormone fluctuations; on the other hand, urinary levels are much more a reflection of hormonal metabolic rates rather than the physiologically active levels though, for estrogens, a good correlation between urinary and plasma levels has been demonstrated (68). The "blood or urine?" question has been well

reviewed by Klopper (69).

Both diurnal variations in hormone excretion rates and day-to-day variations in urine volume can be avoided by collecting 24 hour samples. However, whilst this is practicable as an occasional clinical procedure (hospitalization of the subject is usually required to adequately control the collection problem) it is clearly impracticable in this study where daily collections over long periods are required.

The use of urinary creatinine concentrations as an index of urine volume has been extensively used in the hormonal monitoring of pregnancy (5, 20, 70, 71) and, although not a complete solution to the problem, is an accepted procedure.

Taking all these factors into account, the daily collection of a first-voided urine sample seems to be the best solution. The sleep period is undoubtedly the most consistent period of the day from various points of view, in particular, time, duration, activity (including eating and drinking) patterns. It should be noted that, whilst the diurnal variation in estrogen/creatinine ratios has been observed (72) to be ± 12% of the daily average, the effect of collecting the sample at the same time each day will substantially reduce the variability in results due to diurnal rhythm. Furthermore, the expected variation in hormone levels due to "pregnancy cycles" is expected (5) to be 40-100% (i.e. a two-fold difference between peak and trough) and is, therefore, much greater than the diurnal variation. Thus, it is reasonable to expect that the collection of first-voided urine samples will minimize this confounding variables. The situation will, of course, be kept under careful review both during the collection and analysis of samples and during the evaluation of the data.

Much thought was given to the question of collecting occasional 24 hr urine samples during the course of this study. The principal investigator finds it difficult to envisage how the occasional results thus obtained might justify the inconvenience

to the subject that would be involved, but the possibility of undertaking such a 24 hr collection is not ruled out and allowance has been made for this under "contractual services - additional biomedical services" in the budget.

The age of the subject must be considered as a potential confounding variable. There is no specific evidence in the literature to suggest that very young of very old pregnant women are endocrinologically different from each other or from pregnant women of intermediate age but both age and parity have been shown to affect maternal and perinatal mortality (73). To avoid unnecessary problems, women less than age 18 or older than age 35 will be excluded from the study.

Similarly, there is no specific evidence to suggest that parity affects the endocrinology of pregnancy but women who have had 5 or more pregnancies (including abortions & still-births) will be excluded and primi-gravid subjects will be considered separately.

Duration of Project

1) Purchasing, installing & commissioning new equipment (3-4 months).

2) Sample collection (may ne concurrent with 1) (9-10 month..

3) Sample analyses (concurrent with 2) ((-10 months).

4) Final data evaluation (2 months).

5) Preparation of reports, publication etc. (2 months).

SIGNIFICANCE

This project seeks to discover fundamental information about the patterns of hormone exretion in pregnant, lactating and non-pregnant Bangladeshi women and about the endocrinologic control mechanisms for the regulation of the reproductive pro...

in general and of pregnancy in particular. Such fundamental information may subsequently be applied in maternal and child health care through improved understanding of the physiological and endocrinological bases of pregnancy.

FACILITIES REQUIRED

Attention is drawn to Technical Report No. 578 of the WHO, "The radioimmunoassay of hormones for clinical trials of fertility regulating agents in developing countries".

1) Office Space: Office space in the CRL will be required for the Principal Investigator for the duration of the project. The space currently allocated will probably not be adequate on a long-term basis because (a) the whole of room 216 will be required for the endocrinology laboratory (b) the present arrangement does not provide adequate space for filing, text-books, etc. Office space at Matlab will be required for thw FRP physician for the duration of the project. Access to desk space will be required for 1 yr at both Dacca and Matlab for the Field Assistants to collate their records.

2) Laboratory Space: Laboratory space (70 running feet) is required within the CRL to run the hormone assays. It is envisaged that room 216 will be renovated for this purpose.

3) Hospital Resources: In view of the fact that, at present, there are no suitable facilities for obstetric services in the CRL, subjects who are found to be in need of hospitalization will be treated at the Dept. Obstetrics & Gynaecology of the IPGM. Provision has been made for this in the budget under the headings "Antenatal & Perinatal Health Care" for routine antenatal and perinatal care and "Additional Biomedical Services" for obstetric complications and other health problems which may arise.

4) Animal Resources: are not required.

5) Logistic Support: One car will be required from 9 a.m. to 12:30 p.m. each day (including Saturdays & Sundays) to enable the Lady Health Visitors to visit the subjects and collect the samples.

6) Major Items of Equipment: The project will require extensive use of the two liquid scintillation counters, a refrigerated centrifuge, the atomic absorption spectrophotometer and a visible range spectrophotometer.

COLLABORATIVE ARRANGEMENTS

Dr. F. D'Souza, Physical Anthropologist, U.K. Dr. D'Souza will collaborate in the establishment of the vaginal cytology techniques and in the interpretation of the smears. It is envisaged that Dr. D'Souza will coauthor any publications which include any of the data on vaginal cytology. It is also envisaged that, so far as is practicable (and subject to the approval of the Review Board(s), the CRL will cooperate with Dr. D'Souza in her studies on vaginal cytology in mammalian pregnancy.

Prof. T.A. Chowdhury, Consultant Obstetrician & Gynaecologist, Dacca. Prof. Chowdhury will collaborate in the project both by recommending to the CRL potential subjects from women who attend his clinic for ante-natal care and by providing clinical advice and treatment of the subjects as appropriate. It is envisaged that Prof. Chowdhury will be invited to coauthor any relevant publications arising from this project.

SECTION III BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

Name	Position	% Effort No.days	Annual Salary	Project Requirement TAKA	Project Requirement DOLLARS
Dr. Brian Seaton	Investigator	50%	$ 18907	-	9454 (a)
Dr. Shushum Bhatia	Obstetric consultant	50%	$ 21000	-	1050
Chowdhury M. Huq	Res. Assistant	260 d	Tk.33800	33800	-
To be appointed	Lady Health Visitor	260 d	Tk.16200	16200	-
To be appointed	"	210 d	Tk.16200	13077	-
To be appointed	"	210 d	Tk.16200	13077	-
			Sub Total:	76,154	10504

2) SUPPLIES & MATERIALS

Antisera		250
Radioactive steroids		5(
Non-radioactive steroids		2
Miscellaneous reagents		
Disposable glass/plastic ware		
Scintillator fluid		1
Bottles for sample collection		
Stationery, sample labels, record forms, pencils, note books, etc.	2500	
Sub Total:	2500	61

3) EQUIPMENT

Diluters, Dispensers & Auto-pipettes	-	6000
Ice-making machine	-	250
Ultrasonic cleaning bath & accessories	-	2000
Magnetic stirrers & accessories	-	275
Gas-liquid chromatograph & accessories	-	23000
4.5l Dewar flasks (x5)	-	150
Tissue-Tek II Slide-staining equipment	-	125
Accessories for IEC centrifuge	-	1750
Spectrofluorimeter	-	8000
Vacuum oven & accessories	-	1200
Miscellaneous items	-	50
Sub total:	-	45

		Project Requirements TAKA	DOLLARS	
PATIENT HOSPITALIZATION				
See under contractual services		-	-	(c)
OUTPATIENT CARE				
See under contractual services		-	-	(d)
CRL TRANSPORT				
Mileage - Dacca 25 miles/day for 300 days @ Tk. 1.4/mile		10,500	-	
Travel to Matlab, 10 trips @ Tk. 313/trip attenuated to 25% by sharing.		785	-	
	Sub Total:	11,285	-	
TRAVEL AND TRANSPORTATION OF PERSONS				
LOCAL TRAVEL				
Dacca-Jessore-Ballobhpur Hospital 12 trips @ Tk.500/trip (for collaboration		6000	-	
or Per diems, training)		5000	-	
	Sub Total:	11000	-	
INTERNATIONAL TRAVEL				
London-Dacca return for consultations with Dr. D'Souza 2 trips @ 1250		-	2500	
Per diem, 30 days @ $ 43 (London)		-	1290	
Attendance at meeting		-	2000	
Perdiem, 15 days @ $ 40		-	600	
	Sub Total:	-	6390	
TRANSPORTATION OF THINGS				
Import of supplies, 25% of $ 6100		-	1525	
Import of Equipment, 25% of $ 45,500		-	11375	
	Sub Total:	-	12900	

		Project Requirement	
		TAKA	DOLLARS
9)	RENT, COMMUNICATIONS & UTILITIES		
	Postage, telephone & cables	2500	-
10)	PRINTING & REPRODUCTION	10000	-
11)	OTHER CONTRACTUAL SERVICES		
	Antenatal 2 perinatal health care		
	20 subjects @ Tk. 500 each.	10,000	-
	Additional biomedical services	3,000	-
	Dr. M. Islam, Consultant histopathologist		
	3 hrs/week x 52 weeks @ Tk. 50/hr	7,800	
	Computer time, 4 hrs @ Tk. 650/hr	2,600	
	Sub Total:	23400	
12)	CONSTRUCTION, RENOVATIONS & ALTERATIONS		
	Renovations and alterations to Room 216 to convert it into a working laboratory	35,000	

NOTES

a) Dr. Seaton's salary is paid by the U.K. Government.

b) $ 12,000 towards the cost of the GLC has already been approved under the Biochemistry Branch budget for FY 78.

c) The cost of these items has already been approved under the Biochemistry Branch budget for FY 78.

d) Since there are no facilities for antenatal care at the CRL, all patient care requiring hospitalization will be referred to IPGM on a contractual basis.

e) Since this project has much in common with work being undertaken at Matlab, provision has been made for occassional visits to Matlab for consultations. In general, these trips will make use of transportation requested for other purposes, hence the sum allocated has been attenuated to ¼ of the full cost.

f) Part of the cost of importing equipment has already been approved under the Biochemistry Branch Budget for FY 78 (see c and d above).

B. BUDGET SUMMARY

	Category	PROJECT REQUIREMENTS TAKA	DOLLARS
1)	Personnel Services	76,154	10,504
2)	Supplies	2,500	6,100
3)	Equipment	-	45,500
4)	Hospitalization	-	-
5)	Outpatient Care	-	-
	CRL Transport	11,285	-
	Travel, persons	11,000	6,390
	Transportation, things	-	12,900
	Rent/Communications	2,500	-
	Printing & Reproduction	10,000	-
	Contractual Services	23,400	-
	Construction & renovations	35,000	-
	Sub Total:	171,839	81,394

Conversion rate $ 1 = Tk. 15.5

Total Cost $ 92,480

Attachment 1a

ABSTRACT SUMMARY

1) This project seeks to discover new information about the endocrinological basis of human pregnancy. A human subject population is therefore essential since little information directly applicable to the human situation can be obtained from animal studies. It is also highly desirable to extend the study post-partum, to include the neonate, in order to gain some information about the contribution of the foetus to the endocrinology of pregnancy.

2) There are two potential, albeit minimal, risks in this project (i) irritation of the vagina by the regular taking of vaginal smears; (ii) skin irritation around the genital of the neonate caused by the application of the adhesive patch on the urine collection pouch. The likelihood of these problems arising is extremely small but the Lady Health Visitor will check regularly (see attachment 2) for the occurrence of these problems and, if found, will immediately discontinue the collection of vaginal smears or neonatal urine pending a detailed evaluation of the situation by the consulting physician. Vaginal smears will <u>not</u> be collected during the period of post-partum bleeding due to the increased risk of infection at that time.

3) In view of the fact that the risks mentioned in (2) above are so low, there is no need for special precautions beyond the regular check-ups already mentioned, together with adequate standards of hygiene.

4) Neither the field determinations nor the laboratory analyses will generate any potentially confidential or embarassing data. Nevertheless, subjects records will be kept in a clinic file and data transferred to a computer for analysis will be identified by code number only (i.e. will not include the subject's name).

5) As previously indicated, the potential risks to the subject are virtually non-existent and the information generated by this study would not invade the subject's privacy. Nevertheless the procedures for obtaining signed informed consent from the subjects will be followed.

6) A health status report (see Appendix 2) will be obtained weekly from each subject by a brief interview (of approximately 10 minute's duration) conducted by the Lady Health Visitor in the subject's home.

7) The purpose of this study is to obtain basic information on the endocrinology of pregnancy in normal Bangladeshi women. As such, there is no direct benefit to the subject except that the subjects will receive a higher standard of antenatal and perinatal care than they would have otherwise expected. Subjects will not receive any direct cash benefit from participation in the project but provision has been made in the budget to cover (on the basis of a direct arrangement with IPGM) the cost of delivery fees at IPGM (this is particularly applicable to group 1 subjects) and to provide a baby-care pack for home deliveries (this is particularly applicable to group 2 subjects). It is envisaged that society as a whole will ultimately benefit from the information gained in this study.

8) The project will not require access to hospital or medical records other than those directly related to the pregnancy being studied. Maternal blood (1 or 2 5ml samples), urine (daily samples) and vaginal smears (weekly samples) will be required as will neonatal urine (daily samples where possible

Appendix I

CHOLERA RESEARCH LABORATORY

HORMONAL PATTERNS DURING PREGNANCY

Subject Consent Form

I have been given information about the nature of the research project in which I am going to be a volunteer subject and have been given the opportunity to think the matter over and discuss it with my relatives and friends. I have also been given the opportunity to ask questions and have been given satisfactory answers.

I realise that I shall not receive any direct benefit from participation in this project though I may receive a higher level of antenatal and postnatal care than I would have expected otherwise.

I am willing to participate in this project, to undergo an initial thorough medical examination (including a vaginal examination and the taking of a blood-sample); to collect daily samples of early-morning urine from myself and from my baby when he or she is born; to take (or have taken) each week a vaginal smear and a record of my weight, blood pressure and general health. I will allow these samples to be analysed for substances relevant to this project and I understand that the results will be kept strictly confidential though I may ask to see the results obtained on my own specimens.

I understand that I may withdraw from this project at any time without giving a reason and that, in this event, I will not prejudice my right to receive the usual standard of antenatal care.

I understand that the bottle supplied to me for collecting urine contain a poisonous substance and I will keep them away from children and not allow them to be used for any other purpose.

_____

Name of Subject

_____ _____

Signature or mark of subject Date

In my opinion, this subjects physical or mental well-being will not be prejudiced by participation in this project.

_____

Signature of examining physician

কলেরা গবেষণাগার

অন্তঃসত্ত্বা থাকাকালীন হরমোনের প্রতিরূপ

পরীক্ষণ পাত্রীর স্বীকৃতি ফরম

আমি যে গবেষনা প্রকল্পের জন্য স্বেচ্ছায় প্রণোদিত হয়ে পরীক্ষণ পাত্রী হিসাবে নিয়োজিত হচ্ছি তার প্রকৃতি সম্বন্ধে আমাকে জ্ঞাত করান হয়েছে। আমাকে এ বিষয়ে চিন্তা করার, বন্ধু-বান্ধব ও আত্মীয় স্বজনের সাথে পরামর্শ করার এবং বিভিন্ন ধরনের প্রশ্ন করার ও সন্তোষজনক উত্তর পাওয়ার সুযোগ দেওয়া হয়েছে।

আমি বুঝতে পেরেছি যে আমি আমার প্রসবের পূর্বের ও পরের যত্ন সম্বন্ধে যে ধারনা পোষন করি, এই গবেষনায় অংশগ্রহন করে তার চেয়ে উন্নততর যত্ন পাওয়া ছাড়া আর কোন সরাসরি উপকার পাবনা। আমি এই প্রকল্পে এবং প্রকল্প সম্পর্কীয় নিম্নবর্ণিত কাজে অংশ নিতে ইচ্ছুক, যেমনঃ-

(ক) প্রাথমিক পর্যায়ে নিজের স্বাস্থ্য বিষয়ক পরীক্ষা (স্ত্রী অংগের পরীক্ষা ও রক্ত দেওয়া সহ)

(খ) প্রতিদিন সকালে নিজের ও নবজাতকের প্রস্রাব সংগ্রহ করা।

(গ) প্রতি সপ্তাহে পরীক্ষার জন্য স্ত্রীঅংগ-রস দেওয়া, নিজের ওজন, রক্তচাপ ও সাধারন স্বাস্থ্যের রেকর্ড রাখা।

আমি ঐ সকল প্রস্রাব, রক্ত ও স্ত্রীঅংগ-রসের নমুনাগুলিকে এই প্রকল্পের প্রয়োজনে বিশ্লেষণ করার জন্য অনুমতি দিব। আমি জানি যে ঐ সকল বিশ্লেষণের ফলাফল অত্যন্ত গোপনে রাখা হবে এবং আমি ছাড়া কাউকে জানতে দেওয়া হবেনা। আমি যে কোন সময়ে কোন কারন না দেখিয়ে উক্ত প্রকল্প থেকে নিজেকে সরিয়ে আনতে পারব এবং এও করে প্রসবের যত্ন পাওয়া থেকে বঞ্চিত হবনা।

আমি ইহাও জানতে পারলাম যে, নমুনা সংগ্রহ করার জন্য যে সকল বোতল দেওয়া হয়েছে তাহাতে নমুনা সংরক্ষনের জন্য এক ধরনের বিষ জাতীয় রাসায়নিক পদার্থ দেওয়া আছে এবং সেই জন্য বোতলগুলি আমি শিশুদের নাগালের বাইরে রাখব এবং ঐগুলো অন্য কোন কাজে ব্যবহার করবনা।

পরীক্ষণ পাত্রীর নাম

পরীক্ষণ পাত্রীর স্বাক্ষর বা টিপসহি

তারিখ

আমার মতে এই পরীক্ষণ পাত্রী মানসিক এবং শারিরীক দিক দিয়ে সুস্থ্য এবং তাকে এই গবেষনার জন্য পরীক্ষণ পাত্রী হিসাবে গ্রহন করা যেতে পারে।

চিকিৎসকের স্বাক্ষর

তারিখ

INFORMATION TO SUBJECTS

The purpose of this study is to see (by testing your urine) how the amounts of hormones in your body vary whilst you are pregnancy or breast-feeding your baby. We also want to see how the amounts of the same hormones vary in your baby after he or she is born. The purpose of this study is to learn more about how these hormones regulate your pregnancy. This study will not be of any immediate benefit to you (though we will make sure tha we are able to provide, while you are pregnant), but we hope th by joining in this study you will help us to help you and your relatives and friends to have healthier babies with less risk in the years to come.

Before entering into the study you will be given a thor examination which will include a vaginal examination and the of a sample of blood. This is normal practice in ante-natal and although there is a little discomfort there is no harm don and your body will quickly make fresh blood to replace what we have taken.

If these tests show that you are a suitable subject for our study you will be invited to join in, but even if you are perhap not suitable this does not necessarily mean that there is any thing wrong with you. We will then ask you to collect some of your urine when you wake up each morning (we will provide you with a suitable collecting bottle) and transfer some of it to a little bottle (which we will provide). This bottle will con some special chemicals which could poison you if you eat or d them. The bottle should therefore not be used for any purpos other than collecting urine. You will find this a little inconvenient at first but you will soon get into a routine an think no more of it. When your baby is born we will ask you to collect some of his or her urine each morning as well. We will provide you with a special bag for this purpose.

Very rarely this bag causes a little irritation to the baby's skin. In the unlikely event that this should happen to your baby you should report this to your dai and we will immediately stop the urine collections. These urine will be collected each day by a dai.

Once every week the dai will ask you for a vaginal smear (which needs to be absolutely fresh so it should not be taken until the dai arrives). The dai can do this for you but if you wish, you can be taught to do it yourself. At the same time the dai will ask you some simple questions about your health during the pervious week and take your weight and blood pressure.

The blood sample, the urine samples and the vaginal smear will be taken back to our laboratory and analysed for such things as hormone which give us information about your pregnancy and the development of your baby.

All the information and results that we get from this study will, of course, be kept strictly confidential, though you can see your own records if you wish.

Since you are helping us voluntarily with this study you may withdraw at any time that you wish if you no longer want to continue. You do not have to give any reason for withdrawing and even if you do withdraw this will not prejudice your chances of receiving the normal standard of ante-natal care.

পরীক্ষণ পদ্ধতিকে জ্ঞাত করণ

আপনি যখন অন্তঃসত্ত্বা থাকেন অথবা শিশুকে বুকের দুধ খাওয়ান তখন আপনার শরীরে হরমোনের পরিমানের পরিবর্তন দেখা (আপনার প্রস্রাব পরীক্ষা করিয়া) এই গবেষণার উদ্দেশ্য ।

আপনার নবজাতকের জন্মের পর তার শরীরেও কি ভাবে হরমোনের পরিবর্তন হয় তাহাও আমরা দেখতে চাই । এই গবেষণার আরেকটি দিক হল এই হরমোন গুলি কি ভাবে আপনার অন্তঃসত্ত্বাকে নিয়ন্ত্রিত করে তাহা জানা । এই গবেষনা আপনাকে সংগে সংগে কোন উপকার দিবেনা, (যদিও আমরা আপনাকে নিশ্চিত করতে পারি যে আপনি অন্তঃসত্ত্বা থাকা কালীন উন্নততর স্বাস্থ্য সম্মন্ধীয় যত্ন পাবেন ।) কিন্তু আমরা আশা করতে পারি যে আপনি এই গবেষনায় অংশগ্রহন করে আপনার, আপনার আত্মীয় স্বজনের ও বন্ধুবান্ধবদের আগামী দিনের শিশুদের রক্ত ঝুকিতে ভাল চিকিৎসা করার জন্য আমাদের সাহায্য করলেন ।

এই গবেষনায় যোগ দিবার পূর্বে আপনার স্বাস্থ্য, আপনার স্তন-অংগ ও রক্ত পরীক্ষা করা হবে । এতে করে আপনি সামান্য একটু অসুবিধা অনুভব করতে পারেন, তবে এটা সাধারন ব্যাপার এবং আমরা যে রক্তটুকু নিব তা আপনার শরীর সংগে সংগেই পূরণ করার ক্ষমতা রাখে । যদি এই পরীক্ষা গুলো দ্বারা বোঝা যায় যে আপনি আমাদের গবেষনার জন্য উপযুক্ত তাহলে আপনাকে আমন্ত্রন জানান হবে । আর যদি আপনি আমাদের গবেষনার জন্য উপযুক্ত নাই হন তবে এটাও মনে করার কোন কারন নেই যে আপনার কোন দোষ বা রোগ আছে ।

তারপর আপনি যখন প্রত্যহ সকালে ঘুম থেকে উঠবেন তখন আপনাকে কিছু পরিমান প্রস্রাব ধরতে বলা হবে (আপনাকে প্রস্রাব ধরার জন্য বোতল দেওয়া হবে) এবং সেখান থেকে আরেকটি ছোট বোতলে কিছু পরিমান প্রস্রাব ঢালতে বলা হবে । (এই ছোট বোতলটিও আপনাকে দেওয়া হবে, এই বোতলে নমুনা সংরক্ষনের জন্য বিষ জাতীয় একটি রাসায়নিক পদার্থ দেওয়া থাকবে এবং পদার্থটি খেলে বিষ ক্রিয়া দেখা দিতে পারে । সেইজন্য এই বোতলটি শুধুমাত্র প্রস্রাব ধরা ছাড়া অন্য কোন কাজে ব্যবহার করবেন না ।) প্রথমতঃ হয়ত আপনার একটু অসুবিধা হতে পারে কিন্তু আপনি অভ্যস্ত হয়ে পড়লে আর কোন কিছু মনে হবেনা । আপনার শিশুর জন্মগ্রহনের পর তারও প্রস্রাব প্রত্য ধরতে আপনাকে বলা হবে এবং তার জন্য আপনাকে বিশেষ ধরনের থলে দেওয়া হবে । এই থলে কোন কোন সময় হয়ত শিশুর চামড়ায় চুলকানি বা জ্বালার কারন হতে পারে, যদি আপনার শিশুর বেলায় এমন কিছু ঘটে তবে সংগে সংগে তা আপনার দাইকে জানা এবং আমরা সংগে সংগে আপনার শিশুর প্রস্রাব ধরা বন্ধ করে দিব ।

দাই প্রত্যহ এই প্রস্রাব সংগ্রহ করবে এবং প্রত্যেক সপ্তাহে সে আপনার স্ত্রী-অংগ রস নিতে চাইবে । (এটা অবশ্য দাইয়ের উপস্থিতি ছাড়া সংগ্রহ করা যাবেনা ।) আপনাকে শিখিয়ে দিলে আপনিও দাইয়ের উপস্থিতিতে উহা সংগ্রহ করতে পারবেন । এই সময় দাই আপনার স্বাস্থ্য সম্পর্কে কিছু প্রশ্ন করতে পারে এবং আপনার ওজন ও রক্তচাপ পরীক্ষা করতে পারে । রক্ত, প্রস্রাব এবং স্ত্রীঅংগ-রসের নমুনা সকল আমাদের গবেষণাগারে আনা হবে এবং হরমোনের পরিমান জানার জন্য বিশ্লেষন করা হবে । ইহাতে আপনার অনুঃসত্ত্বা ও শিশুর উন্নতি সম্পর্কে কিছু তথ্য জানা যাবে । এই সকল তথ্য ও ফলাফল অত্যন্ত গোপনীয়তার সাথে সংরক্ষন করা হবে । তবে আপনি জানতে চাইলে আপনাকে জানতে দেওয়া হবে ।

যেহেতু আপনি ইচ্ছাকৃত ভাবে এই গবেষনায় অংশ গ্রহন করেছেন সেইহেতু আপনি ইচ্ছা করলে যে কোন সময় এই গবেষনায় অংশ গ্রহন থেকে বিরত থাকতে পারেন । এরজন্য আপনাকে কোন কারন দেখাতে হবেনা বা আপনি আপনার প্রসবের যত্ন নেওয়া হতেও বঞ্চিত হবেন না ।

Appendix 2

Hormonal Correlates of Pregnancy

Basic Data Sheet

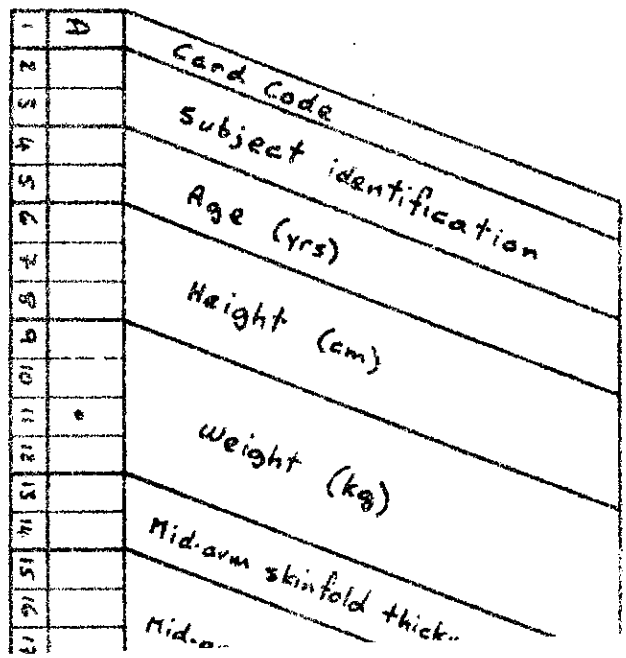
Subject's name

_____

Subject's residence

_____

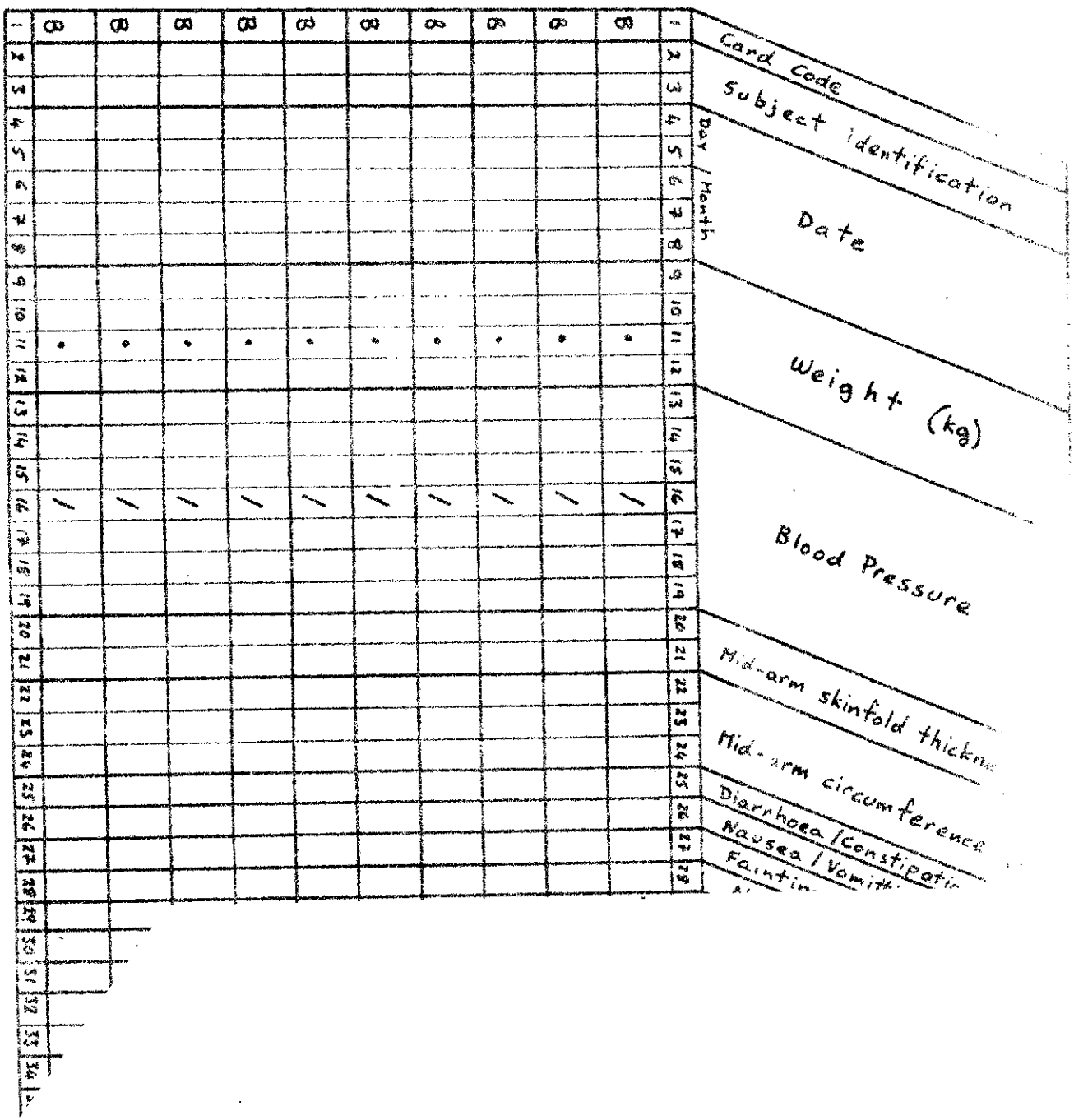
_____



Appendix 2a

Hormonal Correlates of Pregnancy

Weekly Data Sheet



JUSTIFICATIONS FOR THE PURCHASE OF CAPITAL EQUIPMENT

Diluters, Dispensers & Auto-pipettes

This project necessarily involves the processing of large numbers of assays (more than 20000) since multiple hormones will be quantified (in duplicate) on daily or alternate-day samples over long time-periods. Previous experience in this sort of project has shown that: (i) the number of samples envisaged would occupy the whole time of at least two skilled technicians (i.e. not allowing for absences due to illness, etc). (ii) the extremely repetitive nature of the work is highly conducive to operator error (both gross error, e.g. transfer of incorrect sample, and technical error, e.g. inaccurate pipetting). The potentially diastrous consequences of operator error need not be emphasised further. Acquisitions of the automated or semi-automated sample preparation facilities would avoid the need for 1 or 2 additional technicians (i.e. in addition to the research assistant already requested) and eliminate both types of operator error.

Ice-making machine

The radioimmunoassay technique requires that samples be incubated at O 4^{o}C followed by manipulative operations at the same temperature. Ice/water baths are commonly employed for this purpose. The present CRL facilities for producing ice (ice-block trays in storage refrigerators) are not suited to the provision of large quantities of ice on a regular basis and acquisition of an ince-making machine would greatly facilitate this project.

3) Ultrasonic cleaning bath

The rapidly increasing cost of disposable laboratory-ware, together with the costs of shipping them from the USA or the UK means that re-usable glassware has considerable economic advantages over disposable ware. However, it is essential that the washing procedures used are capable of achieving perfect cleanliness every time, particularly in radioimmunoassays where radioisotopes and trace amounts of materials are employed. Ultrasonic cleaning equipment provides a quick, reliable, efficient and economical method of cleaning glassware and the acquisition of an ultrasonic cleaning bath is essential if adequate glasswashing standards for this project are to be reached.

4) Magnetic stirrers & accessories

Magnetic stirrers and accessories are required for running the assays.

5) Gas-liquid chromatograph & accessories

The determination of a wide range of steroids is of immense value in a project like this where one is interested in hormonal patterns since the major steroids (i.e. the ones which are present in the largest quantity) are not necessarily the ones of most significance. Hormone profiling is an extremely laborious technique, very heavily dependent on the technical skills of the operator. Much of the total cost of this item is associated with accessories designed to minimise operational problems. Although essential to this project, a GLC system would also be of considerable value in related projects (e.g. the endocrinology of lactational amenorrhea and in other unrelated projects e.g. in the field of nutrit

6) Dewar flasks are required for the transportation of frozen samples.

7) Tissue-Tek II Staining Equipment is required for the staining of the vaginal smears by the Paponicolaou method.

8) Accessories for IEC refrigerated centrifuge

Radioimmunoassay procedures require access to a refrigerated centrifuge and, in view of the large number of samples envisaged in this project, it is essential that many samples can be centrifuged simultaneously. For this a special head is required which will hold complete racks of tubes (as prepared by the automatic sample preparation unit).

9) Spectrofluorimeter

This is required to assay estrogens by the Kober method (a standard procedure in hospital laboratories all over the world) for comparative purposes. This item has already been authorised under the budget of the Biochemistry Branch for FY78. It will also be of considerable value in other projects including antenatal care and nutritional studies (particularly vitamins).

10) Vacuum oven and accessories

This is essential for sample preparation, both for RIA and for GLC procedures.

Appendix 4

REFERENCES

1. R.B. Heap, J.S. Perry & J.R.G. Challis (1973). Hormonal maintenance of pregnancy. In: Handbook of Physiology; Section 7, Endocrinology; Vol. II, Female Reproductive System, Part 2. (Eds. R.O. Greep & E.B. Astwood) Chap. 41, Pp 217-260. Publ. Am. Physiological Soc.

2. K.J. Ryan (1973). Steroid hormones in mammalian pregnancy. In: Handbook of Physiology; Section 7, Endocrinology; Vol. II, Female Reproductive Syste... Publ. Am. Physiological Soc.

4. O. Swezy & H.M. Evans (1930). Ovarian changes during pregnancy in the rat. Science 71 46.

5. M.P.M. Richards (1964). Cyclical behavioural activity in the pregnant golde... hamster (Mesocricetus auratus Waterhouse). Nature 204 1327-1328.

6. B. Seaton (1978). Patterns of oestrogen and testosterone excretion during pregnancy in a gorilla. J. Reprod. Fertil. In press.

6. C. Bielert, J.A. Czaja, S. Eisele, G. Scheffer, J.A. Robinson & R.W. Goy (1976). Mating in the rhesus monkey (Macaca mulatta) after conception and its relationship to estradiol and progesterone levels throughout pregnancy. J. Reprod. Fertil. 46 179-187.

7. W.T.K. Bosu, E.D.B. Johansson & C. Gemzell (1973). Peripheral plasma levels of oestrogen, progesterone and 17a-hydroxprogesterone during gestation in the rhesus monkey. Acta. Endocr. 74 348-360.

8. F.I. Reyes, J.S.D. Winter, C. Faiman & W.C. Hobson (1975). Serial serum levels of gonodotropins, prolactin and sex steroids in the non-pregnant chimpanzee. Endocr. 96 1447-1455.

9. J.I. Raeside & R.M. Liptrays (1975). Patterns of urinary estrogen excretion in individual pregnant mares. J. Reprod. Fertil. Suppl. 23, 469-475.

10. C.S. Corker, E. Michie, B. Hobson & J. Parboosingh (1976). Hormonal pattern... in conceptual cycles and early pregnancy. Brit. J. Obstet. Gynaec. 83, 48...

11. P. Goebel & E. Kuss. Circadian rhythm of serum unconjugated estriol in lat... pregnancy. J. Clin. Endocrinal. Metab. 39 969-972.

12. R. de Hertogh, K. Thomas, Y. Brietlot, I. Vanderheyden & J. Ferin (1975). Plasma levels of unconjugated estrone, estradiol and estriol and of HCS throughout pregnancy in normal women. J. Clin. Endocrinol. Metab. 40 93...

13. H. Katagiri, W. Distler, R.K. Freeman & U. Goebelsmann (1976). Estriol in pregnancy. Am. J. Obst. Gynec. 124 272-280.

14. M. Levitz, A.J. Slyper & M. Selinger (1974). On the lack of periodicity in plasma estriol in human pregnancy. J. Clin. Endocrinol. Metab. 38 698-700.

15. D.R. Mishell, I.H. Thornycroft, Y. Nagata, T. Murata & R.M. Nakamura (1973) Serum gonodotropin and steroid patterns in early human pregnancy. Am. J. Obstet. Gynecol. 117 631-642.

16. J.B. Brown & N.A. Beischer (1972) Current status of estrogen assay in gynecology and obstrics. Obstet. Gynec. Survey 27 205-235.

17. J.W. Greene & J.C. Touchstone (1963) Urinary estriol as an index of placental function. Am. J. Obstet. Gynec. 85 1-9.

18. S.C. MacLeod, D.M. Mitton & C.R. Avery (1971) Relationship between elevated blood pressure and urinary estriols during pregnancy. Am. J. Obstet. Gynec. 109 375-382.

19. H.J. Osofsky, R.E. Long, E.J. O'Connell & L.D. Marshal (1971). Estrogen excretion during pregnancy in a high-risk population. Am. J. Obstet. Gynec. 109 1-7.

20. L.G.S. Rao (1976) Clinical usefulness of estrogen-creatinine ratios in random samples of urine as a feto-placental function test. J. Endocr. 68 40-41P.

21. J.D. Townsley, N.H. Dubin, G.F. Grannis, L.J. Gartman & C.D. Crystle (19[illegible] Circadian rhythms of serum and urinary estrogens in pregnancy. J. Clin Endocrinal. Metab. 36 289-295.

22. J. MacVicar, R.W. Logan & W.P. Barnard (1973). Fetal maturity assessed from biochemical analysis of the liquor amnii. Scot. Med. J. 18 84-92.

23. D.A. Shutt, I.D. Smith & R.P. Sherman (1974). Estrone, estradiol-17B and estriol levels in human fetal plasma gestation and at term. J. Endocr. 60 333-341.

24. R.B. Heap (1969). The binding of plasma progesterone in pregnancy (Abstr). J. Reprod. Fertil. 18 546-548.

25. H. Ward, H. Rochman, L.A. Varnavides & G.A. Whyley (1973). Hormone and enzyme levels in normal and complicated pregnancy. Am. J. Obstet. Gynec. 116 1105-1114.

26. T.G. Brien & I.J. Dalrymple (1976). A longitudinal study of free cortisol index in pregnancy. Brit. J. Obstet. Gynec. 83 361-367.

27. P. Kumaresan, P.B. Anandarangam, W. Diangon & A. Vasicka (1974). Plasma oxytocin levels during human pregnancy and labour as determined by radioimmunoassay. Am. J. Obstet. Gynec. 119 215-223.

28. P.C. Leighton, Y.B. Gordon, M.J. Kitan, A.E. Leek & T. Chord (1975) Levels of alpha-fetoprotein in maternal blood as a screening test for fetal neural-tube defect. Lancet.

M. Mizuno, J. Lobotsky, C.W. Lloyd, T. Koboyashi & Y. Murasawa (1968). Plasma androsteredione and testosterone during pregnancy and in the newborn. J. Clin. Endocrinal. Metab. 28 1133-1142.

30. H. Pearlman, O. Crepy & M. Murphy (1967). Testosterone-binding levels in the serum of women during the normal menstrual cycle, pregnancy and the post-partum period. J.Clin.Endocrin. 27 1012-1018.

31. A.F. Youssef, Y.A. Naguib, M.M. Fayad & M. Hussein (1971). Prognosis of threatened abortion. Am. J. Obstet. Gynec. 109 8-11.

32. T.G. McCarthy (1975). The relationship of a "short period" to conception. Brit. J. Obstet. Gynec. 82 158-161.

33. In: Pregnancy month by month 2nd Edn. (1969). Publ. Consumers' Association. London. Pg 10.

34. P. Goebel & E. Kuss (1974). Circadian rhythmn of serum unconjugated estriol in late pregnancy. J. Clin. Endocrinol. Metab. 39 969-972.

35. H. Katagiri, W. Distler, R.K. Freeman & U. Goebelsman (1976). Estriol in pregnancy. Am. J. Obstet. Gynec. 124272-280.

36. J.D. Townsley, W.H. Dubin, G.F. Grannis, L.J. Gartman & C.D. Crystle (1973). Circadian rythmns of serum and urinary estrogens in pregnancy. J. Clin. Endocrinol. Metab. 36 289-295.

37. M. Levitz, A.J. Slyper & M. Selinger (1974). On the lack of periodicity in plasma estriol in human pregnancy. J. Clin Endocrinol. Metab. 38 698-700.

38. O.W. Smith (1966). Free and conjugated estrogens in blood and urine before and during porturition in normal human pregnancy. Acta Endocr. Suppl. 104.

39. J.N. Hansen, H.J. Hoffman & G.T. Ross. Monthly gonodotropin cycles in premenorchal girls. Science.

40. E. Diczfalusy & S. Mancuso (1969). Estrogen metabolosm in pregnancy. In: Foetus and Placenta. Eds A. Klopper & E. Diczfalusy. Pgs. 191-248. Publ. Blackwell. Oxford.

41. C. Faiman, F.I. Reyes & J.S.D. Winter (1974). Serum gonodotropin patterns during the perinatal period in man and in the chimpanzee. In: International Symposium on Sexual Endocrinology of the Perinatal Period. INSERM, 1974 INSERM, 1974 32 281-298.

42. G.N. Papanicolaou (1933). The sexual cycle in the human female as revealed by vaginal smears. Am. J. Anat. (1933) 5 519.

43. P.C.T. Chew, S.S. Ratman & J.A. Salmon (1976). Plasma estriol in normal pregnancy in an asian population. Brit. J. Obstet. Gynec. 1976 83 430-433.

44. O.A. Dada & B.K. Adodwob (1975). Total urinary estrogen excretion during normal menstrual cycle and normal pregnancy in Nigerians. Brit. J. Obstet. Gynec. (1975) 82 40-43.

45. M. Jaffe (1886). Uber den Niederschlag, welchen Pikrinsaure in normalen Horn erzeugt and uber eine neue Reaction des Kreatinins. Z. Physiol. Chem. 1886 10 391-400.

46. C. Slot (1965). Plasma creatinine determination. A new and specific Jaffe reaction method. Scand. J. Clin. Lab. Invest. 1965 17 381-387.

47. M.F. Crowley & A. Rosser (1973). Estrogen determination in pregnancy urine using enzymatic hydrolysis of estrogen conjugates. Clin. Chim. Acta. 1973 49 115-122.

48. J. Dray, F. Tillier, F. Dray & A. Ullman (1972). Etude de l'hydrolyse des metabolites urinaires de differentes hormones steroides par la B-glucuronidose de E. coli. Ann. Inst. Pasteur. 1972 123 853-857.

49. V. Graef, E. Furuya & Nishikoze (1977). Hydrolysis of steroid glucuronides with B-glucuronidose preparations from bovine liver, Helix pomatia and E. coli. Clin. Chem. 1977 23 532-535.

50. B. Seaton, J.A. Lusty & J. Watson (1976). A practical model for steroid hormone radioimmunoassays. J. Steroid. Biochem. 1976 7 511-576.

51. R.J. Begue, J. Desgnes, J.A. Gustafsson & P. Padieu (1976). Analyse modulaire des steroides urinaires pendant la gestation humaine. J.Steroid Biochem. 1976 7 211-221.

52. J.F. Van der Calseyde, R.J.H. Scholts & J.J.A. Leizten (1972). Profiling urinary steroids. A reliable procedure. Clin. Chem. Acta 1972 38 103-111.

53. P.J. Brombocher, A.H.J. Gijzan & P.E. Verheesen (1968). Simple and rapid determination of total estrogens in pregnancy urine. Clin. Chim. Acta. 1968 20 360-361.

54. J.B. Brown (1955). A chemical method for the determination of estriol, esterone and estradiol in human urine. Biochem. J. 1955 60 185-193.

55. G. Ittrich (1958). Eine neue Methode zur chemischen Bestimmung der ostrogenen Hormone im Horn. Hope-Seglers Z. Physiol, Chemic 1958 312 1-14.

56. G. Ittrich (1960). Untersuchungen uber die Extraktion des roten Kober-Farbstoffs durch organische Losungsmittel zur Ostrogen-bestimmung im Horn Acta Endocr. 1960 35 34-38.

M.D. Corns, C.M. Corns, A.L. Miller & J.F. Stevens (1973). A comparison of a manual and a simple automated technique for the estimation of total estrogens in pregnancy urine. Clin. Chem. Acta. 1973 48 335-340.

I.R. Hainsworth & P.E. Hall (1971). A simple automated method for the measurement of estrogens in the urine of pregnant women. Clin. Chim Acta 1971 35 201-208.

M. Lever & S.M. Peace (1974). Inter-laboratory variations in urinary estriol estimates. J. Obstet. Gynec. Brit. Comm. 1974 81 97-106.

In: A manual for histologic techniques (1972). A. Preece. Publ. Little, Brown & Co., Boston. pp 159-172.

In: Clinical chemistry: Principles & Techniques, 2nd Edn. 1974. Eds. R.J. Henry, D.C. Cannon & J.W. Winkelman. Harper & Row, Md.

Kit available commercially from Bio-Rad.

Perkin-Elmer. Atomic Absorption Spectrophotometer Instruction Manual.

M. Jacob, T.F. Hunt, O. Dirige & M.E. Swendseid (1976). Biochemical assessment of the nutritional status of low-income pregnant women of Mexican descent. Am. J. Clin. Nutr. 1976 29 650-656.

J.S. Bendat & A.G. Piersol (1971). In: Random data; Analysis and measurement procedures. Publ. Wiley-Intersciences.

A.P. Jeary (1976). Aquisition and analysis of time-varying data. Unpublished report of the Building Research Station.

A.R. Midgley & R.B. Jaffe (1971). Regulation of human gonodotropins: X Episodic fluctuations of LH during the menstrual cycle. J. Clin. Endocrinol. Metab. 33 962-969.

W.P. Black, J.R.T. Coutts, K.S. Dodson & L.G.S. Rao (1974). J. Obstet. Gynec. Brit. Comm. 81 667. Quoted by D.T. Baird (1976) Oestrogens in Clinical practice In: Hormone assays and their clinical application (Eds J.A. Lorraine & E.T. Bell). Publ. Churchill Livingstone.

A. Klopper (1976). The choice between assays on blood or on urine. In Hormone assays and their clinical applications in clinical practice (Eds. J.A. Lorraine & E.T. Bell) Publ. Churchill Livingstone.

R.F. Heys, R.E. Oakey, J.S. Scott & S.R. Stitch (1968). Urinary estrogens in late pregnancy: Estriol excretion as a guide to impending fetal death before term. Lancet i 328-331.

K. Willman & M.O. Polkkinen (1971). reduced maternal plasma and urinary estriol during ampicillin treatment. Am. J. Obstet. Gynaec. 109 893-896.

See ref. 35.

J.B. Lawson (1974). Antenatal care. In: Obstetrics & gynaecology in the tropics and developing countries. Eds. J.B. Lawson & D.B. Stewart. Publ. English Language Book Society (Edward Arrold Ltd.).