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"Simplified Techniques of Fertility Management"

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# PROJECT I

# EVALUATION AND DEVELOPMENT OF IMPROVED PREGNANCY TESTING TECHNOLOGY

# Progress Report of AID Project I

## Evaluation and Development of Improved Pregnancy Testing Technology

This progress report covers the period from January 1 to September 30, 1978

COAL: Improved pregnancy testing for AID projects.

ACTIVITIES: toward this goal

1. Study of the appearance of hCG with simple immunoassays (cap test)

2. Study of the appearance of hCG with 3 different radioimmunoassays (RIA) for confirmation of item 1. Difficulty is that RIA is usually not done with urine.

3. Study on the disappearance of hCG with simple immunoassays (cap test) to explain positive pregnancy tests after termination of pregnancy.

4. Study on the disappearance of hCG with 3 RIA's for confirmation.

5. Analysis of the second series of capillary tests: stressing the low false positive rate.

6. Preparation of preliminary pictorial instructions to break the language barrier for the capillary test with emphasis on handling, performance, and reading the endpoint. Review by AID and then IFRP.

7. Preliminary test of the new instructions.

8. Field trial after new instructions finalized.

9. Design and construction of reading device -- electric.

10. Preliminary attempt to design portable reading device -- non-electric.

11. Continuation of storage stability studies.

INTRODUCTION

We urge a review of our past progress report for background of progress reported here, especially the original objective and justification of current activities. A two-sided copy is enclosed: EXHIBIT 1.

RESULTS OF CURRENT ACTIVITIES

1. The study of the earliest appearance of hCG by the cap test shows detection of hCG 9 days after ovulation and definitely prior to the 1st expected but missed period. It required two years to find sufficient patients presenting themselves before the missed period and with sufficient confirmatory evidence of basal temp charts, timed coitus, or artificial insemination. A full report is enclosed: EXHIBIT 2.

2, 3, and 4. The appearance of hCG by RIA and the disappearance of hCG both by cap test and by RIA are in progress. We hope to have reports on these in the form of exhibit 2 by the next report. The data may generate a mode of managing suspected retained tissue following termination of pregnancy which may help reduce medical procedures and postpartum bleedi
Several hundred pieces of data must now be coordinated for statistical analysis to yield a simple answer as in item 1.

5. The second series of the cap tube is twice as large as the first (2000 cases instead of 1000) with triple the number of negative cases to stress the false positive rate. Lengthiness of the report is now being trimmed for review by Dr. T. King before revision and final drafting. Because some of the results were unexpected and interesting, we are checking clinical outcomes for consistency and validity. This report may be ready by the next progress report.

6. The preliminary pictorial instructions have been written and some pictures have been suggested. The report is currently in the hands of AID. We understand it is then to be discussed with IFRP. A preliminary test of the instructions is then planned. Dr. Edelman of IFRP and Dr. Lau will them coordinate the changes and IFRP is supposed to generate the final graphics. Feed-back from AID is encouraged at this crucial time to make certain the instructions are understandable and will survive translation if any is needed. A copy is provided: EXHIBIT 3.

7. Preliminary test of instructions
We are ready to proceed with this in October.

8. Preparation of cap tubes and readouts for field trial.
Preliminary discussions with David Edelman have been held and the next step awaits the finalization of the instructions.

9. Since the last report a small reading device which enforces proper reading habits for the cap test has been designed, constructed, and readied for four centers. Adaptors have not been purchased because knowledge of the centers and their voltage supply is not yet available.

10. Long term storage stability studies are continuing. Stability in shipping, handling, and storage were covered in the last report and steps to overcome problems were taken.

11. Geographical differences cited in the last report are emphasized again. Please review this. We have started some urinary measurements of different substances to identify geographical differences to explain why not every pregnancy test works everywhere and to assure the optimal performance of the cap test everywhere.

DISPOSITION

1. Confirmation of the appearance of hCG by three RIA's, all new, urinary RIA's which have not been available before, will be completed.

2. The disappearance of hCG by simple immunoassays and by 3 urinary RIA's must be finished and analyzed. A discussion with Dr. Allyn Kimball, professor of statistics, is imminent.

3. The large second series with the cap test must be readied for publication.

4. Portable, non-electric devices for ease in reading the cap tube, although not absolutely necessary, are being conceived for this study to overcome the language difficulties and to make people who are familiar with older simple immunoassays handle this new test properly. We have a prototype, but no finalized model yet.

5. A trip to IFRP to conduct the preliminary trial with the new instructions and modifications of the instructions together with David Edelman is planned. Coordination and approval of all steps with AID is anticipated before final instructions are drafted. Selection of proper graphics is important.

6. Selection of centers must be done.

7. Preparation of reagents and cap tubes for the field trial can then be conducted with quality control tests for shipping, handling, and storage of the present batch.

8. Determination of how the field trial should be conducted taking advantage of the weaknesses and strengths of the preliminary trial should be made.

9. Storage stability studies will continue. Stress testing for specific geographical locations can be conducted if so indicated by choice of center. Ordinary usage at AID centers based on prior experience has been the guideline for these studies; as cited in previous reports modifications were made based on earlier experiments.

SUMMARY

The above represents a considerable amount of new data generated with practical importance for AID projects in both areas of maternal and child health and family planning. The earliest detection before the missed period of pregnancy by simple tests, rather than sophisticated RIA's makes this type of data available to the field worker as well an investigator in a metropolitan center. The use of pregnancy tests after termination of pregnancy likewise can be conducted with simple immunoassays if the new data cited above is forthcoming. The appreciation of the cap test as a new test not to be used like the old simple immunoassay should enhance its practical value as cited by Dr. Whyley of the U.K. A good valid trial of the test outside of Hopkins as is now proposed is essential to evaluating this product (cap tube test) sponsored by the AID.

H. Lorrin Lau, M.D., M.P.H.
October 1, 1978

B. Expected Developmental Problems

These problems included storage, transportation, handling, instruction, reading, and interpretation.

A subjective evaluation should proceed an objective evaluation. Subjective evaluation by people who have never seen or handled the test is important in perfecting the test not only for consumer acceptance and usage, but to increase accuracy in the gathering of hard data. It was never expected that the test would perform elsewhere as it works in a temperature - controlled laboratory in a hospital in the United States, unless modifications and adaptations are made based on feedback from field workers. So far this subjective feedback has been difficult to obtain, and objective evaluations have been attempted before the subjective feedback and modifications have been completed. Specific items are cited chronologically.

Storage and transportation experiments have resulted in a container for the capillary tubes which is an airtight glass vial with padding at both ends to prevent chipping or breakage during shipment and with a color-indicator desiccant. Desiccation has replaced refrigeration. We recommend removing the number of capillary tubes required for a day's use with immediate replacement of the top of the vial to maintain desiccation. Prolonged storage at room temperature is best achieved by storing the glass vials in a vacuum desiccator which has definite advantages over a refrigerator in terms of space and lack of need for an energy source.

Handling mistakes have been discovered. In general it has not been made clear that the new test is different from all previous old tests

EXHIBIT 1

# Progress Report of AID Project I

## Evaluation and Development of Improved Pregnancy Testing Technology

This progress report covers the period July 1 to December 31, 1977.

Currently we are in the midst of:

1. Studying the earliest appearance of HCG in pregnancy and its disappearance following the termination of pregnancy with the capillary tube pregnancy test.
2. Determining the storage stability of the test overseas.
3. Designing pictorial instructions which break the language barrier for the performance of the test and the reading of the endpoint.
4. Constructing an improved apparatus to read the endpoint.
5. Stressing the test for false positive results immediately.
6. Evaluating the capillary test in overseas field trials.

## I. Review of Project I

### A. Original Objective

The original objective was to develop a new pregnancy test to meet the needs of family planning and health services clinics for AID and which meet the requirements of simplicity, sensitivity, no refrigeration, and a storage stability of two years. A secondary objective was to devise a more sensitive, but a more rapid test, than the commercial tests which require several hours rather than minutes to perform. A simple, non-refrigerated, capillary test with a sensitivity of half a unit of HCG per milliliter with a reaction time of ten minutes was developed. Adjustments in the test and its instructions to make it suitable for overseas use and its evaluation, both subjective and then objective, are being carried out.

and must be handled differently. Lack of this knowledge has resulted in odd behavior. One field worker, for example, was observed tapping the contents of the capillary tube test onto a slide for development and reading. An overseas investigator refrigerated the test which wet the reagents and then attempted to use the test even though the color indicator indicated pink which signifies moisture and possible deterioration. A blue desiccant color, indicates integrity of the dry state and integrity of the test reagents.

A breakdown in information concerning the performance of the test may have resulted from the fact that two trained centers, Bombay and Maribor, were bumped and replaced with three untrained centers, London, Sydney, and Taiwan, for the preliminary trials. Only two trained centers, Calcutta and Singapore, were used in the preliminary trials and the preliminary results were based on data from mainly one center, Singapore.

The results from this single center showed that pink or moisture ridden tests were being used in the preliminary trial. Emphasis must be made that like all perishables, freeze-dried materials must be maintained in a dry state to prevent deterioration.

Reading problems were also anticipated and experienced. A natural tendency is to hold the capillary tube up to a strong light source for reading. This "wipes out" the endpoint and makes every test look like a positive test. Emphasis has been made in the written instructions on reading the endpoint against a black background with light striking the back of the tube obliquely. Even though we furnished written instructions to IFRP, it is not known whether these instructions were transmitted with

intactness of meaning after translation. Also, because we doubt that everyone will know how to achieve the proper requirements for reading, we are devising a foolproof reading device that will compel the user to read the test properly.

Interpretation of test results is an important item for the future. Many conditions besides pregnancy can be detected by an abnormal HCG level, which will automatically expand the benefits of the capillary test to improve medical care, such as: pregnancy associated conditions like hydatidiform mole and choriocarcinoma, and nonpregnancy associated conditions like nontrophoblastic tumors.

C. Expected Evaluation Problems

Subjective evaluation and modifications on the test, it was hoped, would be completed before an objective evaluation was begun. Despite failure to obtain subjective feedback and despite a preliminary trial in which wet capillary tubes were used, the interim data showed an overall accuracy of 84.4%, which was 3.7% higher than the Dri-dot slide test when both were used side by side. This is in contrast to an accuracy of 99.65% in an in-house study where the false positive rate was 0.07% and the false negative rate was 0.28%. Besides the difference in how the tests were performed, a difference between histopathological results is noted. The in-house study had fairly complete histopathological data, whereas the overseas interim study, had some dubious histopathological data. We have always stressed that accuracy of a pregnancy test should be determined by comparison of the test result with histopathological data rather than by contrast with another pregnancy test. This is because the greatest variable is the urines themselves, not the test reagents. An intervening

factor in a peculiar urine can cause identical errors in two pregnancy tests.

Understanding of the new capillary test, or rather the lack of understanding, led not only to the mistake in the handling already described (tapping contents onto a slide and erroneous refrigeration), but also to errors in interpretation. Several investigators considered the test wrong when it gave positive results to women who didn't show their pregnancy yet. The high sensitivity of the new test, which approaches and actually intrudes into the range of the radioimmunoassay (RIA), enables the test to detect pregnancies before the missed period. Additionally, the usefulness of the test is extended in detecting pregnancy-related conditions with low HCG levels such as ectopic pregnancy. In the initial data, the capillary tests correctly diagnosed all three cases of ectopic pregnancy.

Performance difficulties were anticipated because the grassroot worker, who actually performs the tests, does not receive the training. The chief overseas investigator usually does. The grassroot workers, not being informed of the advantages and features of the new test, will tend to handle it like all old tests. Proper instruction can remedy this and instructions with photographs might improve the problem in communication.

## II. Work In Progress

### A. Further Evaluation of the Capi lary Test Which Intentionally Stresses the Test for False Positive Results

The first 1486 cases with a false positive rate of 0.07% is the lowest false positive rate reported for any pregnancy test in the

literature. The possible reason for this was that not enough negative cases were included in the first series. Therefore, a new series was started. To obtain more negative cases, urines were collected from the Gynecological Clinic and Fertility Control Center of the Johns Hopkins Hospital. Clinical documentation of negative cases is difficult to obtain, since there is usually no histopathological results. Strict clinical criteria and definitions had to be established. The data have been entered on cards and a preliminary computer print-out is being checked for errors.

B. Improvement in Reading the Endpoint

A new reading device is being designed, but before the design can be finalized, more feedback is needed from the overseas grassroot workers on acceptability. We'd like to obtain feedback about their problems in reading a positive and negative endpoint, and receive their suggestions for improvements. Adjustments then need to be made, not only in the apparatus, but in the instructions before a large scale objective evaluation is conducted.

C. Study on the Disappearance of HCG Following Termination of Pregnancy

Many fertility control centers, both domestic and abroad, have experienced positive results in pregnancy tests following termination procedures. The interpretation of this, whether it be ectopic pregnancy, retained tissue, or normal disappearance, rests on the normal disappearance curve of HCG. Although the literature abounds in HCG disappearance curves by radioimmunoassay, no data exists on the disappearance of HCG with simple tests. An obvious but overlooked problem is that radioimmunoassays are performed on serum and not urine. By contrast, simple

tests are performed on urine and not serum. Why? The much publicized specificity of the radioimmunoassay for the beta subunit of HCG is not valid for urine; the antibody against the beta subunit does cross-react with both LH and FSH in urine. The old simple tests could not be performed with serum because the proteins displaced the hormone from the indicator particle. Therefore, the need exists to study the normal disappearance of HCG following the termination of pregnancy with simple HCG tests which are geared for urine. This will help to improve the management of post-termination cases and will clarify the differential diagnosis of normal positive post-term tests.

D. Study on the Appearance of HCG in Very Early Pregnancy

The development of new reagents for simple pregnancy tests, such as improved latex particles and covalent bonding for more stable reagents, have enabled us to devise a simple, fast, sensitive test for half a unit of HCG for milliliter. This level of sensitivity intrudes into the region of sensitivity for radioimmunoassay (RIA) and radioreceptor assay (RRA) permitting detection of pregnancy prior to the missed period, but without the use of radioactive materials. The earlier a woman confirms that she is pregnant, the earlier she can decide upon a course of medical action. The "earlier is safer" concept pertains to both fetus and mother and to the provision of better medical care. Low dose oral contraceptive users, for example, can be warned to discontinue their medication before limb reduction and other birth defects occur. Early detection of HCG is also useful in identifying cases of blighted ova and missed abortions. Early detection of HCG enforces the concept of good medical care, namely diagnosis first, then treatment.

We are studying the earliest appearance of HCG with the capillary

tube pregnancy test. Our series, using just the last menstrual period (LMP), has shown positive detection prior to the expected period. While this is the type of historical data collected and used in most clinics, it fails to satisfy the purists, who demand hard data. For the latter, we are collecting cases with either timed intercourse or artifical insemination at the Johns Hopkins Hospital and documentation of the LMP from basal temperature charts. It is extremely difficult to find women for testing prior to the time of their expected period, both inside and outside of these clinics. We have placed notices on bulletin boards and in the university hospital newspapers. It has been suggested that we expand our search to the local news media, apartment complexes, and stores to obtain more volunteers and cases.

E. Geographical Differences

An important finding for AID in our work is that not every pregnancy test works everywhere. Geographical differences do exist. The greatest variable in pregnancy testing is not the tests themselves, but the differences between urines. We have found geographical differences in Mexico and in the Far East and in the United States. The differences are greater in the available commercial pregnancy and least with the new covalent capillary tube pregnancy test. The factors causing these differences need to be studied and are being studied to a limited extent in the present project. We are interested in identifying relevant factors only to the extend that modifications can be made in the capillary tube tests to make them optimal.

F. Storage Stability

The storage stability of the capillary tubes in overseas areas are

are expected to be less than ideal compared to optimal storage in isothermal and isohumidity conditions in the Johns Hopkins laboratories. Containers of capillary tubes have been sent overseas and are being returned to Johns Hopkins via IFRP at designated intervals. This study is continuing.

Summary

Storage and transportation problems have been identified and corrected. Improved instructions, preferably with pictures, will overcome the problems encountered in translation and interpretation. An attitudinal and behaviorial change is needed to prevent handling errors; differences between the new tests and all previous older tests must be emphasized. A new reading device is being constructed to obviate problems in reading, especially the temptation to hold the tube up to a window or light and which will facilitate the correct reading of the endpoint. Further education in the interpretation of the results will extend the benefits of this test. More subjective feedback is necessary to make the test optimal. Large scale objective evaluations can then be carried out. Meanwhile, we are completing storage stability tests, are enlarging our series which evaluates the tests, are generating curves for the normal disappearance of HCG following termination of pregnancy, and are studing the earliest appearance of HCG with the capillary tests for the earliest detection of pregnancy.

H. Lorrin Lau MD MA

EXHIBIT 2

ABSTRACT FOR

EARLY DETECTION OF hCG IN URINE BY SIMPLE IMMUNOASSAYS

Data is presented to show that hCG in urine can be detected early in pregnancy by simple immunoassays performed prior to or around the time of the expected but missed menstrual period. Differences between the use of simple immunoassays with urine and radioimmunoassays and radioreceptor assays with serum are discussed.

EARLY DETECTION OF hCG IN URINE BY SIMPLE IMMUNOASSAYS

"Early"detection of human chorionic gonadotrophin (hCG) designates the time before or at the expected or missed period. This study reports the early detection of hCG in urine by simple immunoassays. Early detection of hCG in serum has been reported for radioimmunoassays (RIA) with levels of 0.03 IU per milliliter at 22 days from the last menstrual period (LMP).[1] Early detection of hCG in serum has been reported for radioreceptor assays (RRA) with levels of 0.022 IU per milliliter at 20 days from the LMP.[2]

Data from radioassays in serum cannot be extrapolated for simple immunoassays in urine for several reasons. The RIA and RRA are designed to be specific for hCG in serum, especially in the physiologic range where minimal cross-reaction with pituitary follicle stimulating and luteinizing hormones (FSH, LH) occurs. This specificity of the radioassays for hCG does not hold for urine where considerable cross-reaction occurs between the antibody to hCG and urinary LH and FSH.[3,4] Extra steps are usually necessary to prepare urine for testing by radioassays, but even this does not overcome the lack of specificity. By contrast, the simple non-radioactive immunoassays like the hemagglutination tube, latex slide, and capillary pregnancy tests are designed for urine (low protein content) and cannot be used with serum (high protein content) unless the serum is extracted. Because of these differences between urine and serum and the differences in design and intended use of the radioassays versus simple tests, no reason exists to expect that the concentrations of hCG in urine in very early pregnancies (prior to the expected period) should be the same as concentrations of hCG in serum.

Early detection of hCG in urine by simple immunoassays, therefore, requires

separate study. The difficulty in obtaining and documenting cases of early pregnancy for this type of study is evident by the paucity of reports in the literature where the menstrual, conceptional, and gestational dates are corroborated by basal temperature charts (BTC) and either timed coitus (TC) or artificial insemination (AI).

Twenty-five cases with documentation of dates by BTC and either TC or AI were studied. Two simple immunoassays were used: a capillary tube test[5] with a sensitivity of 0.5 IU of hCG per milliliter of urine and a covalent tube test[6] with a sensitivity of 1.0 IU per milliliter.

The capillary test is a simple, fast, non-refrigerated test in which the tube is used in much the same way as a microhematocrit tube. The tube is dipped into a specimen container and the urine is drawn into the tube by capillary action to a prescribed mark. As the urine ascends, it first dissolves lyophilized antibody, then resuspends latex particles which have been previously deposited within the tube. The end point of either flocculation (negative result) or lack of flocculation (positive result) can be read either after five minutes of tilting the tube back and forth to speed up the interaction between particles or after one hour of standing upright in a tray of clay identical to that used for microhematocrit determinations.

The covalent tube test is performed by adding one milliliter of urine to a tube containing antibody solution, then adding two drops of latex particles. The tubes are placed in a heating block which is provided by the manufacturer and examined at 60 and 90 minutes. Concentrations of 1.0 IU per milliliter or higher gave positive end points at both 60 and 90 minutes. Levels of

sensitivity for each test were confirmed with the United States Pharmocopeial, the Second International, and commercial standards.

Clinical or histopathological confirmation for all cases was obtained. Eighteen cases of the 25 were not pregnant. All failed to show signs or symptoms of pregnancy, resumed menstrual periods within six weeks, and continued their investigations for infertility. All yielded consistently negative pregnancy test results on urines collected daily from midcycle through the next menstrual period, and amounted to approximately 30 days of testing for each subject.

Seven cases were pregnant. Each showed a transition from negative to positive results as shown in Table I. Documentation of the menstrual history was provided by BTC in all seven cases and the conceptional history was documented by insemination by AI in case 3 and by timed coitus in the remaining six cases as shown in Table I. The day of conception was estimated from both the shift in the BTC and by the day of insemination. Two cases gave positive pregnancy test results before the expected but missed period and the other five cases gave positive results around the time of the expected but missed period. The earliest detection of hCG at 0.5 IU per milliliter occurred nine days after the estimated day of conception and 18 days from the last menstrual period (LMP). The earliest detection at 1.0 IU per milliliter occurred 12 days after conception or 21 days from the LMP.

The previously explained and anticipated difficulties (validity and specificity) in performing radioassays on urine specimens preclude the reporting of these results now.

The data show that hCG in urine can be detected prior to or at the time of the expected but missed menstrual period at a level of sensitivity of 0.5 IU per milliliter by simple immunoassays. The earliest detection of hCG in urine by the capillary tube test occurred nine days following the shift in basal body temperature and insemination which compares with the earliest detection of hCG in serum by RIA[1] and RRA[2].

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Table I

Early Detection of hCG in Urine

| Case | Days from LMP | | Days from presumed conception | |
|---|---|---|---|---|
| | Insemination by TC or AI | Shift in BTC (presumed ) (ovulation) | 1st pos result at 0.5 IU/ML | 1st pos result at 1.0 IU/ML |
| 1 | 6,10 | 9 | 9 | 12 |
| 2 | 12,16 | 15 | 13 | 19 |
| 3 | 13 | 14 | 14 | 19 |
| 4 | 13,16 | 17 | 16 | 19 |
| 5 | 13,14 | 13 | 17 | 19 |
| 6 | 9,11,12 | 11 | 21 | 26 |
| 7 | 19,20,21 | 20 | 21 | 26 |

The seven cases who were pregnant are shown. All cases were documented by basal temperature charts (BTC) and timed coitus (TC) or artificial insemination (AI, case 3 only). The day of conception was estimated by both a shift in the basal body temperature and by the dates of TC or AI. The earliest detection of 0.5 IU/ML occurred 9 days after conception or 18 days from the last menstrual period (LMP).

EXHIBIT 3

# Instructions to Perform the Capillary Tube Pregnancy Test

## I. Introduction

The capillary tube test is simpler and more sensitive than other pregnancy tests. Because it is entirely different from other pregnancy tests it must not be used or read like other immunoassays for human chorionic gonadotrophin (hCG). It's sensitivity of 0.5 IU of hCG per milliliter of urine or less brings it into the sensitivity range of the radioimmunoassay (RIA) and radioreceptor assay (RRA) so that special care must be taken to avoid contamination.

Desiccation has replaced refrigeration. Therefore, this test must not be refrigerated but should be kept dry at room temperature in a tightly closed and dry container.

The capillary tubes are designed to be used once only and discarded. They must not be refilled a second time. And they should not be emptied onto a slide or a larger tube to be read like older pregnancy tests.

Used and read properly the capillary test can detect the small amounts of hCG in very early pregnancy, in ectopic pregnancy, and in threatened abortion.

## II. Before Opening the Glass Vial:

Record the color of the desiccant within the vial.

If it is blue (contents are dry), proceed according to instructions below.

If it is pink (contents are moist), do not use the tubes and return them.

If it is partly blue and partly pink (partly moist contents), read Section V on "Moisture and Fragmentation".

III. Collection and Preparation of Urine

A. Collect urine in clean containers.

If testing is not performed immediately, cap the container with a lid which is taped to the container to avoid mix-ups. The same piece of tape can be used to label the specimen with the patient's name and date (Figure 1A).

B. Testing of fresh urines is best.

If more than one hour will elapse before testing, place the specimens in a refrigerator. If a refrigerator is not available, add one drop of a sodium azide solution (36.73gm sodium azide in 1L distilled water) to approximately each 45 milliliter of urine.

C. Before testing mix all urines by gentle swirling, not shaking, since the formation of bubbles in the urine will destroy the hormone.

Allow heavy sediment (if any) to settle for ten minutes before taking a sample from the upper portion of the specimen (Figure 1B).

IV. Testing

A. Count the number of urine samples to be tested and remove the same number of capillary tubes from the glass vial all at once to keep the rest of the vial dry.

Immediately recap the vial tightly.

Place the desired number of capillary tubes on a clean napkin or in a clean container and set aside to prevent contamination from any spilled urine.

B. Hold a capillary tube horizontally between the thumb and middle finger and insert the "red end" into a container of urine which has been tilted as far as possible without spilling (Figure 2A).

Allow the urine to ascend by capillary action until it touches exactly

the front edge of the red mark. Immediately close the opposite end of the tube with the index finger to prevent any more urine from entering the tube (Figure 2B).

With the index finger still held tightly over the end, remove the tube from the container and wipe the outside of the capillary tube with a clean napkin.

C. Reaction with Antibody

Tilt the tube to move the urine into the region between the red and black mark to dissolve the antibody. Do not go beyond the black mark!

Tilt the tube back - and - forth to move the urine between the red end and the black mark at least ten times to dissolve the antibody (Figure 2C). Do not cross the black line!

D. Reaction with Latex Particles

Tilt the tube further to move the urine across the black line into the second half of the tube containing the white latex particles. To suspend all the latex particles, tilt the tube to move the urine back - and - forth at least ten times over the latex particles (Figure 2D).

E. Sealing the Ends of the Tube

Holding the capillary tube between thumb and middle finger, tilt the tube so the urine is between the red and black marks. Tightly close the end opposite to the red mark with the index finger and insert the red end into clay to seal it. This method is similar to the sealing of microhematocrit tubes. Remove the index finger from the open end and insert this end into the clay to seal it.

F. Stand the capillary tube in the tray of clay in the reading device which has been numbered to identify the tubes.

G. Incubation

Allow the tubes to stand in the reading device for one hour at room

temperature before reading the end point. Make a confirmatory reading at two hours.

If the temperature is above $90^{0}F$ ($32^{o}C$) or below $70^{o}F$ ($21^{o}C$), record the temperature of the room.

H. Reading the End Point

1. A negative end point shows clumping, agglutination, or flocculation of the latex particles leaving clear areas of liquid between the clumps or floccules (Figure 3A). A negative end point may be seen in thirty minutes, but for accuracy, the official reading should not be taken until one hour.

   After one hour the clumps or floccules settle to the bottom leaving a region of clear liquid above (Figure 3B).

2. A positive end point shows a homogeneous white mixture without any clear areas of liquid (Figure 3A). Since all tests begin with a positive appearance, a positive end point must <u>never</u> be officially read before one full hour. Even after one hour the reader must be certain a definite positive end point exists before it is recorded.

   After one hour a truly positive end point will remain as a homogeneous white suspension. For this reason, confirmation of a positive end point can and should be made at two hours (Figure 3B).

I. Special Precautions

1. Never hold the tube up against a strong direct light for reading because the scattering of the light rays will give the false impression of a positive end point.

2. Always hold the tube against a dark background with an indirect light source striking the capillary tube from the back or side (Figure 3C). The reading device is designed to give these optimal conditions and

should be used when available.

3. Check the voltage of your electrical supply and use a voltage converter to adapt the reading device (110 volts) to your electrical outlet.

4. Set-up or request a reading device which uses a mirror where a source of electricity is not available.

V. Moisture and Fragmentation

A. The antibody and latex are originally layered evenly around the inside of the tube.

When moisture wets these chemicals an uneven layering results in a "lump" in the tube (Figure 4A).

B. The "lump" causes the urine column to break into fragments which spoils (ruins) the test.

C. To avoid fragmentation, two things can be done:

1. Drying

The tubes can be dried by placing the glass vial in either a large desiccator containing a large amount of drying agent (silica gel or $SiO_2$) or a vacuum jar or a vacuum desiccator until the color of the desiccant in the glass vial changes from pink (indicating "wet") to blue (indicating "dry").

2. Twirling

While the urine is moved up and down the tube by a back - and - forth or to - and - fro motion (Figure 4B), the tube can also be twirled between the fingers as shown in Figure 4C. This coats the liquid evenly inside the tube and prevents fragmentation. This also keeps the front edge of the column of the urine straight as shown in Figure 4D (desirable) and prevents the front edge from becoming distorted as shown in Figure 4E (undesirable).

3. If fragmentation does occur, tapping the tube gently sometimes reunites the urine fragments, but most of the time it is easier to throw away the tube and to begin again.

VI. Dubious Results

Dubious results are rare.

A. Perform a test to check for proteinuria.

B. Centrifuge or filter urines with heavy sediment and use the clear supernatant for testing.

C. Ask the patient about drug intake.

D. Save and send the urine for special analysis.

VII. Special Cases

Ectopic pregnancy, threatened abortion, or early detection of pregnancy (before the first expected or missed period) can be detected with the capillary test.

The end point at one hour may be positive and may change to a negative end point by two hours. This indicates the possible presence of less than half a unit of hCG per milliliter of urine.

# ILLUSTRATIONS

Figure 1A
Container with taped lid

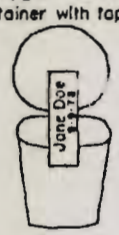



Figure 1B
Avoiding Sediment

Figure 2A
Insertion of Tube

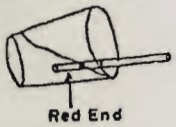



Figure 2B
Index Finger Closing the Opposite End of the Tube

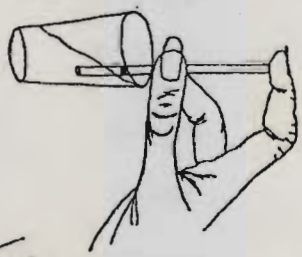


Figure 2C
Tilting Procedure – Antibody Region

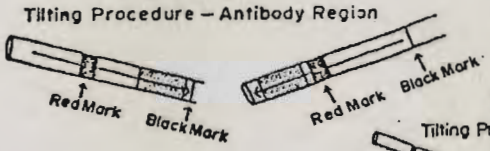



Figure 2D
Tilting Procedure – Latex Region

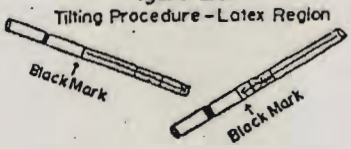



Figure 3A
At One Hour

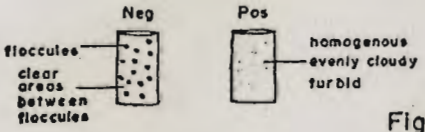



Figure 3B
After One Hour

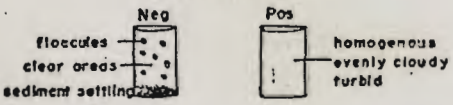



Figure 3C
Viewing Method

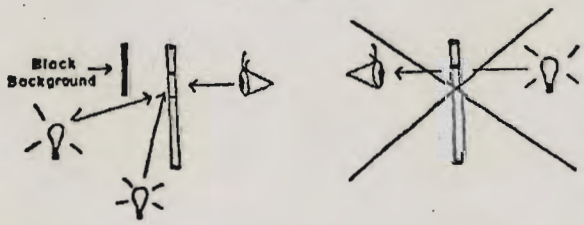



Figure 4A
Moisture Inside Tube

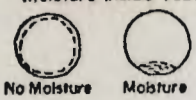



Figure 4B
Tilting

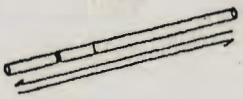


Figure 4C
Twirling

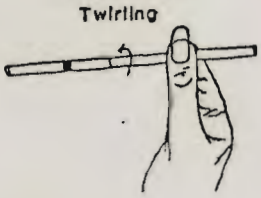


Figure 4D
Straight Edge

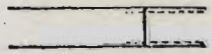
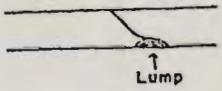


Figure 4E
Distorted Edge



# FURTHER EXPERIENCE WITH A CAPILLARY TUBE PREGNANCY TEST

by

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ABSTRACT

FURTHER EXPERIENCE WITH A CAPILLARY TUBE PREGNANCY TEST

The experience with a simple sensitive capillary tube pregnancy test requiring no refrigeration is described. The performance of the test is compared with histological and clinical documentation. To further stress the test's false positive rate, the number of confirmed negative cases have been tripled (18.6%) from the first series (5.9%). The capillary test, having a sensitivity of 0.5 I.U. per milliliter of urine, had a 99.82% agreement with histopathological and clinical results. No false positive results and a 0.18% false negative rate were obtained; these low unexpected incidences are discussed. HCG was detected in a confirmed pregnancy two weeks from the LMP, which is one week earlier than reported in the first series. The more sensitive capillary test was positive in 52 confirmed cases of pregnancy where at least one of the less sensitive commercial pregnancy tests was negative. The public health aspects of early hCG screening with a half unit test are discussed.

Further Experience with a

Capillary Tube Pregnancy Test

INTRODUCTION

The initial evaluation of a simple, sensitive, nonrefrigerated capillary tube pregnancy test with a series of 1,486 cases was reported.[1] An agreement of 99.65% between the test results and histopathological evidence was obtained with a false positive rate of 0.07% and a false negative rate of 0.28%. Because these false result rates appeared too low, another study with a greater number of cases was initiated which screened more nonpregnant women. In this manner a higher false positive rate might be obtained. The results are reported here.

METHODS AND MATERIALS

The method of performing the capillary tube pregnancy test which has a sensitivity of 0.5 I.U. per of hCG per milliliter of urine has been slightly modified. It is currently performed in the following manner. Both the tube and urine container are held as horizontally as possible. The end with the red mark is dipped into urine which ascends by capillary action to the red mark. After the outside of the tube is wiped, the tube is tilted to allow the column of urine to flow into the region containing the antibody where the urine is rocked back-and-forth ten times over the lyophilized antibody to dissolve it. The urine is not allowed to enter the latex region during this time. After dissolution of the antibody, the tube is tilted further to move the urine column into the latex region at which time the tube is again tilted back-and-forth ten times to suspend the latex particles. The

tube is sealed at both ends by inserting the ends into a tray of clay like that used for microhematocrit determinations. It is then placed vertically in a tray of clay and read at 60 and 120 minutes. The end point is viewed against a black background with a strong light source striking the tube from either the side or back (indirect lighting), but not the front. Holding the tube up against a strong light source (direct lighting) for reading is undesirable as the dispersion of light by the particles may make every end point appear positive. Although faster readings can be obtained by physically agitating the particles on a mechanical rocker for ten minutes, readings at one hour have been found to be clinically practical by permitting the health personnel to set up the test, obtain an adequate history and physical examination, then read the end point of the test. A negative end point of large floccules in a clear solution can be obtained as soon as 25 minutes, but a positive end point of a milky white homogeneous suspension should not be recorded as such until the end of 60 minutes. The test begins with "positive" end point and sufficient time must be permitted for flocculation to occur. Unlike some other immunoassays, the capillary test is not affected by vibration.

The commercially available tests used for comparison and to be described were performed in strict accordance to the manufacturer's instructions.

The sensitivity of both the capillary tube and commercially available tests were confirmed by titration against the United States Pharmacopeial, the Second International Standard Preparation, and commercial standards. Positive and negative controls were run with each batch.

Histopathological results from each case were obtained from the gynecological pathology division of the Johns Hopkins Hospital.

Urine specimens from 2,118 patients were tested: 1,668 patients were from the Fertility Control Center (FCC) from December 16, 1975, through

July 5, 1977, and 450 patients were from the Gynecological Clinic (Gyn) of the Johns Hopkins Hospital from August 13, 1976 through October 31, 1977. When a pregnancy test was required or requested by these clinics, a covalent slide test[2] with a sensitivity of 2.0 I.U. of hCG per milliliter of urine was performed in the clinic. A hemoagglutination tube test[3] with a sensitivity of 0.9 I.U. of hCG per milliliter of urine was also performed in the Gyn Clinic. The urine samples were transferred to a research laboratory and tested with the capillary test within two hours after they were obtained.

A case was categorized as pregnant if the histopathological examination showed decidua and villi and if a fetus and placenta were present. A case with a histopathological report of decidual reaction, Arias-Stella reaction, or "decidua only, no villi" was designated as positive for pregnancy only if it was accompanied by a final diagnosis of incomplete abortion, missed abortion, or ectopic pregnancy. A case was designated as nonpregnant if the histopathological results showed secretory or proliferative endometrium and if two consecutive menstrual periods occurred commencing within six weeks from the last menstrual period (LMP) and if a confirmatory pelvic examination was obtained within six weeks from the LMP.

Cases were eliminated if they lacked any one of the following: histopathological results, medical records, clinical confirmation of outcome with dates, or pelvic examination.

Data was entered on cards, verified for errors, and analyzed by computer. Study numbers were assigned to urine specimens rather than patients as some patients had two or more pregnancy tests.

At no time during the testing did the technician know the results of the histopathology, the history, or the pelvic examination.

RESULTS

A total number of 2,209 tests were performed: 1,721 from the FCC and 488 from the Gyn Clinic. Eliminated for the reasons described were 36 tests, one from the FCC and 35 from the Gyn Clinic. The number of tests with confirmatory evidence was 2,173: 2,162 tests were performed during pregnancy or in the nonpregnant state (Table I) and 11 tests were performed after termination of pregnancy.

The total number of tests which were associated with clinical and histopathological confirmation of pregnancy was 1,759 or 81.4% (Table I, categories 1 - 11). The total number of tests associated with clinical and histopathological evidence of the absence of pregnancy was 403 or 18.6% (Table I, categories 12 - 15). By comparison the first study with the capillary tube pregnancy test[1] included 1,486 confirmed positive tests, or 94.1%, and 88 confirmed negative tests, or 5.9%, showing that the number of negative cases have been tripled in the present study.

Table II a shows that for the capillary tube pregnancy test the overall agreement with the "truth" (histopathological and clinical evidence of pregnancy) was 99.82%, the false positive rate was 0 and the false negative rate was 0.18%. For the tube test (Table II b) with a sensitivity of 0.9 I.U. of hCG per milliliter the overall agreement was 95.98%, the false positive rate was 1.42%, and the false negative rate was 2.60%. For the slide test (Table II c) having a sensitivity of 2.0 I.U. of hCG per milliliter the overall agreement was 97.41%, the false positive rate was 0, and the false negative rate was 2.59%.

A break down of the differences between the tests is provided in Table I. The more sensitive capillary test was positive in 52 confirmed cases of pregnancy or 2.4%, where at least one of the less sensitive tests was negative (Table I, categories 5 - 8, 10, 11). One case in category 10 and two cases

in category 11 were less than 42 days from the LMP where one would expect a more sensitive test to be positive and a less sensitive test to be negative. Category 9 shows four cases which were less than 32 days from the LMP where all pregnancy is improbable or impossible.

The only false positive results were the six encountered with the commercial tube test shown in category 15.

Special attention was paid to early pregnancy testing which is defined as less than 42 days from the LMP. Table III shows these early cases (one test per case) which comprise 10.9% of the total with 8.69% confirmed negatives and 2.22% confirmed positives. The earliest the capillary test detected hCG in a confirmed pregnancy was two weeks from the LMP, which is presumably 10 - 14 days prior to the next expected period and which is one week earlier than reported in the first series.[1] Table III shows 19 cases with a positive capillary test result at two, four, and five weeks from the LMP.

Of public health interest is the confirmation by this second study that teenagers from 15 - 19 years of age receive the most tests, 38.7% as shown in Table IV. Not only did a tenth of this population require pregnancy tests prior to 42 days from the LMP, but 6.1% of the tests were requested less than 28 days from the LMP as shown in Table V.

Ten cases were studied after termination of pregnancy. Four cases were tested within eight days after termination with positive results whereas the other six cases ranging from two to 70 days gave negative results. All three tests were negative for the case with fetal death in utero which was studied two days after termination.

Four cases of ectopic pregnancy were seen and all four had positive capillary test results. In three of these cases the other less sensitive pregnancy tests gave negative results. Also in this series were four cases of twins and one case of hydatidiform mole all of whom had positive test results.

DISCUSSION

Pregnancy testing has become an important public health measure.[4] In planning services it is important to note that 42% of the population requiring pregnancy tests are teenagers, with the largest being the 38.7% in the age group 15 - 19 years. Thus, teenagers, even the 12 - 14 year old age group, should not be denied pregnancy testing because of their age. Also, 10.9% of this population requested pregnancy testing prior to 42 days from the LMP and 6.1% requested pregnancy testing prior to 28 days from the LMP. Therefore, early pregnancy testing should not be denied to persons seeking it prior to their first expected and missed period. This signals the need for family planning, public health, and neighborhood health clinics to provide early pregnancy testing, which indicates the need for simple immunoassays with a sensitivity at least of 0.5 I.U. per milliliter.

The capillary tube test was developed as a simple, sensitive test requiring no refrigeration to meet public health needs. The first study[1] showed the initial requirements of the capillary test had been satisfied; however, too few negative cases were included. To determine whether the false positive rate might be higher the number of confirmed negative cases was tripled from 5.9% to 18.6%. Instead of the number of false positive results increasing from the 0.07% found in the initial study, no false positive results were experienced in this study with the capillary test. The false negative rate fell from 0.28% to 0.18% in the two studies. This improvement in the rate of false results was unexpected. Even though the technicians did not know the results of the histopathology, history, or pelvic examination during the testing procedure, they did gain increased proficiency with the capillary test. Improvements in making the capillary test unit and in reading the end point took place between the two studies. More caution was taken in reading a positive end point because every capillary

test starts off with a positive end point (lack of flocculation) which must change to a negative end point (development of flocculation) in the absence of hCG. Because false positive results are less desirable than false negative results, our practice has been to record a dubious end point as dubious rather than negative or positive. With the capillary test dubious results are rare compared to the commercial tube test (Table I).

A larger false negative rate was expected for the less sensitive commercial pregnancy tests (2.60% and 2.59%, Tables II b and IIc) than for the none sensitive (0.18%, Table IIa). Of the 14 pregnancies studied at 33 days or less from the LMP (Table III) only four were missed by the capillary test and one of these was only five days from the LMP when pregnancy is impossible or improbable.

The simple immunoassays are viewed as complementary to the none sophisticated assays, the radioimmunoassay (RIA)[5-9] and the radio receptor-assay (RRA),[10,11] rather than as opposing measures. Not all neighborhood health centers or family planning clinics have access to centralized facilities which provide a service for RIA or RRA. Simple immunoassays are more widely distributed and can be used to screen for high risk cases which require finer titration by RIA and RRA. Also, the RIA and RRA require serum which is not readily obtainable in many clinics and developing countries for the purpose of pregnancy testing, whereas a urine specimen is more generally available for screening by simple immunoassays. The early detection of hCG to screen for pregnancy is by no means limited to the RIA[5-9] or the RRA[10,11] The capillary tube pregnancy test[1,12] and bioassay[13-16] have all been reported to detect hCG before the first missed or expected period. Requirements for testing and choice of type of test can be expected to vary according to specific needs and geographical location. The capillary test was designed as a simple, non-refrigerated test to meet both general needs and a specific need in early pregnancy testing for population studies. Further

study of the performance and role of the capillary test and other types of tests in both developed and developing countries would be valuable in planning population services.

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Table 1

Categories of Pregnancy

Test Results by Pathology Results

| Category Number | Pathology | Pregnancy Tests | | | Number of Tests |
|---|---|---|---|---|---|
| | | Capillary Test 0.5 I.U./ml | Tube Test 0.9 I.U./ml | Slide Test 2.0 I.U./ml | |
| 1 | P | P | P | P | 147 |
| 2 | P | P | U | P | 1543 |
| 3 | P | P | P | U | 1 |
| 4 | P | P | U | U | 12 |
| 5 | P | P | P | N | 1 |
| 6 | P | P | N | P | 1 |
| 7 | P | P | N | N | 5 |
| 8 | P | P | U | N | 42 |
| 9 | P | N | N | N | 4 |
| 10 | Early P | P | N | N | 1 |
| 11 | Early P | P | U | N | 2 |
| 12 | N | N | N | N | 256 |
| 13 | N | N | U | N | 119 |
| 14 | N | N | U | U | 22 |
| 15 | N | N | P | N | 6 |

Total = 2,162

P = positive; Early P = positive less than 42 days from the LMP; N = negative; U = unknown: missing or dubious

Table 1

Two by Two Tables

Showing

Comparison of the Pregnancy Tests' Results

to

Pathology Results

(a)

| | | Pathology Pos. | Pathology Neg. | |
|---|---|---|---|---|
| Capillary Test 0.5 I.U./ml | Pos. | 1755 | 0 | 1755 |
| | Neg. | 4 | 403 | 407 |
| | | 1759 | 403 | 2162 |

% agreement = 99.82%
% false positive = 0%
% false negative = 0.18%

(b)

| | | Pathology Pos. | Pathology Neg. | |
|---|---|---|---|---|
| Tube Test 0.9 I.U./ml | Pos. | 149 | 6 | 155 |
| | Neg. | 11 | 256 | 267 |
| | | 160 | 262 | 422 |

% agreement = 95.98%
% false positive = 1.42%
% false negative = 2.60%

(c)

| | | Pathology Pos. | Pathology Neg. | |
|---|---|---|---|---|
| Slide Test 2.0 I.U./ml | Pos. | 1691 | 0 | 1691 |
| | Neg. | 55 | 381 | 436 |
| | | 1746 | 381 | 2127 |

% agreement = 97.41%
% false positive = 0%
% false negative = 2.59%

Table III

Number of Early Tests by Days from LMP

with Capillary Results and Pathology Results

| Days from LMP | Number of Tests | | % of Total Tests (2,162) | | Pathology | | % False Results | |
|---|---|---|---|---|---|---|---|---|
| | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. |
| 1 - 3 | 0 | 14 | 0 | 0.647 | 0 | 14 | 0 | 0 |
| 4 - 6 | 0 | 14 | 0 | 0.647 | 1 | 13 | 0 | 0.046 |
| 7 - 9 | 0 | 11 | 0 | 0.509 | 0 | 11 | 0 | 0 |
| 10 - 12 | 1 | 11 | 0.046 | 0.509 | 1 | 11 | 0 | 0 |
| 13 - 15 | 1 | 13 | 0.046 | 0.601 | 1 | 13 | 0 | 0 |
| 16 - 18 | 0 | 14 | 0 | 0.647 | 0 | 14 | 0 | 0 |
| 19 - 21 | 0 | 16 | 0 | 0.740 | 1 | 15 | 0 | 0.046 |
| 22 - 24 | 3 | 17 | 0.139 | 0.786 | 3 | 17 | 0 | 0 |
| 25 - 27 | 0 | 14 | 0 | 0.647 | 1 | 13 | 0 | 0.046 |
| 28 - 30 | 2 | 12 | 0.092 | 0.555 | 2 | 12 | 0 | 0 |
| 31 - 33 | 3 | 9 | 0.139 | 0.416 | 4 | 8 | 0 | 0.046 |
| 34 - 36 | 9 | 13 | 0.416 | 0.601 | 9 | 13 | 0 | 0 |
| 37 - 39 | 13 | 8 | 0.601 | 0.370 | 13 | 8 | 0 | 0 |
| 40 - 42 | 16 | 22 | 0.740 | 1.018 | 16 | 22 | 0 | 0 |
| Total | 48 | 188 | 2.219 | 8.693 | 52 | 184 | 0 | 0.184 |

Table IX

Ages of Patients Receiving Pregnancy

Tests in the FCC and Gyn Clinics

| Age Groups | Number of Tests Patients Requested | % |
|---|---|---|
| 12 - 14 | 79 | 3.65 |
| 15 - 19 | 836 | 38.67 |
| 20 - 24 | 680 | 31.45 |
| 25 - 29 | 306 | 14.15 |
| 30 - 34 | 147 | 6.80 |
| 35 - 39 | 79 | 3.65 |
| 40 - 44 | 25 | 1.16 |
| 45 - 49 | 9 | 0.42 |
| 66 | 1 | 0.05 |
| Total | 2,162 | 100.00 |

Table V

Number of Tests Performed

by Days from the LMP

| Days from LMP | Number of Tests |
|---|---|
| 1 - 15 | 65 |
| 16 - 30 | 76 |
| 31 - 45 | 12[illegible] |
| 46 - 60 | 28[illegible] |
| 61 - 75 | 39[illegible] |
| 76 - 90 | 37[illegible] |
| 91 - 105 | 32[illegible] |
| 106 - 120 | 20[illegible] |
| 121 - 135 | 16[illegible] |
| 136 - 150 | 7[illegible] |
| 151 - 165 | 3[illegible] |
| 166 - 180 | 5 |
| 181 - 195 | 3 |
| 195 | 2[illegible] |
| Total | 2,16[illegible] |

# PROJECT II

# EARLY PREGNANCY TERMINATION

PROJECT II: Improved Means for Termination of Pregnancy in the First Trimester

Study 1: 70% Ethanol

Our experiments on monkeys using 70% ethanol as an early abortifacient are completed. The results have been presented to the American Gynecological Society, San Diego, California, April 1, 1978 and a manuscript has been accepted for publication by the American Journal of Obstetrics and Gynecology. This manuscript is entitled "Intrauterine Ethanol-Induced Pregnancy Termination in Cynomolgus Monkeys (Macaca Fasciculus)" is included in this report.

Our experience with 70% Ethanol in both monkeys and humans has been presented at the PARFR meetings, Nassau, Bahamas, May 1978 and the proceedings of those meetings will be published. That presentation entitled "Research on New Approaches to Pregnancy Termination, Ethanol Abortifacient Activity-Animal and Human Data. A Preliminary Report" is also included in this report.

# INTRAUTERINE ETHANOL-INDUCED PREGNANCY TERMINATION IN CYNOMOLGUS MONKEYS (*Macaca fascicularis*)

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## ABSTRACT

The effectiveness of locally applied ethanol in terminating early pregnancy in cynomolgus monkeys was studied. In a randomized trial ethanol (70%, 1 ml) or saline (0.9%, 1 ml) was injected extraamniotically through a sterile blunt-end needle inserted through the cervix into the uterine cavity. Six of seven monkeys receiving ethanol showed vaginal bleeding beginning 1 or 2 days after treatment. With the one failure, alcohol leakage occurred from the luer-lock needle joint at the time of injection and the amount entering the uterus was unknown. No animal receiving saline showed any vaginal bleeding. The 6 monkeys showing vaginal bleeding were found to have non-enlarged or subnormally enlarged uteri one month after treatment. All monkeys subsequently cycled within one year. Three monkeys became pregnant and delivered healthy, full term offspring. Histological evaluation of uteri from ethanol treated monkeys reveals necrosis of decidua and, to a lesser degree, of the placenta one day after injection. The high efficacy of 70% ethanol in inducing endometrial sloughing and the documentation of normal subsequent pregnancies in 50% of treated monkeys makes this technique worthy of consideration as a menstrual induction agent in humans.

## INTRODUCTION

Despite recent advances, there persists a need for improved methods of fertility control which are effective, safe, easily administered and inexpensive. The contragestational activity of a variety of drugs has been studied using a pregnant rat model and several protein denaturants, including ethanol, were found to be highly effective when injected directly into the uterus prior to implantation.[1] The effectiveness of ethanol in these studies combined with its well described toxicity,[2] antibacterial activity,[3] low cost, and widespread availability encouraged us to test this drug for contragestational activity in a primate model. The effectiveness of locally applied ethanol in terminating pregnancy in cynomolgus monkeys is the subject of this report.

## MATERIALS AND METHODS

Cynomolgus monkeys (*Macaca fascicularis*) were obtained from Mason Research Laboratories (Worchester, Mass.) or Primate Imports (Port Washington, N.Y.). Their diet consisted of Monkey Chow (Charles River), supplemented with fresh fruit. This species was chosen because their cervices are reported to be less tortuous than other monkeys[4] and therefore were expected to be transversed more easily by a straight needle. All monkeys were individually caged except during carefully timed mating periods when a male and female were cohabitated.

### Study 1: Accuracy of Pregnancy Testing

Because subsequent studies would involve treating early pregnant animals, it was necessary to assess our ability to diagnose early pregnancy. Following repeated episodes of cyclic bleeding documented by daily vaginal swabs, females were mated with proven fertile males. There were cohabitated for 8 days beginning 12 days after the onset of vaginal bleeding. Pregnancy, if it occurred, was counted from the midpoint of the cohabitation period. Thus, cohabitation day 5

was counted as pregnancy day 1. Between 18 and 23 days after the midpoint of the cohabitation period, the mated female was subjected to rectal and abdominal bimanual palpation by an experienced monkey caretaker. At the same time, urine was collected from the bladder by urethral catheterization and tested for monkey chorionic gonadotropin (MCG) by a hemagglutination-inhibition test (Ortho Diagnostics Inc.).[5] Pregnancy was later confirmed by the presence of a fetus.

Study 2: Abortifacient Efficacy of 70% Ethanol vs Normal Saline

Mated monkeys which were designated pregnant by palpation or MCG testing were placed on protocol. At 22-42 days of pregnancy the animal was immobilized with an intramuscular injection of 4.4 mg/Kg ketamine hydrochloride (Ketaset®). A 12 cm long, 18 guage sterile blunt-end stainless steel needle was inserted through the cervix into the uterine cavity until resistance was encountered. A 1 ml solution of either 70% (volume/volume) ethanol (ethyl alcohol) or 0.9% (weight/volume) saline was injected. The treatment was randomized and the investigator performing the injection was unaware of which solution was being administered. The monkeys were returned to their cages and observed daily for vaginal bleeding. If bleeding occurred, animals were immobilized with ketamine 1 month after injection and repalpated. Blood samples were taken from a peripheral vein prior to treatment and if bleeding occurred, at the time of repalpation, for blood chemistry (sodium potassium, chloride, urea N., glucose, $CO_2$ concentration) and hematology (white and red cell count and hematocrit). Blood samples were analyzed by the Division of Clinical Laboratories of the Johns Hopkins Hospital.

Monkeys which demonstrated vaginal bleeding following 70% ethanol administration were followed for recurrence of cyclic bleeding and were subsequently remated as described previously.

Study 3: Morphology and Histology

In a separate study, following transcervical insertion of the needle as described in the previous section, one pregnant monkey was anesthetized and laparotomized in order to observe the location of the needle. This monkey was also injected with 70% ethanol containing indigo carmine dye to observe if any ethanol spilled from the fallopian tubes during injection.

Three other pregnant monkeys were injected with 1 ml of 70% ethanol and then subjected to hysterectomy and salpingo-oophorectomy under Halothane anesthesia at 1, 2, or 3 days following injection. The tissues were fixed in Davison's solution, sectioned and stained with hematoxylin and eosin for histological evaluation.

## RESULTS

Study 1: Accuracy of Pregnancy Testing

Following 63 matings, 14 monkeys became pregnant as documented by the presence of a fetus at a later date. Eleven were correctly identified as pregnant by palpation and 8 were correctly identified by urinary MCG-pregnancy testing (Table 1). There were no false positive tests by either method. Thus, for the sample studied, positive tests were 100% accurate either by palpation or by urine MCG testing. Negative tests were 94% accurate by palpation and 88% accurate by MCG testing. Overall, palpation was significantly ($p<.001$ by Chi square analysis) more accurate than the MCG testing in identifying the presence or absence of pregnancy (95% vs 70%). This difference is accounted for by the large number of inconclusive MCG tests.

Study 2: Abortifacient Efficacy of 70% Ethanol vs Normal Saline

Six of the 7 monkeys receiving ethanol showed vaginal bleeding, interpreted as evidence of endometrial sloughing, beginning one or two days after treatment. No animals receiving saline showed any vaginal bleeding. These results for the two treatment groups are significantly different ($p<.01$) by Chi square analysis.

The data are presented in Table 2. Those monkeys showing vaginal bleeding were found to have non-enlarged or subnormally enlarged uteri at the time of repalpation. Concerning the one failure following ethanol treatment, there was some spillage of ethanol from the Luer-lock needle joint of the syringe during the injection and the amount entering the uterus is unknown. This monkey delivered a healthy full-term infant.

Tables 3 and 4 present the blood chemistry and hematology data from the 70% ethanol treated group. There was no difference between blood values before and 1 month after ethanol treatment.

All of the 6 monkeys that aborted following ethanol injection have recycled. Three of these subsequently have become pregnant and delivered healthy, full-term offspring.

Study 3: Morphology and Histology

In an effort to determine the needle location at the time of injection, a needle was inserted transcervically in the manner described previously followed by laparotomy. By palpation of the uterus the needle was located laterally with the tip reaching the fundus. Following injection of 1 ml ethanol-indigo carmine dye, no fluid escaped through the oviducts. An additional 4 ml of ethanol-indigo carmine was then injected but still no fluid escaped. However, a severe hyperemic response was observed on the surface of the uterus. Pressure increased within the uterus as evidenced by 3.5 ml of the administered solution re-entering the syringe when pressure on the plunger was released. The laparotomy was completed and the monkey returned to its cage. Vaginal bleeding began the next day, but the animal appeared otherwise healthy.

Three pregnant monkeys were injected with 70% ethanol and subjected to hysterectomy and salpingo-oophorectomy 1, 2, or 3 days following injection.

When 70% ethanol was injected, decidual necrosis was observed in locations which were not underlying the placenta (Figure 1). The damage was observed at 24 hours after injection. Necrosis was observed in the fundal region, the midregion and the lower uterine segment of the endometrium. Some placental damage was also observed (Figure 2). In the second monkey, observed 2 days after ethanol injection, a cross section of the uterus revealed the presence of a hematoma adjacent to the decidua which had no overlying placental tissue (Figure 3). Damage to the decidua appeared more extensive than to placental tissue.

In the third monkey, the cervix was so tortuous as to prevent passage of the needle into the uterine cavity. In this case an acorn tipped needle was placed firmly against the cervix and ethanol injected. No back flow into the vagina was observed. A cross section of this specimen on day 3 following injection appeared similar to that of the previous monkey which had the needle fully inserted in the endometrial cavity. The ethanol had spread beneath the entire amnion as evidenced by hematoma and decidual necrosis in the fundal as well as lower and mid-uterine regions.

By microscopic examination, there was no damage of the fallopian tubes or ovaries in the animals at the times observed.

## DISCUSSION

Contraception has helped ease population growth in some countries[6] but despite these efforts there is still a high incidence of unwanted pregnancies.[7] In the United States over 1,000,000 legal abortions were performed in 1976[8] and the figure is estimated at 30-55 million worldwide.[9]

First trimester abortions are safer than those performed in the second trimester.[10] Most pregnancy tests are not reliable prior to the 6th week following the last menstrual period. With development of more sensitive and specific pregnancy tests, pregnancy may be documented as early as 3 weeks[11]

allowing for early pregnancy termination if desired. Menstrual extraction has been used in procedures between 4 and 6 weeks of gestation but the procedure requires skilled professionals.[12] The failure rate varies greatly between countries and may be related to professional training.[13] An infection rate of up to 4.8% has been reported.[14] A chemical method for menstrual induction may avoid the potential of cervical trauma and uterine perforation and should be less expensive than a surgical procedure. Intrauterine prostaglandins[15] and analogues[16] have been used successfully for menstrual induction, but because of associated pain, acceptance may be limited.

While the efficacy of ethanol in the rat was only seen when injected prior to implantation, not post-implantation,[1] this did not dissuade the investigation of the drug post-implantation in the primate. The same study in rats revealed hypertonic urea and $PGF_{2\alpha}$ to be non-effective post-implantation leaving uncertain the direct extrapolation from the rat to primate model since both those compounds are known post-implantation abortifacients in humans. Low conception rates, limited number of monkeys, and inability to test for pregnancy prior to implantation prevented us from testing the effect of ethanol that early in gestation.

Ethanol has been shown to induce midtrimester abortions in humans,[17] but was less effective than saline. For this procedure, amniotic fluid was withdrawn and replaced by ethanol similar to the procedure for saline induction.

The present study suggests that menstrual regulation might be effected by a simple intrauterine injection of ethanol. The procedure is inexpensive, and in monkeys has been demonstrated to be effective and reversible. In addition, ethanol toxicity has been extensively studied.[2] Since ethanol is used as an antibacterial agent[3] it might be expected to produce a low order of infection. Systemic ethanol has been used safely in pregnant women to delay labor.[18]

In the present study, 6 of 7 monkeys receiving 1 ml 70% ethanol by the transcervical route showed vaginal bleeding within 48 hours, the exception being one animal in which the amount of ethanol actually injected was questionable. That the abortifacient effect was due to the ethanol and not the trauma of immobilization or needle insertion was documented by the absence of vaginal bleeding in animals receiving normal saline injection. At time of injection of the test substance, the fetal placenta was well developed, bilobed, occupying the anterior and posterior uterine wall. The fetus at this time measured approximately 5 mm in crown-rump length. The cervical canal was convoluted with several blind branches. Usually, the canal was easily transversed with a straight blunt end needle. It appeared that once past the cervix, the needle took the path of least resistance and slid beneath the amnion in the lateral region of the uterus where there was no placenta and continued sliding along the endometrial surface until it encountered the fundus.

Following 70% ethanol injection, there appeared to be immediate tissue necrosis of the surface endometrium in regions that were not covered by placenta. The damage was seen in all regions of the uterus whether the injection site was at the fundal or cervical end of the uterus and indicated that the ethanol spread between the fetal membranes and the endometrium for the entire length of the endometrial cavity. There was some placental necrosis observed also but the damage did not appear to be as extensive as in the endometrium. This may be because the amnion adheres more strongly to the placenta than to the decidua, impeding the suffusion of alcohol to these sites. While vaginal bleeding continued for an average of 6 days no fetal tissue was grossly observable in the discharge. Apparently the necrotic tissue was autolysed prior to discharge.

In the animal injected with ethanol while laparotomized, no spillage was seen in the tube despite the fact that an excess amount was injected. The reason for this is unclear. However, no histological damage was noted in either the tubes or ovaries of the 3 monkeys subjected to hysterectomy and salpingo-oophorectomy.

The endometrial damage appeared to be reversible as indicated by the fact that all 6 of the ethanol-aborted monkeys recycled and 3 became pregnant within the year and subsequently delivered healthy full-term infants. This is further supported by a preliminary study in non-pregnant Rhesus monkeys where intra-uterine ethanol was injected. Histological obsrevation of the endometrium revealed repair beginning at 1 week with normal endometrium seen at 1 month following injection.

The high efficacy of 70% ethanol in monkeys in inducing endometrial sloughing and the documentation of normal subsequent pregnancies in 50% of those animals makes this technique worthy of consideration as a menstrual induction agent in humans.

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TABLE 1

ACCURACY OF PREGNANCY TESTING BY PALPATION AND HEMAGGLUTINATION IN THE CYNOMOLGUS MONKEY

| Test[a] Result | Reproductive State | BY PALPATION Number | BY PALPATION Accuracy of Test | BY MCG HEMAGGLUTINATION TEST Number | BY MCG HEMAGGLUTINATION TEST Accuracy of Test |
|---|---|---|---|---|---|
| Positive | Pregnant[b] (True +) | 11 | 100% | 8 | 100% |
| | Non-pregnant (False +) | 0 | | 0 | |
| Negative | Pregnant (False -) | 3 | 94% | 5 | 88% |
| | Non-pregnant (True -) | 49 | | 37 | |
| Inconclusive | Pregnant | 0 | | 1 | |
| | Non-pregnant | 0 | | 12 | |
| Total Matings Tested | | 63 | 95% | 63 | 70% |

[a]Pregnancy test performed between 18 and 23 days after midpoint of cohabitation period.

[b]As determined by documented presence of a fetus later in gestation.

TABLE 2

EFFECT OF ETHANOL VS. SALINE ON VAGINAL BLEEDING

| Animal No. | PREGNANCY TEST MCG | PREGNANCY TEST Palpation | Pregnancy Day[a] of Injection | Vaginal Bleeding Yes/No | Vaginal Bleeding Begin | Vaginal Bleeding Duration |
|---|---|---|---|---|---|---|
| 70% ETHANOL | | | | | | |
| 91 | - | + | 22 | Yes | 1[b] | 4 |
| 45 | ND | + | 34 | Yes | 2 | 4 |
| p75 | + | + | 31 | Yes | 1 | 7 |
| 15 | + | + | 28 | No[c] | - | - |
| p71 | + | + | 32 | Yes | 1 | 5 |
| 96 | + | + | 25 | Yes | 1 | 8 |
| p67 | + | + | 32 | Yes | 1 | 8 |
| 0.9% SALINE | | | | | | |
| 85 | - | + | 26 | No | | |
| 11 | + | + | 26 | No | | |
| 95 | + | + | 34 | No | | |
| 45 | + | + | 32 | No | | |
| 20 | + | + | 39 | No | | |
| 38 | + | + | 42 | No | | |
| 1 | + | + | 32 | No | | |

ND - not determined

[a]Counted from midpoint of cohabitation period

[b]Days after treatment.

[c]Incomplete injection. (See Text)

TABLE 3

BLOOD CHEMISTRY BEFORE AND AFTER 70% ETHANOL TREATMENT

| | Na mEq/l | K mEq/l | Cl mEq/l | Urea-N mg/dl | Glucose mg/dl | $CO_2$ mEq/l |
|---|---|---|---|---|---|---|
| Before[a] | 144.8 ±1.1 | 4.23 ±.33 | 106.8 ±.5 | 21.6 ±.5 | 53.8 ±8.7 | 24.5 ±1.7 |
| After[b] | 145.7 ±.4 | 3.92 ±.01 | 106.1 ±.6 | 21.2 ±2.6 | 43.7 ±6.4 | 24.3 ±1.0 |

n = 6 in each group.

[a]Prior to injection of ethanol.

[b]One month after ethanol injection.

TABLE 4

HEMATOLOGY BEFORE AND AFTER 70% ETHANOL

| | White Blood Cell Count x $10^3$ | Red Blood Cell Count x $10^6$ | Hematocrit % |
|---|---|---|---|
| Before[a] | 6.9 ± .7 | 5.6 ± .2 | 33.3 ± .6 |
| After[b] | 8.7 ± .8 | 5.3 ± .3 | 31.6 ± 1.2 |

n = 6 in each group.

[a]Prior to injection of ethanol.

[b]One month after ethanol injection.

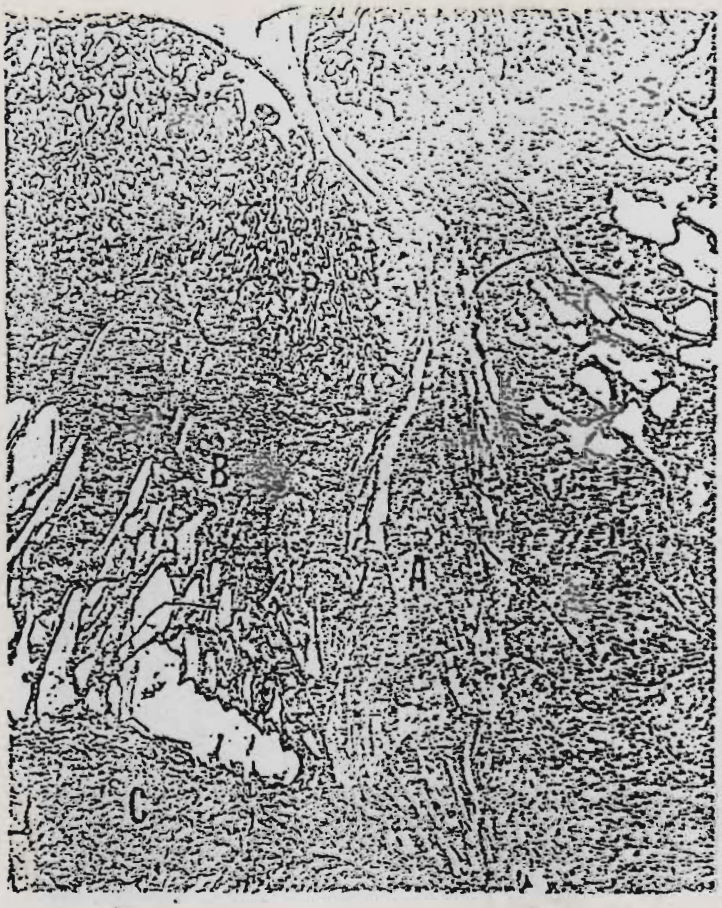


Figure 1: Placenta and uterus of cynomolgus monkey - 24 hours after extra-amniotic injection of 1 ml 70% Ethanol directly in the fundal region of the uterus (29 days pregnant) (P) two lobes of placenta. (A) Necrosis of the decidual tissue not underlying the placenta. Necrotic tissue does not take up stain and appears pale in contrast to normal tissue (B). (C) Normal myometrium (40x).

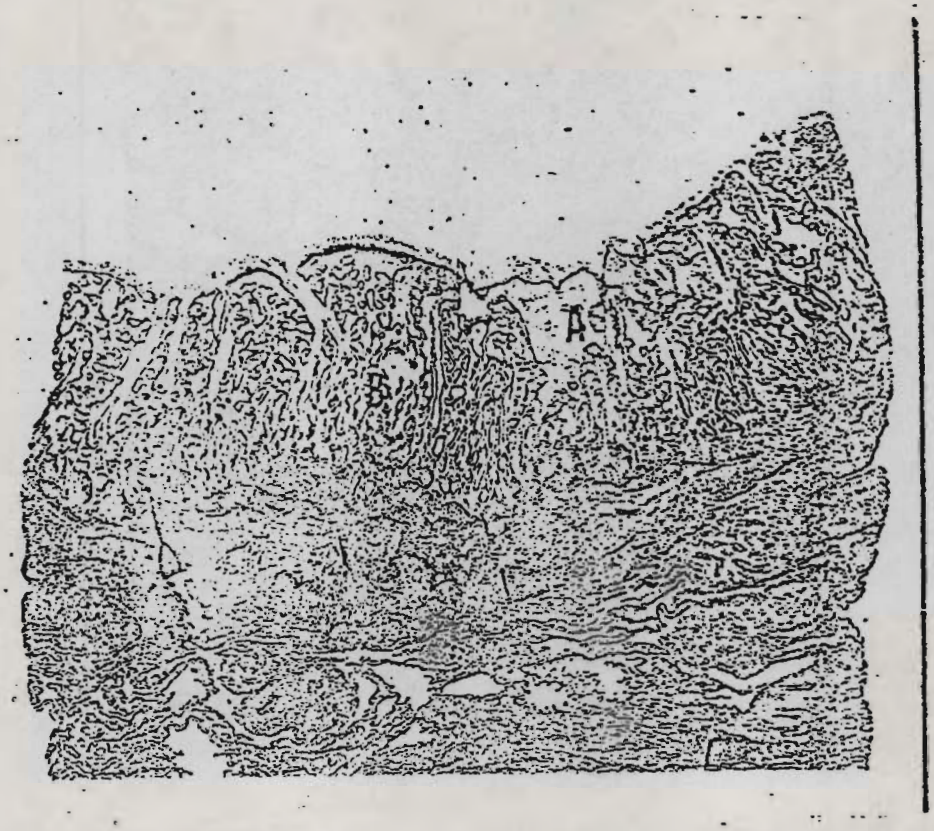


Figure 2: Placenta and uterus of same monkey as Figure 1. (A) Damaged placenta. (B) Normal placenta (40x).

Figure 3: Placenta and uterus of Cynomolgus monkey, 48 hours after extra-amniotic injection of 1 ml 70% Ethanol directly in the fundal region of the uterus (28 days pregnant). (A) Hematoma. (B) Endometrium. (C) Myometrium. (40x)

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# RESEARCH ON NEW APPROACHES TO PREGNANCY TERMINATION

## Ethanol Abortifacient Activity - Animal and Human Data. A Preliminary Report.

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Since 1976, an estimated one million legal abortions are performed yearly in the United States (1) and over 30 million per year occurred worldwide (2). In general, the earlier pregnancy termination is performed, the safer the procedure (3). Menstrual extraction has been used between 4 and 6 weeks of gestation but the procedure requires skilled professionals (4). The infection (5) and failure rate varies greatly between countries and the latter complication may be related to professional training (6). A chemical method for menstrual induction may avoid the potential of cervical trauma and uterine perforation and should be less expensive than a surgical procedure. Intrauterine prostaglandins and analogues have been used successfully for menstrual induction (7), but because of associated pain, acceptance may be limited.

A variety of chemicals has been tested in rats as locally applied contragestational agents (8). Efficacy has been determined both preimplantation and postimplantation by application Day 3 and Day 7 of dated pregnancies. Ethanol, a protein denaturant, was a highly effective contragestational agent in rats at 100%, 80%, 70% and 63% concentrations when administered into the uterus preimplantation.

The present study describes the effectiveness of locally applied ethanol in terminating pregnancy in cynomolgus monkeys. In addition, initial results of FDA approved Phase I studies in humans are presented.

## MATERIALS AND METHODS

### Monkey Studies

Cynomolgus monkeys (*Macaca fascicularis*) were obtained from Mason Research Laboratories (Worchester, Mass.) or Primate Imports (Port Washington, N.Y.).

Their diet consisted of Monkey Chow (Charles River), supplemented with fresh fruit. All monkeys were individually caged except during carefully timed mating periods when a male and female were cohabitated. Following repeated episodes of cyclic bleeding documented by daily vaginal swabs, females were cohabitated with proven males for 8 days beginning 12 days after the onset of vaginal bleeding. Pregnancy, if it occurred, was counted from the midpoint of the cohabitation period. Between pregnancy day 18 and 23, the mated female was subjected to rectal and abdominal bimanual palpation by an experienced monkey caretaker. At the same time, urine was collected from the bladder by urethral catheterization and tested for monkey chorionic gonadotropin (MCG) by a hemagglutination-inhibition test (Ortho Diagnostics Inc.) (9). If tests were positive, the animal was immobilized with an intramuscular injection of 4.4 mg/Kg ketamine hydrochloride (Ketaset®) on one occasion between 22 and 42 days of pregnancy. A 12 cm long, 18 guage sterile blunt-end stainless steel needle was inserted through the cervix into the uterine cavity until resistance was encountered. A 1 ml solution of either 70% (volume/volume) ethanol (ethyl alcohol) or 0.9% (weight/volume) saline was injected. The treatment was randomized in 14 animals and the investigator performing the injection was unaware of which solution was being administered. The monkeys were returned to their cages and observed daily for vaginal bleeding. If bleeding occurred, animals were immobilized with ketamine 1 month after injection and repalpated.

Monkeys which demonstrated vaginal bleeding following 70% ethanol administration were followed for recurrence of cyclic bleeding and were subsequently remated as described previously.

Three pregnant monkeys, not in the randomized study, were injected with 1 ml of 70% ethanol and then subjected to hysterectomy and salpingo-oophorectomy under Halothane anesthesia at 1, 2, or 3 days following injection. The tissues were fixed in Davison's solution, sectioned and stained with hematoxylin and eosin for histological evaluation.

Another monkey, not in the randomized study, received an intrauterine injection of 1 ml of 35% ethanol at 35 days of pregnancy.

Human Studies

Under an F.D.A. approved I.N.D., Phase I human studies have been initiated. Following obtainment of informed consent, 3 patients scheduled for hysterectomy for indications other than endometrial pathology were placed on protocol. A polyethylene PE90 catheter was threaded intracervically into the uterine cavity and 2 ml of 35% or 70% ethanol was injected into the endometrial cavity. Blood samples were obtained prior to and 30 minutes following ethanol injection for ethanol determination by an automated head space gas chromatographic technique and performed at the Medical Examiners Toxicological Laboratories in Baltimore, Maryland. Patients subjective reactions to injected ethanol were recorded.

Hysterectomy and salpingo-oophorectomy were performed 1 or 24 hours after ethanol injection and histological examinations were completed on the uterus, fallopian tubes and ovaries.

## RESULTS

Monkey Studies

No animal receiving 1 ml intrauterine injection of normal saline showed vaginal bleeding immediately following injection, although the outcome of these pregnancies was variable (Figure 1).

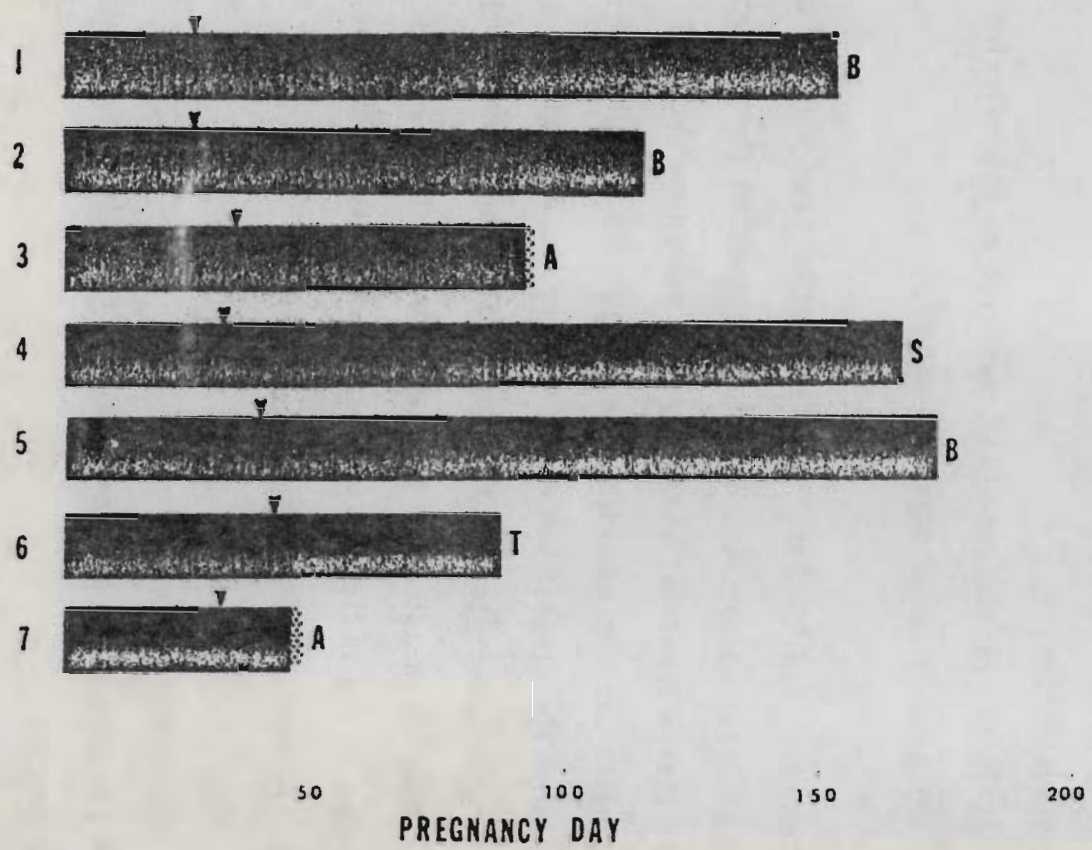


Figure 1: Pregnancy outcome following intrauterine injection of 1 ml normal saline. Arrow indicates time of injection. Stiples indicate vaginal bleeding.

B - birth of healthy infant.
A - abortion
S - stillbirth
T - maternal death; pathology report unable to confirm cause of death.

Six monkeys receiving ethanol showed vaginal bleeding, interpreted as evidence of endometrial sloughing, beginning one or two days after treatment (Figure 2). These monkeys were found to have non-enlarged or subnormally enlarged uteri at the time of repalpation. There was some spillage of ethanol from the Luer-lock needle joint of the syringe during the injection of one monkey in the ethanol group. This monkey, which delivered a normal healthy infant, was considered an ethanol failure; however, it is possible that little if any ethanol entered the uterus. Figure 3 describes the bleeding patterns during the month following ethanol administration in the ethanol-aborted monkeys. All showed an initial episode of bleeding within 1 or 2 days following ethanol administration. In some animals other episodes of bleeding occurred throughout the month. It is not possible to interpret whether the bleeding during the 3rd or 4th weeks is in response to ethanol administration or represent the reoccurrence of cyclic bleeding. If bleeding during the first month is disregarded, the latest that cyclic bleeding was reestablished was 95 days (Table 1). Four of the 6 ethanol-aborted monkeys became pregnant within 1.5 years after treatment. Three of these delivered healthy full term offspring. The other pregnancy was artificially terminated in another experiment.

In the three pregnant monkeys injected with 70% ethanol and subjected to hysterectomy and salpingo-oophorectomy 1, 2, or 3 days following injection, the following observations were made. When 70% ethanol was injected, decidual necrosis was observed in locations which were not underlying the placenta. The damage was observed at 24 hours after injection. Necrosis was observed in the fundal region, the midregion and the lower uterine segment of the

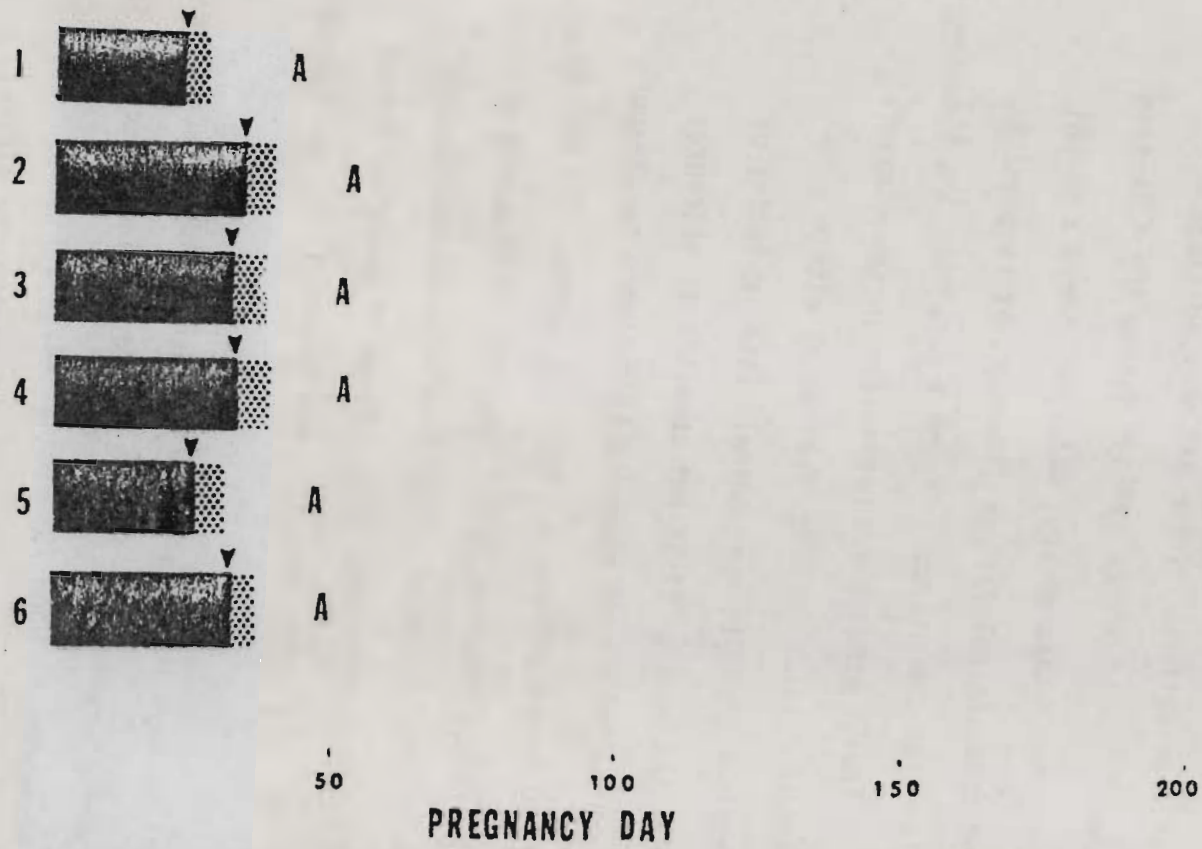


Figure 2. Pregnancy outcome following intrauterine injection of 1 ml 70% ethanol. One animal receiving unknown amount of ethanol not included in figure (see text). Arrows indicate time of injection. Stiples indicate vaginal bleeding.

| Animal No. | Week 1[a] | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|
| 1 | X X S X _ _ _ | _ S S X S S X | _ _ _ _ _ _ _ | _ _ S X _ S S |
| 2 | _ X X X S _ _ | S S _ X X S S | _ _ _ X S _ _ | _ _ _ _ _ _ _ |
| 3 | X X S S S X S | _ _ _ _ S S S | _ _ _ _ _ _ _ | _ _ _ _ _ _ _ |
| 4 | S X X S S _ _ | _ _ _ S _ _ _ | _ _ _ _ _ _ S | X X _ _ _ _ _ |
| 5 | X X X X X X X | S _ _ _ _ _ _ | _ _ _ _ _ _ _ | _ _ _ _ _ _ _ |
| 6 | X S X X X X X | S _ _ _ _ _ _ | _ _ _ _ _ _ _ | _ _ _ _ _ _ _ |

Figure 3. Vaginal bleeding during the month following 70% ethanol-induced pregnancy termination in cynomolgus monkeys.

S - blood on cotton tip following vaginal swabbing.
X - blood at bottom of cage as well as on cotton.

[a]After ethanol injection.

TABLE 1

TIME OF REOCCURRENCE OF CYCLIC BLEEDING FOLLOWING 70% ETHANOL-INDUCED PREGNANCY TERMINATION IN CYNOMOLGUS MONKEYS

| Animal No. | 15-30 days | >30 days |
|---|---|---|
| 1 | 27* | 69 |
| 2 | 18 | 49 |
| 3 | 0 | 49 |
| 4 | 21 | 95 |
| 5 | 0 | 75 |
| 6 | 0 | 74 |

*First day of vaginal bleeding following ethanol injection which might represent cyclic bleeding, expressed as days after injection.

endometrium. Some placental damage was also observed. In the second and third monkeys hysterectomized 2 and 3 days after ethanol injection, cross section of the uterus revealed the presence of a hematoma adjacent to the decidua which had no overlying placental tissue (Figure 4). Microscopic examination, revealed no damage to the fallopian tubes or ovaries in the animals at the times observed.

The monkey injected with 35% ethanol showed vaginal bleeding beginning 2 days after injection and lasting for 6 days and it was determined 1 month later that pregnancy was terminated.

Human Studies

Observations from the patient studies are summarized in Table 2. Patient 2 described a "tingling" sensation in the vagina immediately following administration of 35% ethanol. The other patients did not describe any unusual sensations. In all patients superficial necrosis of the zona compacta and to a lesser extent of the spongiosa of the endometrium was observed (Figure 5). Damage was not distributed evenly throughout the endometrium in these uteri.

Upon histological evaluation, no damage was observed in the fallopian tubes or ovaries.

Ethanol was not detected in the plasma in any of the patients following intrauterine administration of the drug.

## SUMMARY AND CONCLUSION

Following intrauterine injection of 70% ethanol, there appeared to be immediate necrosis of the superficial endometrium in regions that are not covered by placenta. The damage was seen in all regions of the uterus whether the injection site was at the fundal or cervical end of the uterus,

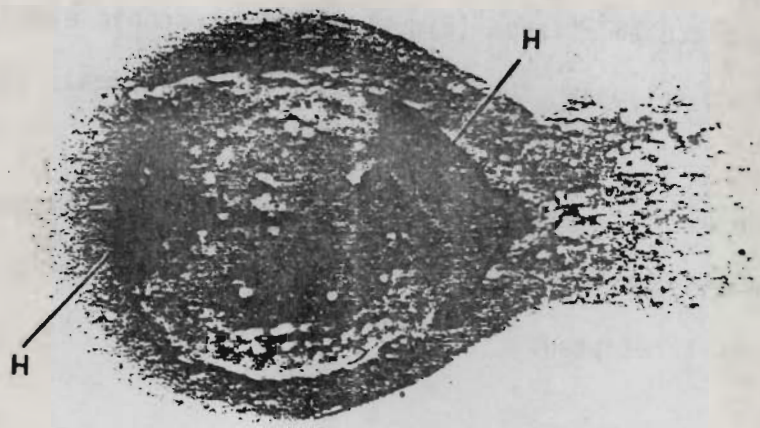


Figure 4. Mid-longitudinal section through uterus of cynomolgus monkey which received 1 ml 70% ethanol intrauterine injection on pregnancy day 28, 3 days prior to hysterectomy.

H - hematoma.

TABLE 2

NON-PREGNANT PATIENT PERCEPTION AND ENDOMETRIAL

RESPONSE FOLLOWING INTRAUTERINE ETHANOL ADMINISTRATION

| Patient | ETOH Concentration | Time to Hysterectomy | Patient Perception | Endometrial Findings |
|---|---|---|---|---|
| 1 | 35% | 1 hr. | None | Superficial Necrosis |
| 2 | 35% | 24 hrs. | Vaginal "Tingling" | Superficial Necrosis |
| 3 | 70% | 1 hr. | None | Superficial Necrosis |

Figure 5. Human endometrium demonstrating superficial tissue necrosis hours after intrauterine ethanol injection.

indicating that the ethanol spreads between the fetal membranes and the endometrium for the entire length of the endometrial cavity. There was some placental necrosis observed but the damage did not appear to be as extensive as that noted in the endometrium. No histological damage was noted in either the tubes or ovaries of the 3 monkeys subjected to hysterectomy and salpingo-oophorectomy.

The endometrial damage appeared to be reversible as indicated by the fact that the 6 animals with ethanol-induced pregnancy termination showed vaginal bleeding which might be construed as cyclic bleeding by at least 95 days after injection. Furthermore, within one and a half years after ethanol injection, 4 became pregnant.

Intrauterine instillation of 2 ml of 35% or 70% ethanol appeared to be well-tolerated in non-pregnant human subjects. Subsequent histological evaluation revealed superficial necrosis of the endometrium between 1 and 24 hours after injection. Similar studies in our laboratory, in which intrauterine 70% ethanol was administered to non-pregnant rhesus monkeys revealed repair of endometrial tissue beginning at 1 week with normal endometrium seen 1 month following alcohol injection.

The present study suggests that menstrual regulation might be effected by a simple intrauterine injection of ethanol. Such an approach would be inexpensive, and in monkeys has been demonstrated to be effective and reversible. In human subjects, intrauterine injection of ethanol appears to be well-tolerated.

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Study 2: Effect of Intrauterine 1% Silver Nitrate on Pregnancy in Cynomalgus Monkeys.

Female monkeys were cohabitated with males from day 12-20 after the onset of vaginal bleeding and tested for pregnancy approximately 2 weeks later. If pregnant, the monkey received an intrauterine injection as described in detail in our previous studies with ethanol (see enclosed manuscript). The monkey received either 1 ml. normal saline or 1% silver nitrate. Treatment was randomized.

Current results are shown in Table 1. Four animals received saline. Bleeding patterns following injections were variable. One passed tissue 9 days after injection. Two others gave birth to normal infants. One other is still pregnant. Six monkeys received silver nitrate. Bleeding occurred consistantly during the week following injection. Uterine growth was subnormal upon palpation one month after injection indicating resorption. Four have recycled. One has subsequently become pregnant and delivered a normal healthy infant.

| Animal 1% Silver Nitrate | Pregnancy Day of Injection | Week 1[a] | Week 2 | Week 3 | Week 4 | Pregnancy Test 1 month after After Injection | Follow Up |
|---|---|---|---|---|---|---|---|
| 1 | 43 | XXXXXXX | ------- | ------- | -XXXX-- | - | Cycle >160d |
| 2 | 30 | XXXXS-- | ------- | ------- | ------- | - | + |
| 3 | 30 | SXSSX-- | ------- | ------- | ------- | - | Cycle >79d |
| 4 | 24 | SSXX--- | ------- | ------- | ------- | - | Cycle >112d |
| 5 | 27 | XXXS--- | ------- | ------- | ------- | - | Cycle >43d |
| 6 | 46 | -SXXXSS | ------- | ------- | ------- | - | No cycle >33d |
| 0.9% Saline | | | | | | | |
| 1 | 41 | -----XX | *<br>-X----- | ------- | ------- | - | |
| 2 | 32 | X------ | ------- | ------- | ------- | + | Baby Born |
| 3 | 37 | SS-SXXX | XXXS--- | ------- | ------- | + | Baby Born |
| 4 | 39 | ------- | ------- | ------- | ------- | + | Still Pregnant |

Figure 1. Vaginal bleeding during the month following intrauterine injection of silver nitrate 1% or 0.9% saline.

a - Week after injection

* - Tissue found at bottom of cage

S - Blood on cotton tip following vaginal swabbing

X - Blood at bottom of cage as well as on cotton

Study 3:

A problem concerning the effect of intraluminal injections of vehicle led to a detailed study of our experimental model. Interesting results have been obtained and are included in this report as an early draft of a manuscript entitled "Effect of Intrauterine Normal Sterile Saline on Implantation and Fetal Survival".

EACA

Plasminogen activators convert plasminogen to plasmin, a fibrinolytic enzyme (see figure 1). Migratory cells, such as malignant cells (Ossowski, et. al., Expl Med 137:112, 1973) and macrophages, (Unkeless, et. al., Expl Med 139:834, 1976) produce plasminogen activators. Such cells have the property of degrading connective tissue and invading this tissue. Fibrinolytic activity has been reported in cultured mouse trophoblast (Sherman, et. al, Cancer Research, 36:4208, 1976) and first appears on Day 6 and peaks on Day 9 corresponding to the time of trophoblast invasion in the mouse. Fibrinolytic activity has also been reported in the rat (Liedholm and Astedt, Int J Fertil 20:24, 1975), however, most activity was detected during the time of ovum passage through the tube with activity disappearing at the time of blastocyst attachment to the uterus.

Epsilon-amnio caproic acid (EACA) is a drug which blocks conversion of plasminogen to plasmin (i.e., fibrinolysin). Assuming that trophoblast invasiveness does depend upon fibrinolytic activity, it should be possible to block development of the blastocyst by EACA.

Based upon our study of the effect of intralumeral injections of normal saline and our previous experience with EACA, the following experiment was done. Pregnant rats received intralumeral injections on the morning of Day 6 with 10 ul volumes or normal saline or EACA in doses of 0.3, 0.6 or 3 mg. There was no effect on the number of implantations, however, the % variability was significantly decreased by EACA injection (Figure 2) and the effect appeared dose related. In addition, injecting 0.5 CU plasmin simultaneously with 3 mg EACA appeared to reverse the effect.

Figure 1: The Fibrinolytic System

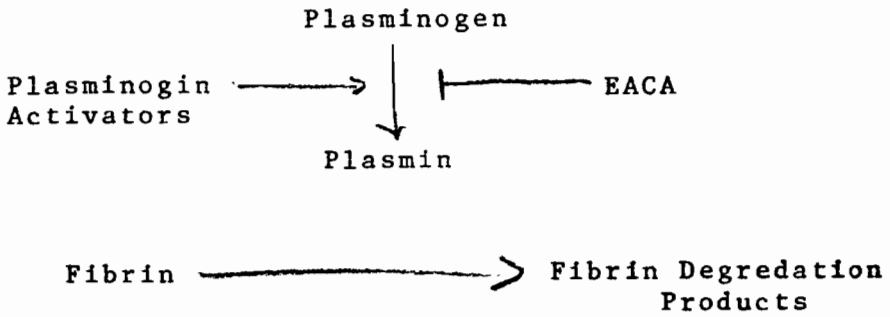
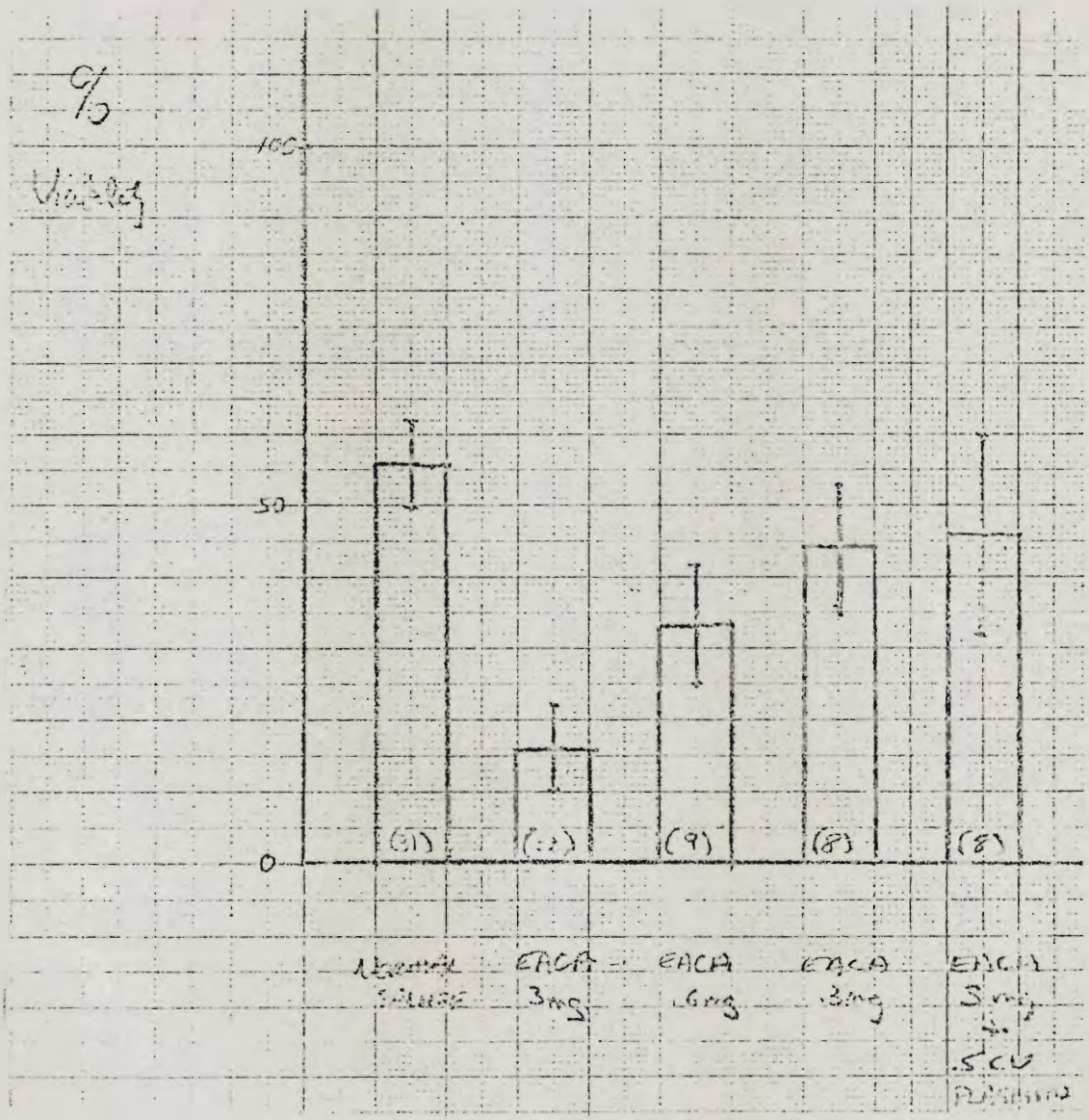


Figure 2: The Effect of Epsilon Amnio Caproic Acid (EACA) Fetal Viability in the Pregnant Rat



Study 4: Thyroid Analogs

This study was completed and reported in the 1977 Annual Report.

Study 5:

Results of initial studies in 1977 Annual Report. Because of technical problems discussed in Study 3 and low priority rating of this project, efforts were put into Study 3, which looks more promising.

# EFFECT OF INTRAUTERINE NORMAL STERILE SALINE ON IMPLANTATION AND FETAL SURVIVAL

N. H. Dubin, N. A. Barcs, T. M. King

## INTRODUCTION

The rat has long been used as a model to study physiological controls of implantation and fetal survival and the pharmacologic interference with these processes. One approach is to inject various substances directly into the uterine horn (D'Amour and Kevin, 1935, Batta and Martini, 1975, Bo et al, 1976, Conners et al, 1976). A major problem has been the variable results reported for control injections. The present report describes in a detailed manner the effect of intrauterine injection of normal saline on implantation and fetal survival. The data indicates that as little as 0.1 ml of injected saline can cause complete fetal resorption when injected on day six of pregnancy.

## MATERIALS AND METHODS

~~Sprague Dawley~~ Rats were purchased timed pregnant from Sprague Dawley, Madison, Wisconsin. The day after cohabitation was defined as Pregnancy Day 1. On various days of pregnancy, groups of rats were lightly anesthetized with ether and laparotomized abdominally under asceptic conditions. The uterine horn to be injected was grasped lightly with forceps near the cervical end and a sterile 1 cc tuberculine syringe equipped with a 25 g needle was inserted through the uterine wall into the lumen. Normal sterile saline was injected into the lumen. Light pressure was placed on the forceps during the injection and for 10 seconds after removal of the needle to prevent fluid back flow.

The abdominal wall was sutured and the skin closed with wound clips. The rat was then returned to its cage until autopsy on Day 15 unless otherwise noted. At autopsy, animals were decapitated. Corpora lutea, implantation sites and viable fetuses (based on size) were counted. Percent implantation was determined by dividing the number of implantations by the number of corpora lutea and percent viability was determined by dividing the number of viable fetuses by the number of implantation sites.

Study 1: Effect of Day of Injection.

Groups of rats were injected in one horn with 0.1 ml normal sterile saline on either Days 3, 4, 5, 6, 7, 8 or 9. These groups were compared to untreated controls. All rats were autopsied on Day 15.

Study 2: Effect of Volume.

All rats were injected on Day 6. Groups of rats received various volumes ranging from 10 to 100 µl. One group laparotomized and had the needle inserted into the uterine lumen, but received no injection and was considered as zero volume.

Study 3: Effect of Various Treatments.

The following comparisons of % implantation and % fetal viability were made for uterine horns of the following groups: Untreated control (right and left horns analyzed separately); sham (zero volume) injected; norman sterile saline (0.1 ml); distilled water (0.1 ml); normal sterile saline (0.1 ml); distilled water (0.1 ml); normal sterile saline (0.1 ml) injected from the ovarian end. Horns contralateral to those receiving 0.1 ml normal sterile saline at the cervical end.

Study 4: Histological Study.

Rats receiving intrauterine injections of 0.1 ml normal sterile saline on Day 6 were autopsied on Day 9. The uteri were removed and histological sections were made at the implantation sites. Uninjected horns were examined at the same time for comparison.

## RESULTS

Study 1: Effect of Day of Injection.

Intrauterine injection of 0.1 ml normal sterile saline on day 4 resulted in a 62.6% implantation rate compared to 81.7% for untreated controls (Figure 1). This was the only day that intrauterine injection appeared to affect the implantation rate; however, by analysis of variance, even this was not significant. Fetal viability was reduced when uteri were injected with 0.1 ml saline on Day 4 through 8 (P .001). The greatest effect was with injection on Day 6 when 4.8% were viable compared to 95.0% for untreated controls. This represented complete resorption of embryos in 27 of 28 uterine horns. Complete resorption could be effected in either 1 horn or both if both horns were injected on Day 6 (Figure 2).

Study 2: Effect of Volume.

No volume of normal sterile saline affected implantation but fetal viability was adversely affected and was a function of the volume injected (Figure 3).

Study 3: Effect of Various Treatments

In control horns, no difference in implantation or fetal viability was seen between right and left horns (Figure 4). Saline injected at the

ovarian end on Day 6 resulted in 16.6% viability compared to 4.8% in those injected at the cervical end but the difference was not significant. When saline caused resorption in the injected horn there was no contralateral effect on untreated horns. Distilled water had the same effect as saline on fetal viability. Implantation rates were the same for all the groups stated.

Study 4: Histological Studies.

Histological examination revealed well developed decidual reaction on Day 9 in both saline injected and control horns. The embryo at this time was well differentiated with embryonic and extraembryonic ectoderm, endoderm and ectoplacental cone readily identifiable. In the saline injected horn an implanted blastocyst is also apparent, however, the tissue lacked cellular organization and was infiltrated by maternal red blood cells, indicative of a resorbing embryo.

## DISCUSSION

A useful postcoital contraceptive might be a substance which may safely be administered to women and selectively toxic to the embryo (Segal and Atkinson, 1973). An early report by D'Amour and Kevin, 1935, demonstrated that physiologic saline (0.1 or 0.2 ml) injected directly into the uterus on Day 4 resulted in 6 of 9 horns having the fetuses resorbed. On day 7 only 2 or 24 had all resorbed. Our study extends these findings showing that the greatest embryotoxic effect is achieved by injection on Day 6 where 4.8% of fetuses were resorbed representing complete resorption in 27 of 28 horns.

Since intrauterine injections in laboratory animals have been used

to study physiologic (Batta and Martini, 1975, Bo et al,1976) controls of implantation and decidualization and since models also have been used to study pharmacologic agents which might inhibit implantation (D'Amour and Kevin, 1935; Conners et al, 1977) it is important to carefully define the limits of this model. It would appear that volumes greater than 0.1 ml saline could not be used in drug testing on Days 4-6 because of the high resorption rate due to the vehicle alone. However, if Day 6 were of particular interest, volumes of 10 ul or less might be used.

No effect on implantation was seen when saline was injected on Day 3, probably because the ova have not yet entered the uterus (Enders and Schlafke, 1967). The lower implantation rate observed on Day 4 following saline injection may be due to the fact that the blastocyst does not yet adhere to the uterine wall.

The implantation process of the rat begins on Day 5 when most blastocysts lose their zona pellucida and by Day 7 the trophoblast cells are in intimate contact with the uterine stroma (Enders and Schlafke, 1967). Decreased fetal viability following intraluminal saline was observed to the greatest extent on Days 4, 5, and 6. Mechanical disruption may be involved.

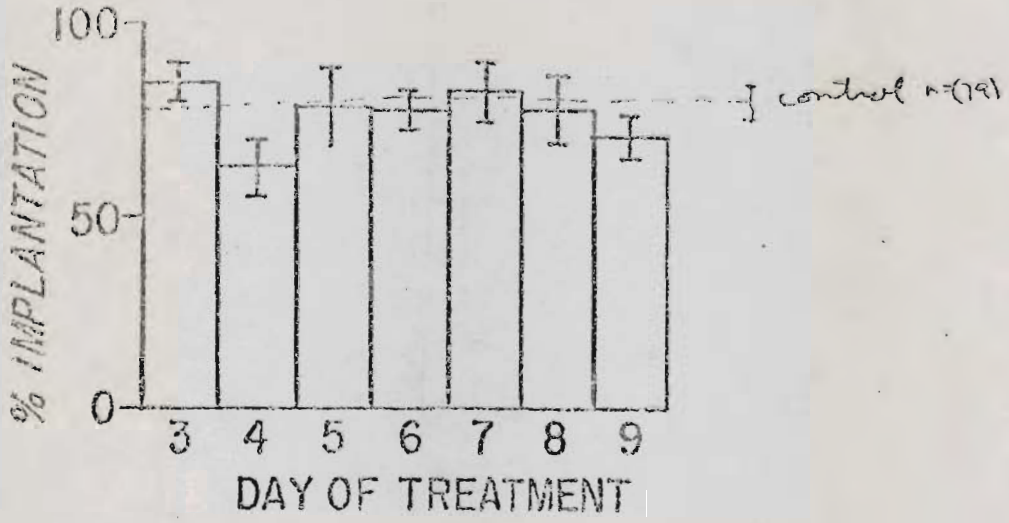
It has been suggested that closure of the uterus (clasping) on the blastocyst is a prerequisite for the interaction of blastocyst with the uterus (Hedlund, Nilsson, Reinius and Aman, 1972). Removal of uteri luminal fluid may be essential for clasping to take palce (Enders and Nelson, 1973; Parr and Parr, 1974). Possibly injection of fluid into the lumen at this crucial time interferes with the clasping phenomenon and subsequent viability of the blastocyst.

Alternatively, several substances are present in the uterine lumen which may be important in the implantation process or subsequent development of the embryo including hormones (Eiler et al, 1977; Bhatt and Bullock, 1974), hormonally-induced proteins (Pratt, Rosenfeld and Joshi, 1977), ions (Borland, 1977), immunoglobins (Wira and Sandoe, 1977); and neutrophils (Bo et al., 1976). Addition of saline may dilute an essential milieux at this crucial time. Since adherence of the blastocyst initiates the decidual reaction, it is possible that saline on Day 4-6 is washing out the blastocyst and what were interpreted as a resorption site consists only of uterine tissue. However, it was histologically demonstrated that the blastocyst is present within the decidual tissue on Day 9; however, its histological structure was disorganized and maternal blood cells were present typical of a regressing blastocyst.

Injection of saline (0.5 ml) from the ovarian end is reported not to affect fetal viability (Batta and Martini, PG, 10: 1075, 1975). In contrast, our studies indicate that 0.1 ml saline injected from the ovarian end caused almost as much fetal resorption as from the cervical end.

In conclusion, the present study illustrates some limits of the rat model regarding intrauterine injections during pregnancy. In addition, the results suggest that a "non-toxic" substance has introceptive properties in the rat. Whether a similar effect could be rendered in humans might be explored. Finally, since injection of 0.1 ml saline in both uterine horns on Day 6 usually results in complete resorption of fetal tissue, this model might be considered analogous to fetectomy and be used to study fetal control of various processes during pregnancy.

Figure 1


100
50
0
% IMPLANTATION
3 4 5 6 7 8 9
DAY OF TREATMENT
control n=(19)

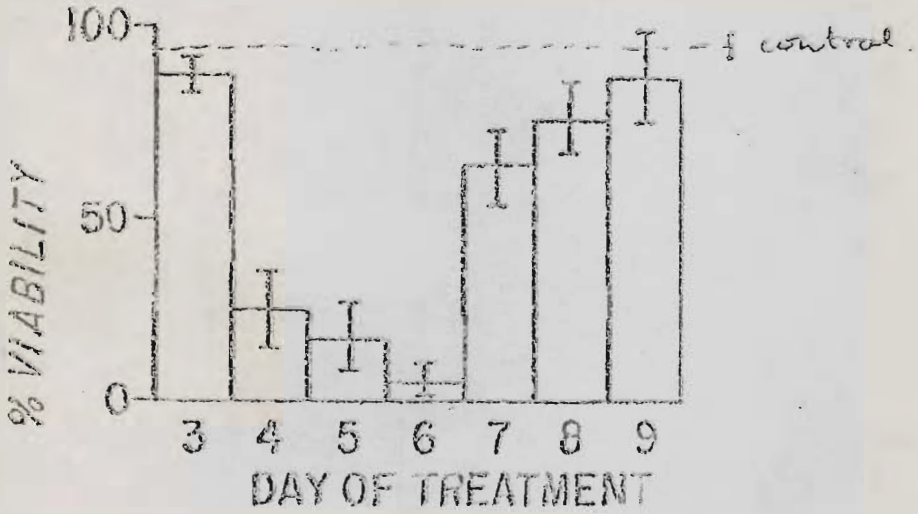

100
50
0
% VIABILITY
3 4 5 6 7 8 9
DAY OF TREATMENT
control

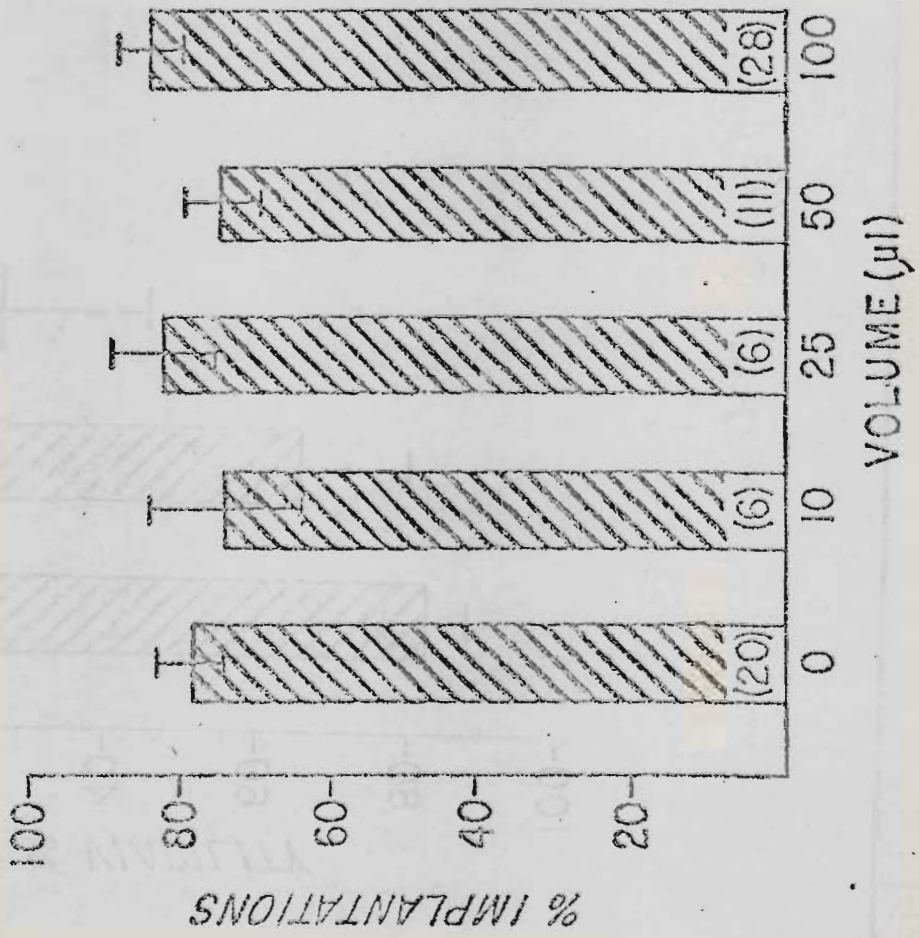
Figure 2

A. Control uterus Day 15 pregnancy

B. Right horn injected with 0.1 ml saline on Day 6

C. Both horns injected with 0.1 ml saline on Day 6

Figure 3 (A)


% IMPLANTATIONS
100
80
60
40
20
(20)
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(11)
(28)
0
10
25
50
100
VOLUME (µl)

Figure 3 (B)

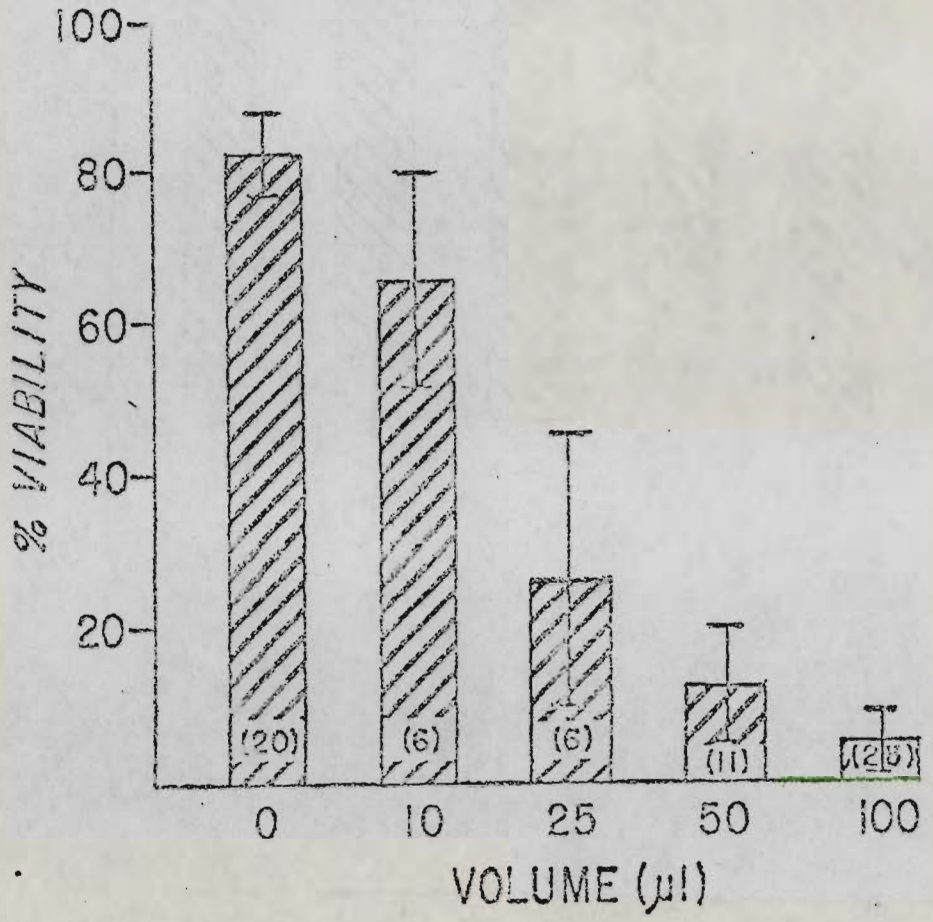
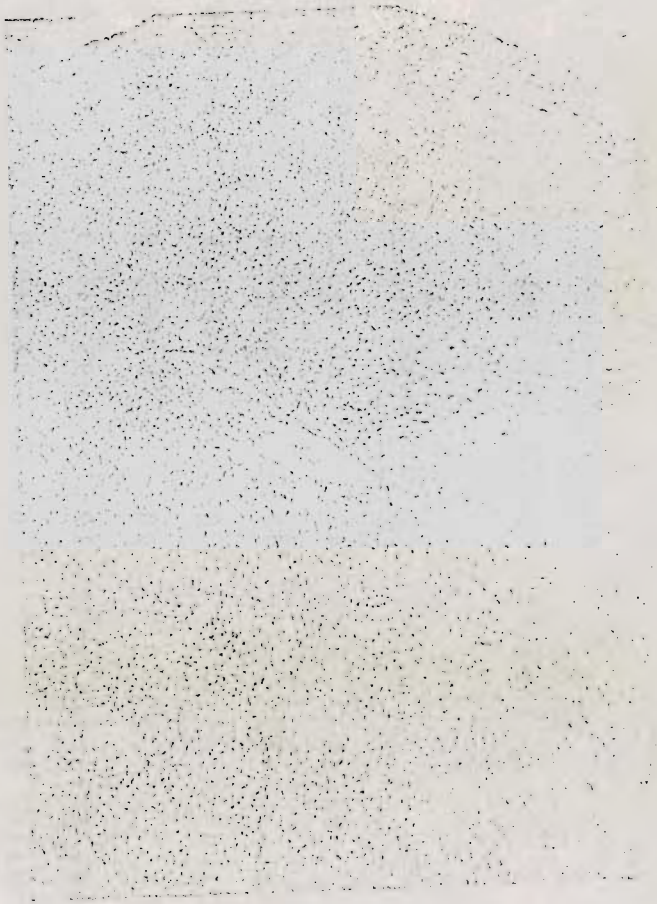


Figure 4

Control - Day 9 of pregnancy showing normal implanted Blastocyst



Saline injected on Day 6 - Section shows Day 9 of pregnancy with Blastocyst implanted, but degenerating.

Table 1

Effect of Various Treatments on

Implantation and Fetal Viability

| | n | % Implantation | % Viable |
|---|---|---|---|
| Control (Left Horn) | 42 | 80.1±5.5 | 92.7±2.1 |
| Control (Right Horn) | 42 | 85.0±2.6 | 95.4±1.7 |
| Sham Injection[a] | 8 | 78.8±7.9 | 84.6±6.3 |
| 0.1 ml Saline | 28 | 83.6±3.9 | 4.8±3.7* |
| 0.1 ml Saline (Ovarian end) | 12 | 95.5±2.3 | 16.6±6.4* |
| 0.1 ml Distilled $H_2O$ | 6 | 82.4±9.0 | 3.3±8.1* |
| Contralateral to Saline | 8 | 86.4±4.4 | 94.8±3.5 |

[a]All injections at cervical end unless otherwise noted. All injections on morning of Day 6. Autopsies on Day 15

*$P < .001$ compared to untreated controls

n - Number of uterine horns

# PROJECT III

# IMPROVED MEANS FOR TERMINATION OF PREGNANCY IN THE SECOND TRIMESTER

Project III

Study 1:

Two papers have been published this year entitled "Intra-amniotic Urea and Prostaglandin $F_2a$ for Midtrimester Abortion. Clinical and Laboratory Evaluation" and "Uterine Activity and Prostaglandin Production Following Intraamniotic Hyperosmolar Urea". Reprints are included in this report. Another paper entitled "13, 14 Dihydro 15 Keto Prostaglandin $F_2a$ Concentrations in Human Plasma and Amniotic Fluid" has been accepted for publication by the American J. Obstetrics and Gynecology and is also included.

Since the last report, a study which evaluated a reduction in the use of oxytocin augmentation in patients undergoing urea plus $PGF_2a$ (5 mg) midtrimester abortion was completed. The data from this study was presented at the American Association of Planned Parenthood Physicians in October 1977 and the paper has been accepted for publication by Advances in Planned Parenthood. The paper is included for review.

Four other manuscripts concerning clinical studies utilizing hyperosmolar urea are also enclosed. The first paper was presented at the American Association of Planned Parenthood Physicians in October 1977. This study showed that oxytocin augmentation for multiparous patients was useful, particularly if membrane rupture occurred within four hours of initial injection.

The second report details our comparison of a 1 mg prostaglandin augmentation dose verses a 5 mg dosage form with hyperosmolar urea. This study also reported on the use of laminaria tents in nulliparous patients. The major conclusions were that 1 mg prostaglandin is effective as an augmenting agent, though slightly less so than the 5 mg dosage. In addition, the study clearly shows that laminaria tents in nulliparous patients reduced injection-abortion intervals to approximately 12 hours.

The third report reviews our experience with hyperosmolar urea. This was presented at the PARFR meetings in Nassau, 1978.

Another manuscript describes placental histopathology following second trimester pregnancy termination is also included.

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# Intra-amniotic urea and prostaglandin $F_{2\alpha}$ for midtrimester abortion: Clinical and laboratory evaluation

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The clinical management of the elective midtrimester abortion continues to be unsatisfactory as judged by either national mortality or morbidity rates. This report documents the results of a randomized series of 16 midtrimester abortions induced by either intra-amniotic hyperosmolar urea and 5 mg. of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or intra-amniotic hyperosmolar urea alone. Pertinent clinical characteristics and biochemical determinations were compared between these two groups. A series of 150 patients were then treated with urea and 5 mg. of $PGF_{2\alpha}$. The clinical results of this series of patients are presented and compared with a previous group who had urea and 10 mg. of $PGF_{2\alpha}$. These studies demonstrate that 5 mg. of $PGF_{2\alpha}$ with 80 Gm. of urea achieves injection-abortion intervals that are less than 24 hours. (AM. J. OBSTET. GYNECOL. 129: 817, 1977.)

INTRA-AMNIOTICALLY administered hyperosmolar urea as a midtrimester abortifacient has been characterized in terms of efficacy and incidence of complications.[1-3] Since hyperosmolar urea has increased relative safety compared to similarly administered hypertonic sodium chloride,[4] urea is the agent of choice for patients requesting midtrimester abortion who have medical conditions contraindicating the use of intra-amniotic prostaglandin $F_{1\alpha}$ ($PGF_{2\alpha}$). Also, because of the markedly reduced likelihood of the abortion of live fetuses, hyperosmolar urea may even supersede intra-amniotic $PGF_{2\alpha}$ as the agent of choice in midtrimester pregnancies of 18 to 22 weeks' duration. The major disadvantage of hyperosmolar urea is the requirement for intravenous oxytocin or intra-amniotic $PGF_{2\alpha}$ as augmenting agents to ensure predictably short injection-abortion intervals of less than 24 hours.[1, 5]

*From the Fertility Control Clinic of The Johns Hopkins Medical Institutions, Department of Gynecology and Obstetrics.*

*Presented by invitation at the One Hundredth Annual Meeting of the American Gynecological Society, Litchfield Park, Arizona, April 13-16, 1977.*

*Reprint requests: Dr. Theodore M. King, Fertility Control Clinic, Department of Obstetrics and Gynecology, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205.*

Although clinical studies have shown efficacy when 20[2] and 10[3] mg. of intra-amniotic $PGF_{2\alpha}$ are utilized in combination with hyperosmolar urea, the minimal effective dose of $PGF_{2\alpha}$ has not been determined.

This report documents the clinical characteristics and biochemical determinations in a randomized series of midtrimester abortions induced by either intra-amniotic hyperosmolar urea and 5 mg. of $PGF_{2\alpha}$ or intra-amniotic hyperosmolar urea alone. The number of patients in this study was limited to 16, the number of patients necessary to demonstrate a statistically significant difference between the injection-abortion intervals of the two study groups.

In addition, the clinical outcome of 150 patients at midtrimester pregnancy who were subsequently treated with urea and 5 mg. of $PGF_{2\alpha}$ will be reported.

## Methods

Consenting multiparous patients seeking termination of midtrimester pregnancy participated in the randomized series. The methods of abortifacient administration have been described previously.[1-3] The following constituted the variations from our previously reported techniques: After intra-amniotic injection of 80 Gm. of urea dissolved in 135 ml. of 5 per cent dextrose and water (59.7 per cent), patients

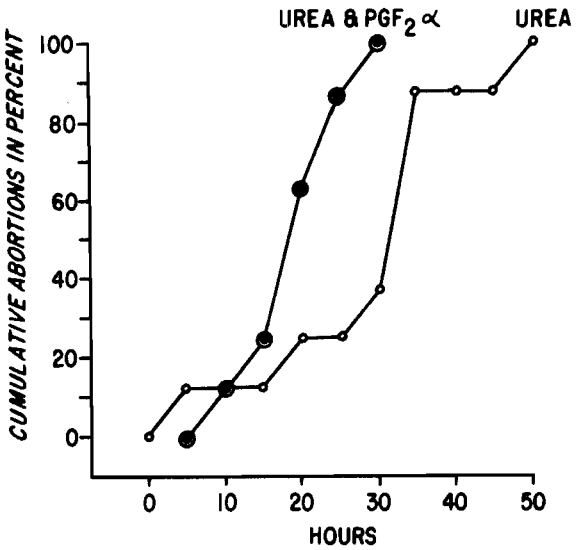


**Fig. 1.** Injection-abortion interval in hours expressed as cumulative abortions in percentages.

**Table I.** Clinical characteristics of study groups

| *Factors* | *Urea + PGF$_{2\alpha}$* | *Urea* |
|---|---|---|
| No. | 8 | 8 |
| Mean age (yr.) | 22.4 (18-29*) | 22.3 (19-30) |
| Mean parity | 2.1 (1-4) | 1.6 (1-3) |
| Mean duration of gestation (wk.) | 18.5 (16-22) | 18.1 (16-22) |
| Fluid removed at amniocentesis (ml.) | 201.3 (200-210) | 190.0 (130-230) |

*Ranges are given in parentheses.

were randomized. One group of patients received 5 mg. of intra-amniotic PGF$_{2\alpha}$ infused slowly over one to two minutes and the other group received no further intra-amniotic medications. An intra-amniotic polyethylene PE-90 catheter was inserted after injection of medication and left in place for amniotic fluid sampling as well as for intra-amniotic pressure recording. Also, a heparin lock was inserted into a peripheral vein for collection of blood samples.

For both the randomized series patients and the 150 patients treated with intra-amniotic urea and 5 mg. of PGF$_{2\alpha}$ (urea-PGF$_{2\alpha}$), intravenous oxytocin at 332 mU. per minute was employed for three indications: failure to abort within 24 hours of intra-amniotic injection for patients showing no evidence of labor, four hours after spontaneous rupture of membranes in instances of no active uterine contractions, and after incomplete abortion.

A number of clinical and biochemical parameters were determined on all patients in the randomized series. The presence or absence of fetal heart tones was documented by Doptone examination at 20 minute intervals following intra-amniotic injection. Intra-amniotic pressure recordings with a pressure transducer* and a polygraph† were obtained at least five minutes every hour for the first 10 hours following intra-amniotic injection. Also, data were collected concerning injection-abortion intervals, failed procedures, incomplete and complete abortions, and complications. Failed procedures were defined as instrumental removal of all products of conception because of failure to undergo abortion within 48 hours or because of the occurrence of hemorrhage or infection. A complete abortion was noninstrumental passage of both fetus and placenta within two hours of each other. An incomplete abortion was a failure to pass all or part of the placenta within two hours of abortion of the fetus.

Amniotic fluid samples for determination of urea nitrogen, alpha fetoprotein (AFP), PGF$_{2\alpha}$, and prostaglandin metabolites were collected at the following times: immediately prior to intra-amniotic injection and at 15 minutes and one, four, eight, 12, and 24 hours after intra-amniotic injection. Blood samples for determination of serum sodium, potassium, chloride, carbon dioxide–combining power ($CO_2$), urea, nitrogen, PGF$_{2\alpha}$, prostaglandin metabolites, and AFP were also obtained at the same time intervals.

Serum was assayed for sodium, potassium, chloride, $CO_2$, and urea nitrogen by the Division of Clinical Laboratories of The Johns Hopkins Hospital. Amniotic fluid urea nitrogen was determined with a blood urea nitrogen kit‡ after a dilution of the amniotic fluid that would give a value that was within the working range of the standard curve was determined. The serum AFP concentration was determined by a method described by Waldmann and McIntire.[6]

The immunoassay method for measuring PGF$_{2\alpha}$ and prostaglandin metabolites and the results obtained for these patients are the subject of a separate report.[7]

Statistical significance between the two groups was tested with the use of Student's t test. When timed samples were taken, two-factor (time and treatment) analysis of variance was employed and the F test was used to test the significance of main effects and detailed comparisons. A total of eight patients in each group was required in order to show a statistically significant difference in injection-abortion intervals between the two study groups in the randomized series ($P < 0.05$).

In a series of 150 nulliparous and multiparous patients who were treated with intra-amniotic urea and 5

*Statham Instruments, Inc., Oxnard, California.
†Model 260, Brush Instruments Div., Cleveland, Ohio.
‡Harleco, Philadelphia, Pennsylvania.

**Table II.** Characteristics of abortion

| | *Hours after intra-amniotic injection* | | *P value* |
|---|---|---|---|
| | *Urea + $PGF_{2\alpha}$* | *Urea* | |
| Injection-abortion interval (hr.) | 18.3 ± 2.8* (3.8-28.0†) | 28.8 ± 4.2 (6.3-31.8) | <.05 |
| Spontaneous rupture of membranes (hr.) | 7.8 ± 2.6 (2.3-24.8) | 19.3 ± 4.2 (6.3-31.8) | <.001 |
| Loss of fetal heart tone | 1.8 ± .4 (0.3-3.2) | 1.7 ± .8 (0.3-7.0) | N.S.‡ |

*Means ± standard errors.
†Ranges are given in parentheses.
‡Not significant at the 5 per cent probability level.

**Table III.** Effect of treatment of uterine activity

| | *Treatment* | *Hours after treatment* | | | |
|---|---|---|---|---|---|
| | | *0.25* | *1* | *4* | *8* |
| Tone (mm. Hg) | Urea | 5.3 ± 0.8 | 5.8 ± 0.9 | 8.6 ± 1.2 | 6.7 ± 2.4 |
| | Urea + $PGF_{2\alpha}$ | 11.4 ± 0.9* | 15.4 ± 2.2† | 16.3 ± 1.1‡ | 13.8 ± 2.8 |
| Frequency (contraction/hr.) | Urea | 0 | 0 | 26.2 ± 9.5 | 20.1 ± 7.0 |
| | Urea + $PGF_{2\alpha}$ | 0 | 87.1 ± 6.8‡ | 60.8 ± 6.1* | 62.0 ± 15.6 |
| IIUP (mm. Hg · min./min.) | Urea | 5.2 ± 1.1 | 6.0 ± 1.1 | 9.7 ± 1.8 | 8.4 ± 3.1 |
| | Urea + $PGF_{2\alpha}$ | 11.9 ± 1.0‡ | 20.2 ± 2.2‡ | 23.2 ± 1.4‡ | 21.9 ± 3.5* |

*$P < 0.05$.
†$P < 0.01$.
‡$P < 0.001$.

mg. of $PGF_{2\alpha}$, the following data were collected: injection-abortion intervals, failures, complete and incomplete abortions, and complications.

## Results

### Randomized series.

*Clinical characteristics of the randomized group.* Table I characterized the two groups of patients. There was no significant difference observed between any of the parameters.

*Characterization of abortion.* The mean injection-abortion interval, time of spontaneous rupture of the membranes, and the hour of fetal heart tone loss are given in Table II. The injection-abortion interval with its associated rupture of membranes was significantly shorter in the urea-$PGF_{2\alpha}$–treated group. The fetal heart tones in both patient groups were lost early in the procedure.

Fig. 1 displays the cumulative per cent of abortions at five-hour intervals after intra-amniotic injection for the two groups of patients. Eighty-six per cent of the urea-$PGF_{2\alpha}$ patients were aborted within 25 hours in contrast to the group receiving urea alone in which only 25 per cent were aborted within the same time interval.

Table III depicts uterine tone, contraction frequency, and integrated intrauterine pressure (IIUP) for the two study groups. Uterine tone and the IIUP were significantly higher in the group receiving urea-$PGF_{2\alpha}$ as compared to those treated with urea alone at every point of measurement. Before contractions were observed, the IIUP was equivalent to the uterine tone. One hour after intra-amniotic injection, contractions had begun in the urea-$PGF_{2\alpha}$–treated patients but not in patients receiving urea alone.

In the urea-$PGF_{2\alpha}$–treated group, two patients failed to pass the placenta within two hours of abortion and they underwent curettage. In addition, one patient underwent curettage because of persistent bleeding and another, because of fever at the time of abortion. In the group treated with urea alone, seven patients underwent curettage. In four, curettage was undertaken because of failure to pass the placenta and in three, because of failure to deliver the complete placenta.

In the urea-$PGF_{2\alpha}$–treated group, because of absence of labor, four patients were given oxytocin four hours following membrane rupture and two patients were given oxytocin 24 hours after intra-amniotic injection. In the group treated with urea alone, all patients received oxytocin. Five patients received oxytocin because of absence of labor 24 hours after intra-amniotic injection, one patient, following membrane rupture, and two patients had oxytocin started following incomplete abortion.

*Laboratory results.*

SERUM ELECTROLYTES. The mean serum electrolytes

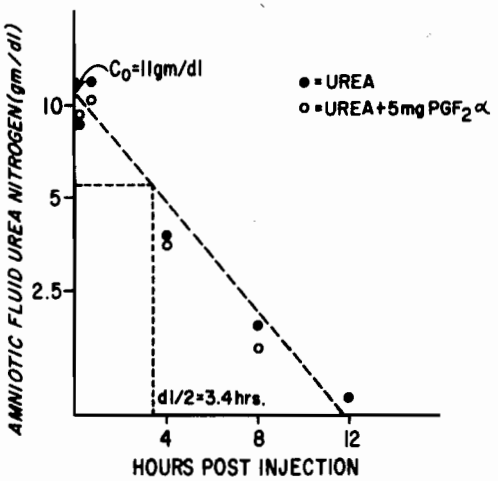


**Fig. 2.** Amniotic fluid urea nitrogen following intra-amniotic injection of 80 Gm. of urea. $C_0$ = Concentration extrapolated to time zero. $d\frac{1}{2}$ = half life. Ordinate is plotted logarithmically.

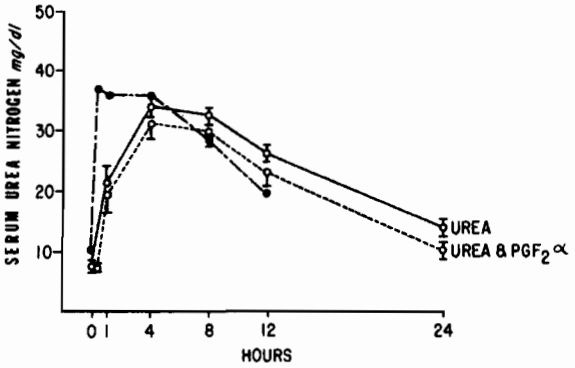


**Fig. 3.** Serum urea nitrogen following intra-amniotic injection of 80 Gm. of urea with and without 5 mg. of $PGF_{2\alpha}$. •- - -• Represents concentrations in one patient who experienced an intravascular injection of urea.

are summarized in Table IV. There were no significant alterations in the serum sodium, potassium, and $CO_2$ values. Both treatment groups demonstrated decreases ($P < 0.05$) in serum chloride at 12 hours compared with the initial values. However, at no time was the chloride value below levels considered normal in our laboratory.

Urea nitrogen determinations. Fig. 2 depicts amniotic fluid levels of urea. As shown, the half life of urea in this compartment was approximately three hours. No significant difference in urea clearance was noted between the two treatment groups. The apparent intra-amniotic distribution volume for urea was determined by dividing the dose of urea administered by the theoretical concentration at time zero and was found to be 725 ml.

The serum urea nitrogen determinations for the two treatment groups are given in Fig. 3. The additional curve documents the serum urea nitrogen levels of one patient in the urea-$PGF_{2\alpha}$–treated group who experienced an inadvertent intravascular injection of urea (see "Complications"). As is characteristic of intra-amniotic urea infusions, values increased to a maximum at four hours and gradually returned toward pre-injection values. No significant differences existed between the two treatment groups.

AFP determinations. Table V presents the AFP determinations in amniotic fluid and plasma in micrograms and nanograms per milliliter, respectively. There was a progressive increase in amniotic fluid and plasma AFP following intra-amniotic injection. There were no significant differences observed between the two treatment groups for either plasma or amniotic fluid values. At the time of disappearance of fetal heart tones in both groups, no significant elevations in amniotic fluid AFP were found. Similarly, at the time of abortion, no differences were noted in serum AFP levels between the two treatment groups.

*Complications.*

Immediate. There were no failures in the urea-$PGF_{2\alpha}$–treated group, but one patient in the group treated with urea alone underwent vaginal evacuation because of hemorrhage prior to abortion. The hemorrhage was attributed to partial separation of the placenta.

The amounts of estimated blood loss (mean ± S.E.M.) for the two treatment groups were 239 ± 76 ml. and 466 ± 118 ml., respectively, for the groups receiving urea-$PGF_{2\alpha}$ and urea alone. However, this difference in blood loss was not statistically significant. One patient in the urea-$PGF_{2\alpha}$–treated group and three patients in the group treated with urea alone experienced blood losses in excess of 500 ml. However, no patient in this study received transfusion.

Emesis occurred in five of the urea-$PGF_{2\alpha}$ treated group and three of the those receiving urea alone. Only one patient experienced diarrhea in the urea-$PGF_{2\alpha}$–treated group and no patient experienced diarrhea in the group given urea alone. Of note was the finding that emesis in the urea-$PGF_{2\alpha}$–treated patients appeared to occur at the time of significantly increased uterine tone and contraction frequency whereas in the group treated with urea alone emesis occurred at approximately 20 hours and did not appear to coincide with increased uterine tone.

One patient experienced an inadvertent intravascular spill. During infusion of urea, the patient noted a burning sensation in the pelvis and complained of feeling warm. At this point, the operator noted loss of free flow of amniotic fluid from the amniocentesis needle. After re-establishment of free flow, the remaining urea

**Table IV.** Serum blood chemistry determinations following induction of abortion with urea or urea + $PGF_{2\alpha}$

| | *Hours after injection* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *0* | *0.25* | *1* | *4* | *8* | *12* | *24* |
| *Sodium (mEq./L.)* | | | | | | | |
| Urea | 138.9 ± 1.3 | 136.1 ± 0.7 | 137.3 ± 1.2 | 136.6 ± 0.3 | 136.4 ± 0.8 | 135.9 ± 0.6 | 137.0 ± 1.9 |
| Urea + $PGF_{2\alpha}$ | 137.8 ± 0.8 | 137.1 ± 0.8 | 136.8 ± 0.7 | 137.9 ± 1.3 | 136.4 ± 0.5 | 135.4 ± 1.4 | 136.8 ± 1.7 |
| *Potassium (mEq/L.)* | | | | | | | |
| Urea | 3.87 ± 0.13 | 3.83 ± 0.10 | 3.98 ± 0.14 | 3.88 ± 0.14 | 3.67 ± 0.09 | 3.75 ± 0.09 | 3.87 ± 0.09 |
| Urea + $PGF_{2\alpha}$ | 3.93 ± 0.16 | 3.83 ± 0.09 | 3.78 ± 0.08 | 3.89 ± 0.17 | 3.61 ± 0.10 | 3.60 ± 0.13 | 3.53 ± 0.19 |
| *Chloride (mEq./L.)* | | | | | | | |
| Urea | 104.4 ± 0.7 | 103.8 ± 0.7 | 102.8 ± 1.5 | 103.8 ± 0.5 | 102.1 ± 0.4 | 101.5* ± 0.7 | 103.7 ± 0.8 |
| Urea + $PGF_{2\alpha}$ | 102.5 ± 1.0 | 104.1 ± 1.1 | 104.3 ± 0.9 | 103.1 ± 0.8 | 101.8 ± 0.8 | 101.3* ± 0.3 | 101.4 ± 1.3 |
| *$CO_2$ (mEq./L.)* | | | | | | | |
| Urea | 24.0 ± 0.7 | 22.8 ± 0.4 | 23.3 ± 1.4 | 23.0 ± 0.7 | 22.5 ± 0.7 | 21.7 ± 0.9 | 23.1 ± 0.5 |
| Urea + $PGF_{2\alpha}$ | 23.6 ± 0.8 | 23.4 ± 0.6 | 23.0 ± 0.8 | 22.9 ± 1.0 | 22.0 ± 1.0 | 23.2 ± 0.9 | 21.0 ± 1.0 |

*Time 12 versus 0: $p < 0.05$ by F test.

**Table V.** AFP in amniotic fluid and plasma following induction of abortion with urea or urea + $PGF_{2\alpha}$

| | *Hours after injection* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *0* | *0.25* | *1* | *4* | *8* | *12* | *24* |
| *Amniotic fluid (μg/ml.)* | | | | | | | |
| Urea | 17.8 ± 3.4* | 5.9 ± 3.5 | 13.9 ± 3.5 | 44.8 ± 4.8 | 59.0 ± 9.4 | 68.5 ± 9.4 | — |
| Urea + $PGF_{2\alpha}$ | 11.8 ± 2.6 | 10.5 ± 3.8 | 11.1 ± 2.2 | 25.1 ± 6.0 | 44.1 ± 13.8 | 65.0 ± 26.9 | — |
| *Plasma (ng./ml.)* | | | | | | | |
| Urea | 52 ± 16 | 73 ± 34 | 227 ± 81 | 380 ± 92 | 602 ± 105 | 532 ± 107 | 1,351 ± 352 |
| Urea + $PGF_{2\alpha}$ | 37 ± 10 | 42 ± 6 | 83 ± 17 | 422 ± 118 | 677 ± 172 | 875 ± 229 | 1,568 ± 233 |

*Mean ± standard error of the mean.

was administered and 5 mg. of $PGF_{2\alpha}$ was then given. The patient's symptoms disappeared within 10 minutes. The patient aborted uneventfully 17 hours after initial injection. The associated serum urea levels are present in Fig. 3.

A 23-year-old patient, para 3, without a history of epilepsy, in the urea-$PGF_{2\alpha}$–treated group convulsed approximately 30 minutes after injection of urea and $PGF_{2\alpha}$. The convulsion was grand mal in nature and lasted one minute. There was no urinary or fecal incontinence or tongue biting but the patient was depressed after the seizure. The only other physical abnormality was mild bradycardia. Serum electrolytes in blood samples drawn immediately following the injection of the abortifacient were within normal limits and the serum urea level was 6 mg. per cent. An electrocardiogram demonstrated a second-degree heart block with Wenckebach's phenomenon. An electroencephalogram demonstrated a focus of abnormal discharge in the left anterior cerebral area. The patient was treated with atropine which converted the cardiac rhythm to a normal sinus rhythm. The patient aborted completely approximately four hours after initial injection and was discharged on the fourth hospital day to be followed in the Neurology Clinic.

One patient in the urea-$PGF_{2\alpha}$–treated group developed fever four hours after abortion. Since an extrauterine site of infection could not be documented, curettage was performed and the patient was treated with parenteral antibiotics. She became afebrile within 24 hours and was discharged on the third hospital day receiving oral antibiotics. Endometrial cultures were positive for group A beta hemolytic streptococci.

DELAYED. No delayed complications or readmissions to the hospital occurred in either group.

**Series of 150 urea-$PGF_{2\alpha}$ (5 mg.)–treated patients.** The injection-abortion intervals, clinical outcome, and complications of this patient group are presented in Table VI and compared to 180 patients receiving urea-$PGF_{2\alpha}$ (10 mg.).

## Comment

The randomized series demonstrates that utilization of 5 mg. of $PGF_{2\alpha}$ with 80 Gm. of urea achieves a mean

**Table VI.** Injection-abortion intervals and clinical outcome of urea plus 5 mg. of $PGF_{2\alpha}$

| | *Nulliparous patients* | *Multiparous patients* |
|---|---|---|
| No. | 82 | 68 |
| Injection-abortion interval (hr.) | 18.4 ± 1.0* | 16.4 ± 1.1* |
| Complete abortion (%) | 66.0 | 60.0 |
| Incomplete abortion (%) | 34.0 | 40.0 |
| Failures (%) | 1.2 | 0.0 |
| Hemorrhage† (%) | 6.1 | 10.3 |
| Infection‡ (%) | 2.4 | 1.5 |
| Cervical laceration§ (%) | 1.2 | 0.0 |
| *Gastrointestinal (%)* | | |
| Nausea and vomiting | 72.0 | 53.0 |
| Diarrhea | 0.0 | 0.0 |

*Mean ± standard error of mean.

†Blood loss in excess of 500 ml. No transfusions required.

‡Treatment with antibiotics for presumed endometritis as either inpatient or outpatient.

§Laceration was a 2 cm. vertical laceration.

**Table VII.** Comparison of urea plus 10 mg. of $PGF_{2\alpha}$ with urea plus 5 mg of $PGF_{2\alpha}$

| | *Urea + 10 mg. $PGF_{2\alpha}$* | *Urea + 5 mg. $PGF_{2\alpha}$* |
|---|---|---|
| No. | 180 | 150 |
| Injection-abortion interval (hr). | 16.3 ± 0.7 | 17.5 ± 0.7 |
| Complete abortion (%) | 55.0 | 63.0 |
| Incomplete abortion (%) | 45.0 | 37.0 |
| Failure (%) | 0.6 | 0.7 |
| Hemorrhage† (%) | 7.8 | 10.1 |
| Infection‡ (%) | 6.7 | 2.0 |
| Cervical laceration (%) | 3.3§ | 0.7 |
| *Gastrointestinal (%)* | | |
| Nausea and vomiting | 70.0 | 63.0 |
| Diarrhea | 1.1 | 0.0 |

*Data from reference 3.

†Blood loss in excess of 500 ml. No transfusions required.

‡Treatment with antibiotics for presumed endometritis as either inpatient or outpatient.

§A total of six lacerations: two transverse and four vertical.

injection-abortion interval that is less than 24 hours. Use of $PGF_{2\alpha}$ resulted in an earlier onset and a greater frequency of contractions when compared to hyperosmolar urea only. Furthermore, tone and IIUP were also greater in the urea-$PGF_{2\alpha}$–treated group. The greater uterine activity in this group was associated with the occurrence of early rupture of the membranes and shorter injection-abortion intervals when compared to the group treated with urea alone. Since it was thought that oxytocin should be used in the absence of labor 24 hours after intra-amniotic injection or after membrane rupture in order to minimize the risk of endometritis or salpingitis, which may be associated with prolonged injection-abortion intervals, intravenous oxytocin still was required for a significant proportion of patients. However, the total time of oxytocin infusion is usually less than that found with urea-oxytocin as previously reported,[1, 3] thus reducing the risk of fluid and electrolyte imbalance associated with this medication. As demonstrated in the current study, serum electrolytes showed very little change.

In amniotic fluid, urea has a half life of approximately three hours. The apparent volume of distribution was 725 ml., a volume much greater than the total volume of amniotic fluid in midtrimester gestations. It is theorized that urea most likely is rapidly distributed to the fetus with a much slower relative uptake by the mother. Such findings have been documented in pharmacokinetic studies in the rhesus monkey.[8] Furthermore, urea uptake is probably toxic to the fetus since fetal heart tones disappeared prior to appearance of significant contractions. AFP changes in amniotic fluid and plasma found in this study substantiate a case report of progressively increasing AFP levels in hyperosmolar urea–induced abortion.[9]

It has been previously shown that mean injection-abortion intervals of less than 20 hours can be obtained for patients receiving intra-amniotic hyperosmolar urea augmented with either 20[2] or 10 mg. of $PGF_{2\alpha}$[3]. The present study demonstrated that the minimal effective dose of $PGF_{2\alpha}$ as an augmenting agent with intra-amniotic hyperosmolar urea is no more than 5 mg. The mean injection-abortion interval of less than 20 hours observed in the eight multiparous patients in the urea-$PGF_{2\alpha}$ group of the randomized series was substantiated in the 150 cases in which more than half of the patients were nulliparous. Table VII compares the previously reported series of cases in which urea and 10 mg. of $PGF_{2\alpha}$ were utilized to the currently reported series.[3] Utilization of 5 mg. of $PGF_{2\alpha}$ as an augmenting agent does not appear to be associated with an increased occurrence of failures or complications. The only complication that may be decreased with this reduced dosage of $PGF_{2\alpha}$ appears to be cervical laceration. In order for this finding to be substantiated, a larger number of patients will require study. However, elimination of posterior cervical laceration or detachment as well as vertical laceration in association with midtrimester abortion would be a significant improvement.

Urea has recently been approved by the United States Food and Drug Administration as an abortifacient. However, its effective use requires an augmenting agent. This study suggests that 5 mg. of $PGF_{2\alpha}$ serves that role when administered intra-amniotically in the manner described. Other factors of importance

would include a concentrated urea solution approaching 60 per cent, removal of amniotic fluid in volumes greater than 100 ml., and utilization of intravenous oxytocin for the indications and at the dosage levels described.

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## Discussion

**Dr. Lawrence Hester,** Charleston, South Carolina. The number of elective abortions in the United States probably exceeds one million per year,[1] of which approximately 15 per cent are done in the second trimester or midtrimester.[2] It is also a fact that midtrimester interruption of pregnancy is associated with increased maternal mortality and morbidity rates. The death-to-case ratio for legal abortion is 0.4/100,000 for gestations of eight weeks or less and increases to 19.7/100,000 for pregnancies of 16 to 20 weeks' gestation.[3] Thus, in terms of risk of death, legal abortion, even in the midtrimester, is a relatively safe procedure when compared with such commonly performed operations as tonsillectomy or appendectomy, which have a death-to-case ratio of 5/100,000 and 352/100,000 procedures, respectively. From the standpoint of maternal mortality and morbidity rates as well as fiscal and other considerations, publicity should be given to the safety of first-trimester abortion. Hence, those with unwanted pregnancies could have an abortion by suction curettage, with midtrimester abortion performed for medical or genetic reasons. In 1974, in Japan and Denmark, 3.2 and 2.7 per cent, respectively, of all abortions were performed during the midtrimester.[2] If we could reduce the number of midtrimester abortions in the United States to 3 per cent, then approximately 30,000 would be done yearly. What a saving in hospital beds! What a saving in money! What a saving in psychic trauma! What a saving of physician's time and frayed nerves!

Dr King's studies showed that the combination of urea and $PGF_{2\alpha}$ is effective in inducing midtrimester abortions in less than 20 hours. Also, he showed that there were no significant alterations in serum electrolytes even though both treatment groups demonstrated decreases in serum chloride at 12 hours as compared with initial values.

It is noted that in the urea-$PGF_{2\alpha}$–treated group, six of the eight patients received oxytocin augmentation prior to abortion, whereas in the group receiving urea alone six patients also received oxytocin augmentation prior to abortion. However, in the urea-$PGF_{2\alpha}$ group four of eight patients (50 per cent) received oxytocin following rupture of the membranes as compared to only one patient (16.5 per cent) in the group receiving urea alone. Dr. King's study showed that in the urea-$PGF_{2\alpha}$–treated group the injection-abortion interval was significantly shorter, 18.3 hours as compared with 28.8 hours in the group receiving urea alone. However, spontaneous rupture of the membranes occurred 7.8 hours after urea-$PGF_{2\alpha}$ injection and 19.3 hours after intra-amniotic injection of urea alone, with abortion occurring approximately 10 hours later in both groups. Thus, it appears that the injection-abortion interval of 18.3 hours may occur because of early spontaneous rupture of the membranes in the urea-$PGF_{2\alpha}$ group followed by oxytocin augmentation. Could Dr. King tell us if this pattern of early membrane rupture followed by oxytocin augmentation was present in the other studies involving urea and 10 mg. of $PGF_{2\alpha}$ or urea and 5 mg. of $PGF_{2\alpha}$? Also, does he believe that oxytocin augmentation significantly decreases the injection-abortion interval in the urea-$PGF_{2\alpha}$ groups?

The complications of midtrimester abortion include gastrointestinal side effects, uterine hemorrhage, infection, respiratory and cardiovascular problems, and grand mal seizures; maternal death occurs rarely. In addition, uterine rupture and cervical laceration do occur as a result of the increased uterine tone and the

integrated contractile force. The late complication of incompetence of the cervix or endocervix associated with midtrimester abortion should be appearing in our clinics. Could Dr. King inform us of the experience at The Johns Hopkins Medical Institutions with endocervical or cervical incompetence associated with therapeutic interruption of pregnancy, particularly related to midtrimester abortion?

Dr. King's studies reinforce my belief that midtrimester abortion should be reserved for medical or genetic indications. As obstetricians-gynecologists we should encourage and participate actively in sex education, including contraceptive advice, in our schools. It is hoped that with time the number of unacceptable or unwanted pregnancies will be decreased. In case there is contraceptive or social failure, the "unacceptable" pregnancy should be terminated before the twelfth week of gestation by suction curettage.

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**Dr. Burkman** (Closing). I certainly think none of us would argue with the comment that there is a need to reduce the frequency of midtrimester elective abortions in this country. With legalization in 1973, about 20 per cent of the elective abortions were of the midtrimester variety. This figure has been reduced to about 10 to 12 per cent, but again, as mentioned, this converts to about 100,000 women annually requiring the procedure in this country.

Unfortunately, most of these women are young, primigravid patients and constitute a high-risk group.

One would welcome any approach to reduce the requirement for midtrimester abortion. I think, however, we will reach a number that is irreducible, and there will still be a need for safe, efficacious procedures for handling these patients.

Regarding the use of oxytocin and rupture of the membranes, we selected this study design in order to compare the results with previous studies which utilized 10 or 20 mg. of $PGF_{2\alpha}$. However, early spontaneous rupture of the membranes may indeed explain some of the reduced injection-abortion intervals in the patients.

Does oxytocin augmentation increase the injection-abortion interval? I think it does not. Currently we are undertaking a study where we will withhold oxytocin until 24 hours and eliminate the use of oxytocin following membrane rupture. Therefore, this modification should answer that question.

What is the frequency of late or midtrimester abortion as a long-term sequela of cervical laceration following these procedures? I think this question is unanswered at this point in time. The rates of both laceration and later spontaneous abortion vary highly from center to center and are dependent upon whether patients were examined routinely after abortion to detect these lacerations.

Our rate of laceration is from 1 to 3 per cent, depending on the procedure, and there does not seem to be a common denominator at this point in time to identify patients at risk or to explain the occurrence physiologically. I think it is still too early to determine how many cervical complications will occur in the future since most of these patients have not yet experienced a subsequent pregnancy. However, I think that patients who undergo midtrimester abortion and have this complication will require close follow-up.

Regarding the abortion of live fetuses, to our knowledge there has only been one abortion of a live fetus in association with the administration of urea in this country. Of the patients from our center over the past several years, which now involve a number in excess of 1,900, we have never had abortion of a live fetus. I think this supports the contention that the use of urea amnioinfusion may be the method of choice in pregnancies of 18 to 22 weeks.

Regarding studies suggesting that dilatation and evacuation may be a safer procedure, when compared with saline, prostaglandin, or urea, most of the data come from a study by the Center for Disease Control, based on material from 32 different centers, which ended in June, 1975.

This was not a well-designed clinical trial. Patients were not assigned at random to different methods of abortion and more importantly, the methodology varied. In addition, the management of complications from center to center showed a wide degree of variation.

Furthermore, study groups had a wide variation in numbers of patients studied. For instance, there were approximately 1,200 patients in the prostaglandin group compared to approximately 10,000 in the saline category.

Many of the complications such as fever, infection, and hemorrhage were placed into large groups for the purpose of analysis and comparison.

I think it would be important to examine a stratification of the particular complications before anyone can really draw any significant conclusions.

Therefore, there is a need for some type of prospective study to determine whether dilatation and evacuation are better than our current methods.

# UTERINE ACTIVITY AND PROSTAGLANDIN PRODUCTION FOLLOWING INTRAAMNIOTIC HYPEROSMOLAR UREA

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## ABSTRACT

The relationship between endogenous prostaglandin (PG) production and uterine activity was studied in hyperosmolar urea induced abortion patients. Polygraphic recordings of intraamniotic pressure were obtained at periodic intervals following intraamniotic injection of 80 gm urea. At 0, 0.25, 1, 4 and 8 hours amniotic fluid and blood samples were obtained for PGE, PGF and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) analysis by radioimmunoassay. Blood was also sampled at time of abortion. In eight patients studied, uterine tone was elevated by 0.25 hour although no rhythmic contractions were observed by 1 hour. At 4 hours, amniotic fluid PGF concentration increased significantly ($P<.01$) over the pre-injection value and continued to increase at 8 hours. Amniotic fluid PGE, PGFM and all plasma PG's showed no change during the 8 hour period following urea administration. At time of abortion the plasma PGFM concentration was significantly greater than at the time of injection (238 $\pm$ 54.4 vs. 86.7 $\pm$ 7.3 pg/ml). There was no significant difference between pre-injection and abortion plasma PGF or PGE concentrations. In the present study, there is no evidence that increased prostaglandin production precedes urea induced contractions. The possible role of PG's in uterine contractions is discussed.

## ACKNOWLEDGEMENTS

The authors thank Dr. S. M. M. Karim for his generous gift of anti-rabbit $PGF_{2\alpha}$ and Dr. J. H. Robinson of Merck, Sharp and Dohme, Rahway, N. J. for indomethacin. The secretarial assistance of Ms. Patricia Gottal is sincerely appreciated.

## PROSTAGLANDINS

Hyperosmolar urea has been demonstrated to be as effective as hypertonic saline for induction of midtrimester abortions and the advantages over saline have been discussed (1). The mechanism by which hyperosmolar agents act is not known although hypertonic saline inductions are associated with increases in prostaglandins (PG) at abortion (2) and PG production has been postulated as the mediating mechanism (3). This hypothesis was studied by simultaneous measurements of uterine activity and PG levels during midtrimester pregnancy termination induced by hyperosmolar urea.

### MATERIALS AND METHODS

After obtaining informed consent, eight multiparous women desiring midtrimester pregnancy termination were selected for study. Eighty grams of urea dissolved in 135 ml 5% dextrose in water was injected intra-amniotically as reported previously (4) in women with pregnancies 16 to 22 weeks in duration. An intraamniotic polyethylene PE 90 catheter was inserted after injection of urea and left in place for amniotic fluid sampling and for intraamniotic pressure recording utilizing a Statham pressure transducer and Gould-Brush 260 polygraph. Frequency of uterine contraction and uterine tone were determined at periodic intervals following urea administration. A heparin lock was inserted in a peripheral vein for collection of blood samples. Blood and amniotic fluid were sampled at 0, 0.25, 1, 4, and 8 hours and in some cases at 12 hours. Blood was also drawn at the time of abortion. Blood samples were collected on ice in heparinized tubes containing indomethacin (5 μg/ml blood) and the plasma was immediately separated by centrifugation and frozen until assayed. Prostaglandins were measured in plasma and amniotic fluid by radioimmunoassay. PGF was measured by the method of Salmon and Amy (5). PGE was measured in an analogous manner. Specificity for the two antibodies used were such that neither cross reacted greater than 1% with the other series. 13,14-Dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) was measured by a method described in another manuscript (6). Coefficient of variation was <10% within assay and <15% between assays for each of the above assays.

When multiple timed samples from individual patients were analyzed, two factor (patient and time) analysis of variance was employed and significance was assessed by F test. When comparing pre-injection vs. abortion plasma levels of PG a paired t-test was used to establish significance.

### RESULTS

Following 80 gm urea, uterine tone was elevated at 0.25 hour although rhythmic contractions were not observed by 1 hour (Figure 1). At 4 hours, the average rate of uterine contractions was 26.2 ± 9.5 contractions per hour. Induction-abortion intervals for these patients ranged from 8.3 to 48.4 hours following urea injection (see reference 4 for clinical details).

In amniotic fluid, PGF concentration increased significantly ($P<.01$)

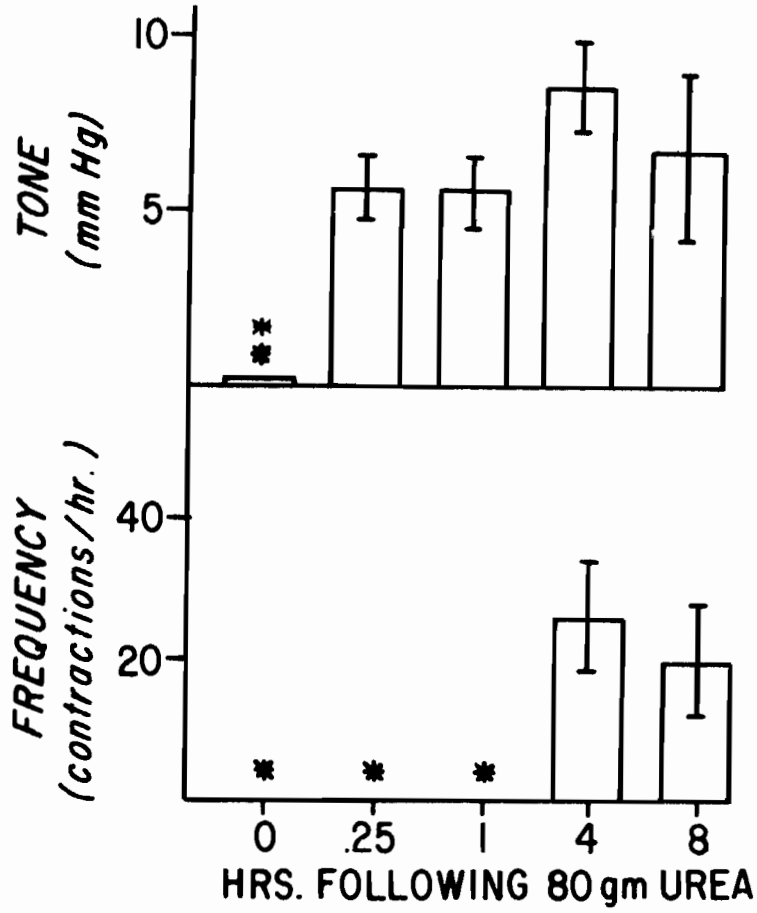


Figure 1. Uterine tone and frequency of contractions. following intraamniotic injection of 80 gm urea. Vertical bars represent ± standard error (n = 8).

** - standardized to zero mm Hg at this time.

* - no rhythmic contractions at these times.

by 4 hours compared to the pre-injection sample (time zero) although PGE and PGFM remained unchanged during the 8 hour period of sampling (Figure 2). Plasma PG levels did not change significantly for the 8 hour period following urea administration (Figure 3). At the time of abortion, plasma PGFM concentration was significantly higher than the concentration at the time of injection (238 $\pm$ 54.5 vs 86.7 $\pm$ 7.3 pg/ml, $P<.01$, Figure 4). There was no significant difference between injection and abortion plasma PGF or PGE concentrations.

Two of the eight patients aborted in less than 29 hours. Patient A, who aborted in 8 hours, demonstrated a rapid increase in amniotic fluid PGF compared to the mean value for late (>29 hours) aborters (Figure 5) beginning at 15 minutes after injection of urea. At 4 hours, her contraction frequency was 60 per hour, higher than any other patient at this time. The amniotic fluid PGF levels remained elevated until abortion. Patient B aborted in 16 hours. Her amniotic fluid PGF levels were initially low, but increased dramatically at 8 hours and reached the highest of any value observed for any patient at 12 hours. This patient demonstrated rhythmic contractions (30 per hour) at 4 hours and this rate remained essentially the same at 8 and 12 hours.

In the eight patients studied, the injection-abortion interval was not significantly correlated with the pre-injection PG levels in amniotic fluid or plasma.

## DISCUSSION

There is abundant evidence that $PGF_{2\alpha}$ and $PGE_2$ are pharmacologically potent oxytocic agents and administration of these drugs by a variety of routes has been used to induce uterine contractions and abortion (7). Spontaneous abortion (8) and hypertonic saline-induced abortion (2) are both associated with increases in PG concentrations in amniotic fluid. Lengthening of the injection-abortion interval following administration of PG synthetase inhibitors has been reported for saline-induced abortions (9) and urea-induced abortion in nulliparous patients (10). It has been hypothesized that hyperosmolar agents act by causing lysosomal breakdown in the decidua, releasing phospholipase enzymes and increasing the precursor pool of arachidonic acid for PG production (3). To support this hypothesis, it should be possible to demonstrate that PG levels increase prior to a change in uterine activity. In the present study population, uterine tone increased within 15 minutes of intra-amniotic injection of hypertonic urea while there was no elevation observed in the mean amniotic fluid PGF, PGE or PGFM by 1 hour. Rhythmic uterine contractions were observed at 4 hours, as was an increase in amniotic fluid PGF suggesting a temporal relationship. More frequent sampling times between 1 and 4 hours may have defined this relationship more precisely. However, one patient who aborted at 8 hours demonstrated a rapid increase in amniotic fluid PGF beginning 15 minutes after injection of urea, yet no rhythmic contractions were observed by 1 hour, although within 4 hours she had the highest frequency of contractions noted for all the patients at that time. Another patient, who had an induction-abortion interval of 16 hours had low amniotic fluid PGF levels initially

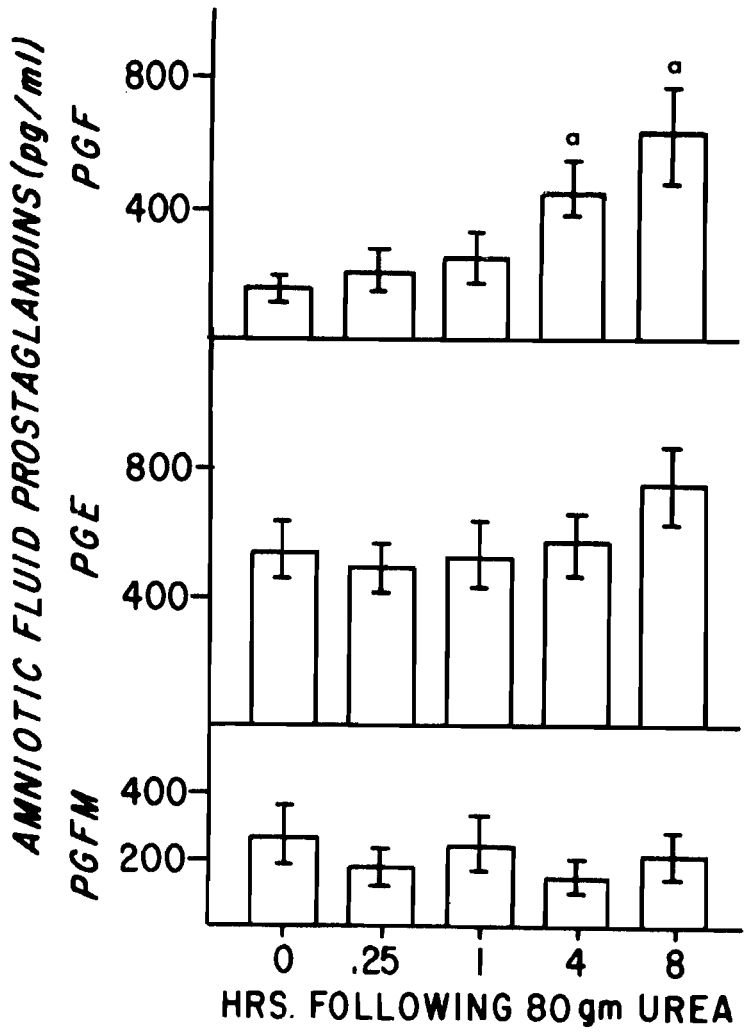


Figure 2. Amniotic fluid prostaglandins following intraamniotic injection of 80 gm urea. Vertical bars represent ± standard error (n = 8).

a - F test P<.01 vs. time 0.

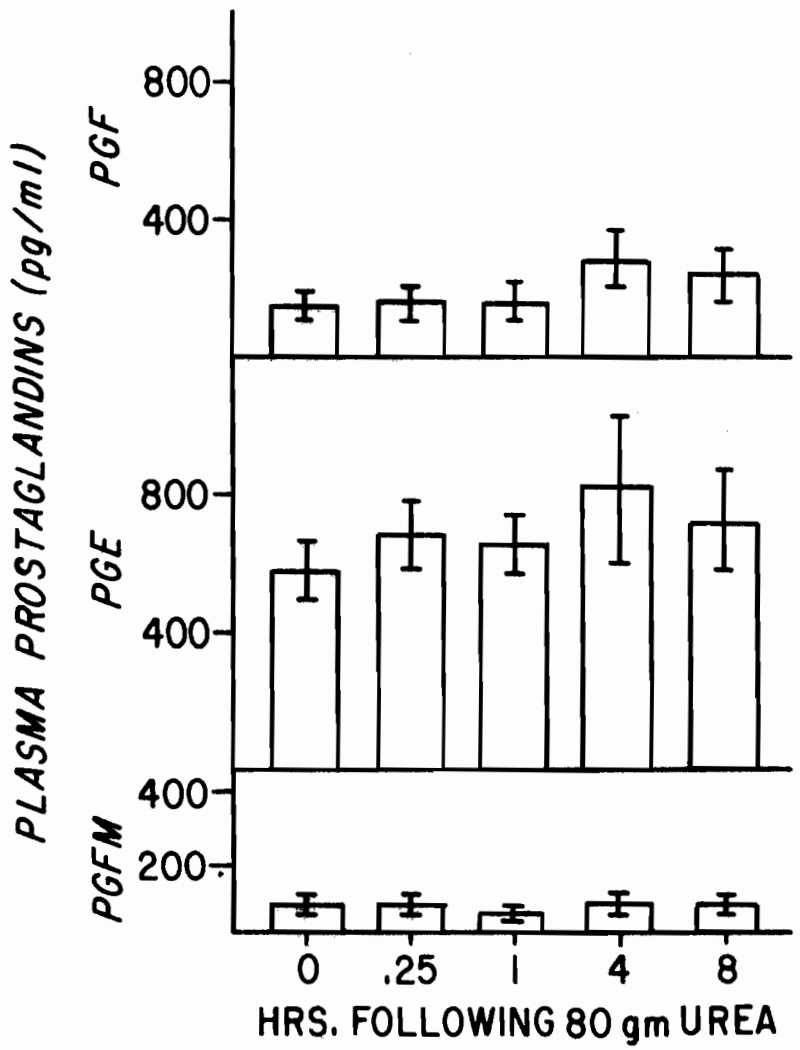


Figure 3. Plasma prostaglandins following intra-amniotic injection of 80 gm urea. Vertical bars represent ± standard error (n = 8).

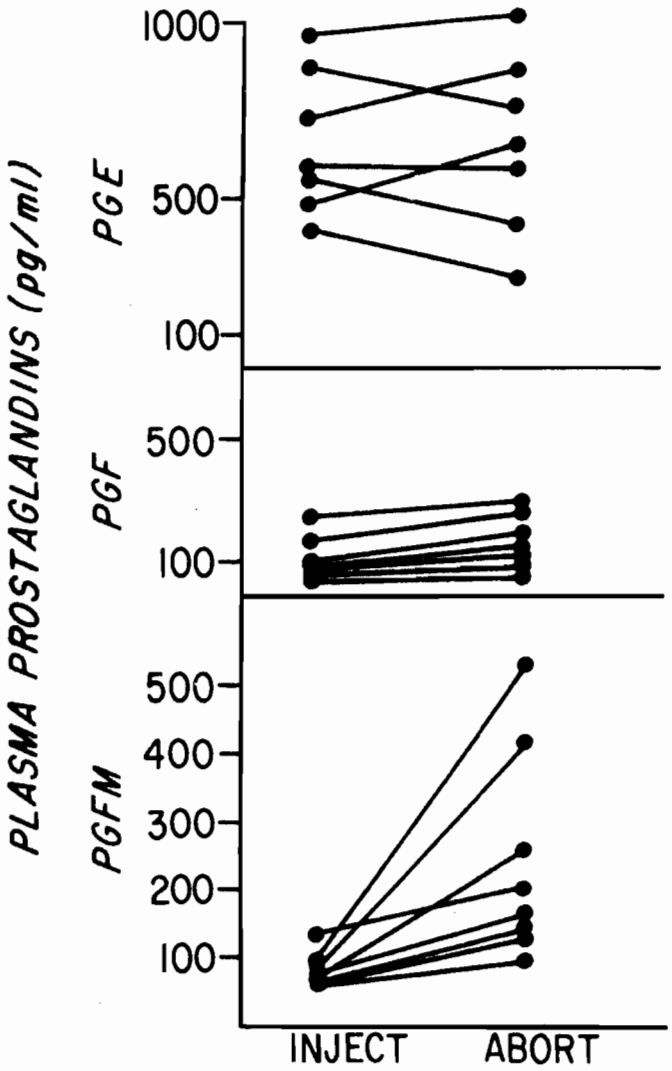


Figure 4. Plasma prostaglandins at time of injection and abortion. Lines join concentrations from individual patients.

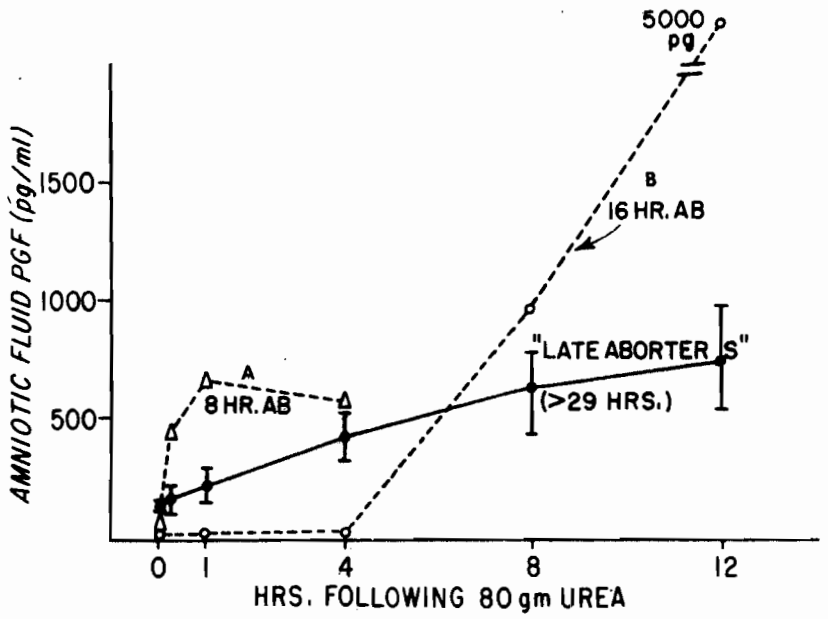


Figure 5. Amniotic fluid PGF concentration in two individual "early aborters" compared to the mean ± standard error of "late aborters" (n = 6). The injection-abortion interval for patient A was 8 hrs., for patient B, 16 hrs. The interval was >29 hours for "late aborters".

but showed a dramatic increase in levels at 8 hours and had the highest concentration of all patients studied at 12 hours. It should be noted, however, that this patient had rhythmic contractions (30 per hour) at 4 hours, that is, before an increase in PGF was observed. These results suggest that the increase in PG formation was the result rather than the initiator of uterine contractions.

There were no changes in mean plasma concentrations of PGF or PGE even at times when amnitoic fluid PGF concentrations were elevated. Since PGF is rapidly metabolized by lung and other tissues (11) and since artifactual formation of PGs may occur during the sampling process (12) it has been suggested that changes in production of PGF may best be monitored by measuring its major circulating metabolite, PGFM. In fact, when $PGF_{2\alpha}$-Tromethamine is injected intraamniotically, there is a greater per cent increase in PGFM than PGF in the maternal plasma (6). Also, in that study, an increase in PGFM was observed in advanced spontaneous labor (>7 cm cervical dilatation) but not earlier, suggesting that increases in circulating PGFM are the result of vigorous contractions. Consistent with those findings, the present study reveals no increase in plasma PGFM prior to induced abortion, although the mean concentration of plasma PGFM was greater at the time of abortion compared to the concentration prior to urea injection.

While the present data demonstrate some correlation between amniotic fluid PGF production and uterine contractions, there is no consistent increase in PG levels in plasma or amniotic fluid prior to the onset of uterine activity. It has been demonstrated in vitro that hyperosmolar urea can increase uterine activity or pregnancy rat uteri even when PG production is depressed by indomethacin (13). In that situation, PG production is not obligatory in the mechanism of action of hyperosmolar urea. In the present study it is possible that enhanced prostaglandin production in the uterus occurred earlier than detectable changes in amniotic fluid. Alternatively, other PG-related compounds (e.g. thromboxanes) (14) may initiate contractions. Once initiated, contractions may cause increased release of PG which in turn positively feedback upon the uterus. Thus, PG's may be important regarding the injection-abortion interval, without being the primary mechanism by which hyperosmolar urea acts. Apropos this postulate, augmentation of hyperosmolar urea with 5 mg PGF significantly shortens the injection-abortion interval (4).

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Revised

# 13,14-DIHYDRO-15-KETO PROSTAGLANDIN $F_{2\alpha}$ CONCENTRATIONS IN HUMAN PLASMA AND AMNIOTIC FLUID

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## ABSTRACT

An antiserum to 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}M$) was prepared and evaluated in various reproductive states. $PGF_{2\alpha}M$ plasma concentration was 63.6 $\pm$ 10.3 pg/ml (Mean $\pm$ SEM) in cycling women. The concentration fluctuated throughout the menstrual cycle and pregnancy, but no discernible patterns were noted. $PGF_{2\alpha}M$ concentrations were elevated at the time of urea + oxytocin induced abortion (238 $\pm$ 54 pg/ml) and during late stages of normal labor (352 $\pm$ 107 pg/ml) but were not elevated during labor prior to 7 cm dilatation. Following intra-amniotic instillation of 5 mg of $PGF_{2\alpha}$ tromethamine into the amniotic sac $PGF_{2\alpha}M$ concentration increased in the amniotic fluid. In the plasma of these patients there was an 18 fold rise in plasma $PGF_{2\alpha}M$ concentration compared to a 3.5 fold rise in $PGF_{2\alpha}$ at 1 hour, suggesting changes in $PGF_{2\alpha}M$ may be more easily detected than the parent compound. While $PGF_{2\alpha}M$ may be a useful index of $PGF_{2\alpha}$ production, it is clear that $PGF_{2\alpha}M$ is of no value in predicting the occurrence of uterine contraction.

## INTRODUCTION

Circulating physiologically active prostaglandins have a short half-life due to rapid metabolism by lung, liver and other organs (1). There is also evidence that prostaglandins are metabolized to inactive forms within the cells of origin prior to secretion (2,3). It has been suggested, therefore, that measuring the metabolite rather than the parent compound might better reflect changes in production of prostaglandins (4). The major circulating metabolite of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}M$) (1). This paper describes $PGF_{2\alpha}M$ plasma concentrations in various reproductive states and compares amniotic fluid and plasma $PGF_{2\alpha}$ with $PGF_{2\alpha}M$ during midtrimester abortions induced with intraamniotic hyperosmolar urea and $PGF_{2\alpha}$

## MATERIALS AND METHOD

### Antiserum Preparation

Antiserum was prepared by coupling $PGF_{2\alpha}M$ to bovine serum albumin (BSA) by the carbodiimide method and rabbits were immunized as previously described (5). An average of 29 molecules of $PGF_{2\alpha}M$ were coupled to each molecule of BSA as determined by retention of $PGF_{2\alpha}M$ trace following dialysis. Blood, obtained by cardiac puncture, contained a peak titre at one month, and the titre remained high for 10 months at which time the rabbit was sacrificed and blood collected.

### Radioimmunoassay

A standard curve was prepared in tricine buffered saline (TBS) containing gelatin (1 mg/ml) for the range of 0 to 320.0 pg $PGF_{2\alpha}M$. Antiserum was diluted

with TBS (1:20,000) and 0.1 ml of this dilution was added to a tube containing 0.1 ml unextracted plasma, 0.1 ml standard or 0.1 ml TBS (zero tube) and was incubated for 30 minutes at room temperature. (5,6,8,11,12,14 (n) - $^3$H) 13,14-dihydro-15-keto $PGF_{2\alpha}$ (specific activity; 85 Ci/mMol) was obtained from Amersham Searle Corporation, Chicago, Illinois. A stock solution containing 10 μCi/ml in ethanol:water (7:3) was stored at -20$^o$ C. On the day of assay, 25 μl were removed, dried under nitrogen and redissolved in 10.6 ml of TBS. 0.1 ml volume (approximately 2500 c.p.m. with a mass of 10 pg) was added to each tube and incubated overnight at 4$^o$ C.

The next day the antibody-bound complex was precipitated by addition of 1.0 ml of 68% saturated ammonium sulfate suspension containing 4% calcium sulfate as described previously (6). The contents were mixed and allowed to stand at 4$^o$ C for 10 minutes. The tubes were centrifuged for 30 minutes at 2,000 r.p.m. at 4$^o$ C. 0.8 ml of supernatant (free phase) was added to 10 ml of toluene based scintillation cocktail and counted for 10 minutes. The corresponding values were read from the standard curve and expressed in terms of $PGF_{2\alpha}M$ concentration, pg/ml of sample.

The minimum of mass of $PGF_{2\alpha}M$ necessary to cause a displacement of $^3$H-$PGF_{2\alpha}M$ from the antibody which was signfiicantly different from the zero tube was 1.25 pg and the working range extended to 100 pg. "Charcoal stripped" plasma gave blank values that averaged 2.1 pg. The correlation coefficient between added and observed mass of $PGF_{2\alpha}M$ was 0.99 and the slope of the regression line was 0.89 with the Y intercept close to Y = 0. The within assay coefficient of

variation was 5% and the between assay coefficient of variation was 16%. The cross reaction of related prostaglandins was tested for their relative ability to displace the $^3$H-PGF$_{2\alpha}$M from the antibody relative to PGF$_{2\alpha}$M. The results found are as follows: 13,14-dihydro-PGF$_{2\alpha}$, 3%; 15-keto-PGF$_{2\alpha}$, 1%; PGF$_{2\alpha}$, .01%; 13,14-dihydro-15-keto-PGE$_2$, 1%; 15-keto-PGE$_2$, <.01%.

PGF$_{2\alpha}$ was measured by the method of Salmon and Amy (7). The anti-serum was provided by Dr. S. M. M. Karim, Singapore. The antibody shows some cross reaction with other prostaglandins of the F series, but cross reaction with E series is less than 1%. Interassay coefficient of variation was 15% and intraassay coefficient of variation was 9%.

Clinical Studies: One blood sample was obtained from each patient and plasma PGF$_{2\alpha}$M was determined and compared in the following groups of adult women:

a) those in the follicular stage of the menstrual cycle

b) those on a combined oral contraceptive (5 mg norgestrel and 50 μg ethinyl estradiol)

c) those with uncomplicated pregnancies, 17-20 weeks after the last menstrual cycle. This group was further separated into those less than and those greater than 7 cm dilated.

d) elective abortion patients, 17-20 weeks pregnant, at the time of abortion. These patients were injected with intraamniotic hyperosmolar urea (80 gm) followed by intravenous oxytocin (332 mUnits/min)

The following patients had multiple blood samples drawn. Daily plasma samples from 3 women throughout the menstrual cycle and sequential samples from 2 pregnant women were also assayed. Eight multiparous patients over 18 years of age seeking termination of a midtrimester pregnancy and who gave informed consent, participated in the following study. An 18 gauge butterfly needle

was placed in the patient's right antecubital vein for collection of blood samples prior to and at time intervals (t = 0, 0.25, 1, 4, 8 and 12 hours) following abortifacient injection. Polyethylene tubing was inserted into the amniotic cavity through which amniotic fluid was withdrawn and replaced by 134 mls of 5% dextrose containing 80 gm urea and 5 mg $PGF_{2\alpha}$ tromethamine. This initiation of the abortion process was considered time zero (t = 0). This procedure is discussed in detail in another publication (8). The tubing was left in place so that amniotic fluid could be sampled at intervals corresponding to the blood sampling prior to rupture of membranes. Urea added to prostaglandin standards was found not to affect the radioimmunoassay displacement curves.

All blood samples were collected on ice in heparinized tubes containing indomethacin (5 μg/ml blood) and the plasma was immediately separated by centrifugation and frozen until assayed. All samples were assayed in duplicate.

## RESULTS

Plasma $PGF_{2\alpha}M$ concentrations for women in various reproductive states are listed in Table 1. Levels of $PGF_{2\alpha}M$ at time of induced abortion were significantly higher than pregnant women not in labor at the same stage of pregnancy (65.5 ± 8.6 SEM vs 238.3 ± 54.5 pg/ml). There was no detectable increase in $PGF_{2\alpha}M$ in early labor (<7 cm), however, there was a significant increase at 8 cm to one hour post-partum (Table 1). Although $PGF_{2\alpha}M$ values fluctuated throughout the cycle, there appeared to be no discernible pattern (Fig. 1). Values fluctuated in individual patients throughout pregnancy, but again there appeared to be no pattern (Fig. 2).

Following $PGF_{2\alpha}$ instillation into the amniotic sac, amniotic fluid concentration of $PGF_{2\alpha}$ increased from 0.15 ± .01 to 6250 ± 1300 ng/ml in 15 minutes. After one hour, there was a decline in $PGF_{2\alpha}$ concentration which

reached 1370 ± 970 ng/ml by 12 hours. The disappearance half-life (time for concentration to fall to 50% of the back-extrapolated time zero concentration) was 4.5 hours (Fig. 3). The apparent volume of distribution of PGF was calculated by dividing the injected dose of $PGF_{2\alpha}$ (5 mg) by the extrapolated concentration of $PGF_{2\alpha}$ at time zero (7000 ± 1500 ng/ml) and found to be 710 ± 150 ml. $PGF_{2\alpha}M$ increased in amniotic fluid within 15 minutes of injection from .24 ± .05 ng/ml to 53.7 ± 24.8 ng/ml. These levels continued to rise until 8 hours when the concentration reached 377 ± 103 ng/ml.

In the plasma, $PGF_{2\alpha}$ concentration increased by 1 hour after intraamniotic administration of $PGF_{2\alpha}$ from .32 ± .03 ng/ml to 1.09 ± .27 ng/ml. There was no significant change in these levels over the next 12 hours. $PGF_{2\alpha}M$ increased from .07 ± .01 to .76 ± .31 ng/ml plasma in 15 minutes and remained above the preinjection values for 12 hours.

The average concentration in $PGF_{2\alpha}M$ in plasma increased 18 fold at 1 hour post amniotic injection of $PGF_{2\alpha}$ compared to a 3.5 fold rise in average $PGF_{2\alpha}$ concentration (Fig. 4).

## DISCUSSION

Changes in the pattern of plasma $PGF_{2\alpha}$ have not been shown during the menstrual cycle (9) despite the fact that increased levels have been shown in endometrial tissue (10) and jet washes of endometrium (11) during the luteal phase. In the present study, while there was a day to day variation in plasma $PGF_{2\alpha}M$, no obvious pattern in plasma $PGF_{2\alpha}M$ was apparent during the menstrual cycle of any of the three subjects studied. No difference was seen between women on combined oral contraceptives and control women in the follicular stage of their cycle (61 ± 7.4 vs 63.6 ± 10.3 pg/ml).

Following intraamniotic administration of 5 mg $PGF_{2\alpha}$, the amniotic fluid disappearance rate of $PGF_{2\alpha}$ was found to be 4.5 hours. The volume of distribution was 710 $\pm$ 150 ml. This volume agrees well with that found for urea in the same patients (8). These results suggest that the $PGF_{2\alpha}$ was distributed to an apparent space larger than amniotic fluid, the volume of which was estimated to be about 200-300 ml based on the amount removed at time of injection. The other compartments to which the $PGF_{2\alpha}$ is most likely distributed includes the fetus and placenta as demonstrated pharmacokinetically for urea in Rhesus monkeys (12).

Following intraamniotic injection of $PGF_{2\alpha}$, amniotic fluid $PGF_{2\alpha}M$ concentration increased gradually with time indicating some enzymatic degradation occurring in the fetal compartment and/or maternal conversion and transfer back to the fetus. The relative change in $PGF_{2\alpha}M$ at 1 hour post injection was greater (peak = 18 x initial value) compared to $PGF_{2\alpha}$ (3.5 x initial value), suggesting changes in $PGF_{2\alpha}M$ may be more easily detected than $PGF_{2\alpha}$. The patterns of $PGF_{2\alpha}$ and $PGF_{2\alpha}M$ concentration with time are similar to 3 patients reported previously by Cornett et al. (3) despite the fact that a 40 mg dose of $PGF_{2\alpha}$ without urea was injected intraamniotically compared to our 5 mg dose. That study also differed in that the initial $PGF_{2\alpha}M$ levels were about 10 fold greater than in this report which may be related to the specificity and sensitivity of the antibody. Plasma levels of $PGF_{2\alpha}M$ reported in the literature range from 38 (4) to 1400 pg/ml (3) as measured by radioimmunoassays. Our values are among the lower range and agree in magnitude with values obtained by the tedious but more precise methods of gas chromatography-mass spectrophotometry (13).

Prostaglandin $F_{2\alpha}$ is a potent oxytocic agent which is endogenously produced in increasing amounts by actively contracting uteri (14,15). Since circulating $PGF_{2\alpha}$ is rapidly metabolized by prostaglandin dehydrogenase and $\Delta^{13}$ reductase (1) it has been suggested that $PGF_{2\alpha}M$ may more accurately reflect changes in production of $PGF_{2\alpha}$ (4). Also, in vitro $PGF_{2\alpha}$ production has been documented after blood is collected (16) whereas this seems not to be the case for $PGF_{2\alpha}M$ yielding an additional advantage of $PGF_{2\alpha}M$ measurements.

In this study, a 3.6 fold increase in plasma $PGF_{2\alpha}M$ levels was found in induced abortion patients at time of high uterine activity as compared to controls of the same gestational age not in labor. In early labor, there was no detectable rise in plasma $PGF_{2\alpha}M$ until after 7 cm dilatation at which time levels were significantly increased. While $PGF_{2\alpha}M$ may be a good index of $PGF_{2\alpha}$ production it is clear that $PGF_{2\alpha}M$ is of no value in predicting the occurrence of uterine contractions.

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TABLE 1

CONCENTRATION OF $PGF_{2\alpha}M$ IN FEMALE PLASMA

| | Number | $PGF_{2\alpha}M$ (pg/ml) |
|---|---|---|
| Cycling (follicular stage) | 6 | 64 ± 10[a] |
| Oral Contraceptives | 7 | 62 ± 7 |
| Pregnant (17-20 weeks) | 10 | 66 ± 9 |
| Abortion (17-20 weeks) | 8 | 238 ± 54* |
| Labor (<7 cm) | 8 | 47 ± 6 |
| Labor (>7 cm to 1 hr. post-partum) | 6 | 352 ± 107* |

[a]Standard Error of the Mean

*Significantly greater (P<.01) than all values without asterix (by F test following analysis of variance)

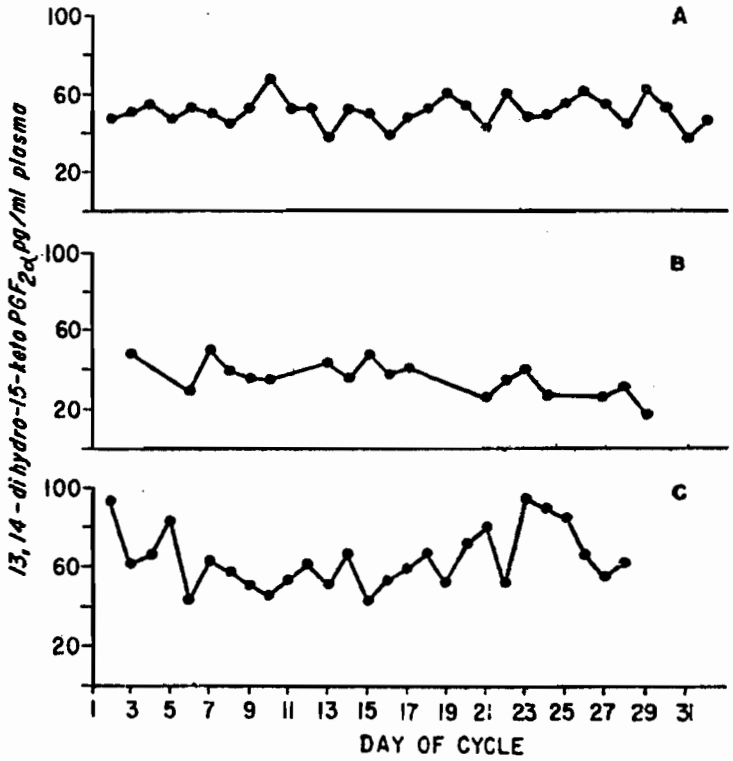


Figure 1. Plasma $PGF_{2\alpha}M$ concentrations in 3 normal women followed serially throughout the menstrual cycle. Day 1 is the first day of menstrual bleeding.

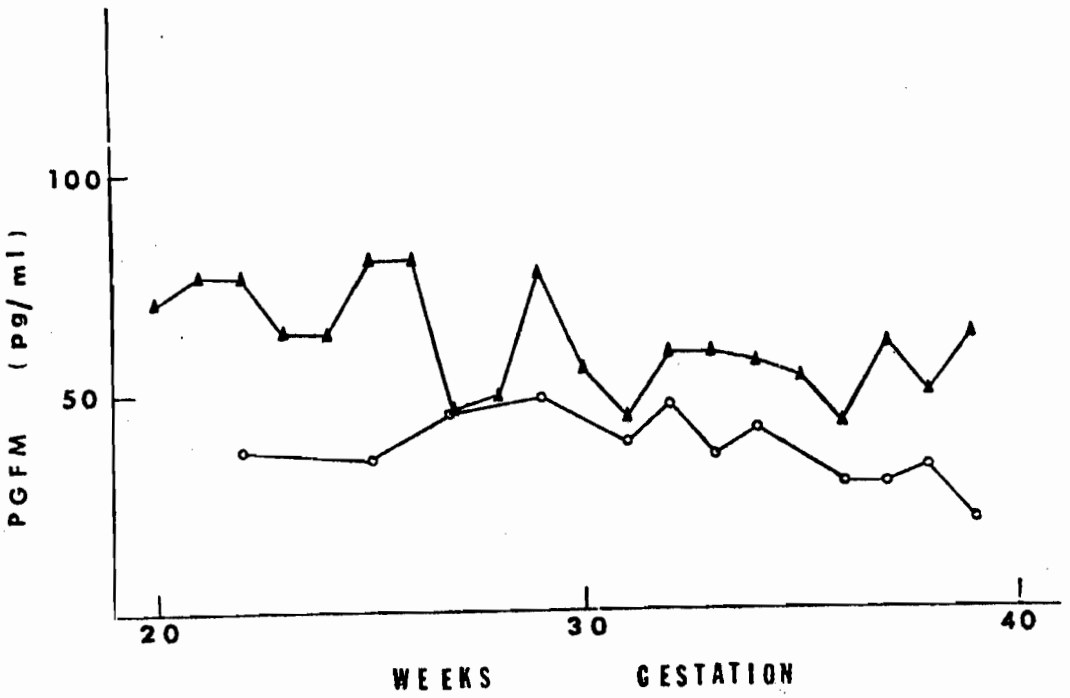


Figure 2. Plasma $PGF_{2\alpha}M$ concentrations in 2 women followed serially throughout pregnancy.

CONCENTRATION OF PGF AND PGFM IN AMNIOTIC FLUID
FOLLOWING INTRAAMNIOTIC PGF ADMINISTRATION

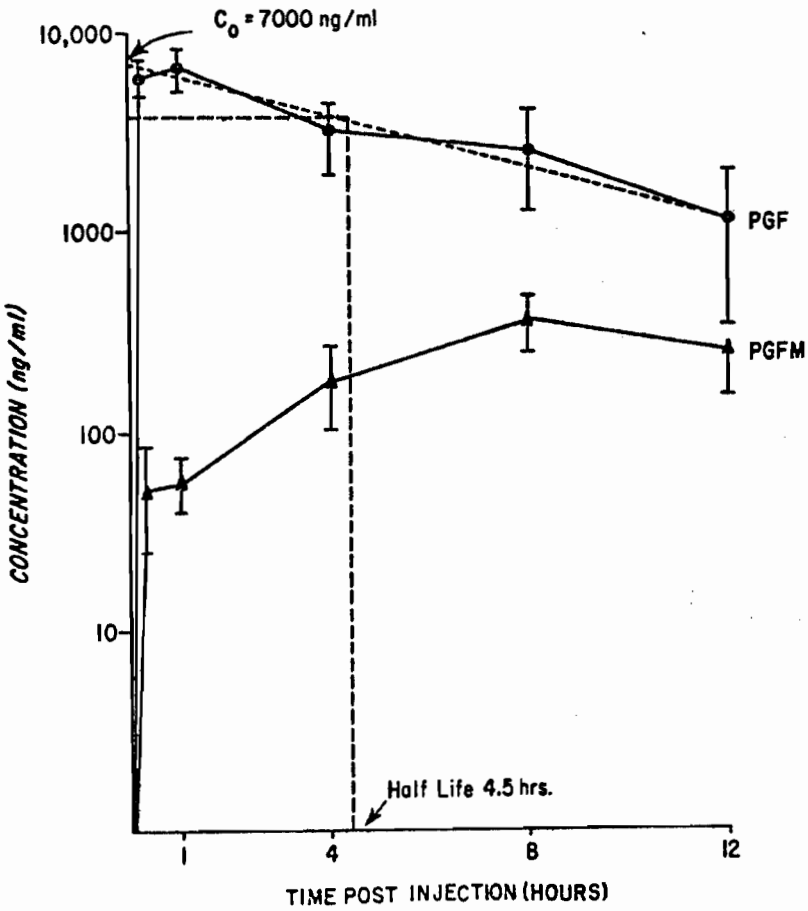


Figure 3. Concentration of $PGF_{2\alpha}$ and $PGF_{2\alpha}M$ in amniotic fluid following intra-amniotic $PGF_{2\alpha}$ (5 mg) administration. $PGF_{2\alpha}$ was administered along eith 80 gm urea to induce abortion. Dotted line through $PGF_{2\alpha}$ curve represents the regression line (fitted to an exponential decay curve) for $PGF_{2\alpha}$ extrapolated to time 0 to estimate the concentration at time 0 ($C_0$). Number of patients = 8. Verticle line indicates standard error of the mean.

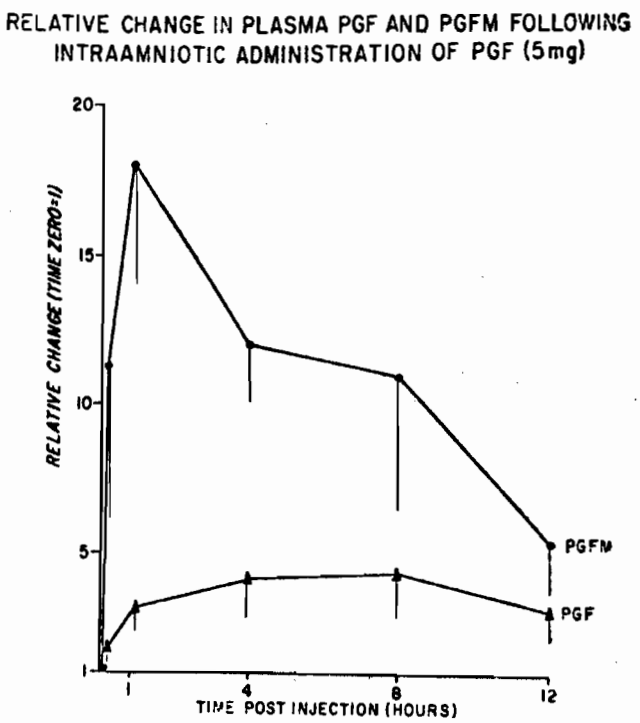


Figure 4. Relative change in plasma $PGF_{2\alpha}$ and $PGF_{2\alpha}M$ following intra-amniotic administration of 5 mg $PGF_{2\alpha}$ . Time 0 relative change was defined as 1. $PGF_{2\alpha}$ was administered along with 80 gm urea to induce abortion. Number of patients = 8. Verticle line indicates standard error of the mean.

ACKNOWLEDGEMENT

These studies were carried out under a contract AID/csd-3627. The secretarial assistance of Ms. Patricia Gottal is sincerely appreciated. We wish to thank Dr. John Pike, Upjohn Company, Kalamazoo, Michigan 49001, for the kind gift of prostaglandins and Dr. J. H. Robinson of Merck, Sharp and Dohme, Rahway, New Jersey for indomethacin.

# AUGMENTATION OF HYPEROSMOLAR UREA FOR MIDTRIMESTER INDUCED ABORTION: COMPARISON OF TWO METHODS

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ABSTRACT

Hyperosmolar urea, although safe and efficacious as a midtrimester intra-amniotic abortifacient, requires augmentation with oxytocic agents such as intra-amniotic prostaglandin $F_{2}a$ ($PGF_{2}a$) or intravenous oxytocin in order to achieve injection-abortion intervals of 24 hours or less. The present study compared two groups of 150 patients each who underwent infusion with intra-amniotic urea plus $PGF_{2}a$ (5 mg) initially and who both had intravenous oxytocin (332 mu/min) started in instances of incomplete abortion and in the absence of labor 24 hours after initial injection. For comparison, one group additionally received oxytocin in the absence of labor four hours after membrane rupture. While both groups had injection-abortion intervals of less than 24 hours, parous patients appeared to benefit from oxytocin use when membrane rupture occurred within the first four hours after urea injection.

## Introduction

Hyperosmolar urea has been found to be safe and efficacious as an intra-amniotic midtrimester abortifacient.[1] However, in order to achieve predictable injection-abortion intervals of 24 hours or less, an oxytocic agent is required for augmentation.[2]

At the Fertility Control Center of the Johns Hopkins Hospital, studies have been completed utilizing intravenous oxytocin (332 mu/min)[3] and 20 mg[4], 10 mg[5], and 5 mg[2] of intra-amniotic prostaglandin $F_2a$ ($PGF_2a$) as the initial augmenting agent in conjunction with urea. Although high dose intravenous oxytocin is effective, there is concern for fluid retention and associated electrolyte abnormalities. Prostaglandin $F_2a$ reduces one's concern for such problems, however, intravenous oxytocin has still been used in addition to prostaglandin $F_2a$ for specific situations which include: the absence of labor four hours after membrane rupture, in the absence of labor at 24 hours after initial injection, and following incomplete abortion. Although such usage of oxytocin is associated with a decreased time of oxytocin infusion, an additional reduction in time of infusion would further minimize the risk of water intoxication. Therefore, a study comparing our standard method of oxytocin use versus a reduced dosage method was carried out in patients presenting for elective midtrimester abortion.

## Materials and Methods

The methods for the administration of hyperosmolar urea have been described previously.[3-5] An initial group of 150 patients undergoing abortion with urea plus $PGF_2a$ (5 mg) had intravenous oxytocin infused at 332

mu/min for three indications: four hours after membrane rupture in the absence of labor, 24 hours after initial injection in the absence of labor, and following incomplete abortion. For comparison, this group was labeled as the standard oxytocin group.

After study of the standard oxytocin group was completed, an additional 150 patients underwent abortion with urea plus $PGF_2a$ (5 mg) utilizing intravenous oxytocin infused at 332 mu/min for only two indications: 24 hours after initial urea injection in the absence of labor and following incomplete abortion.

Data regarding composition of study groups, outcome, injection-abortion intervals, requirement for oxytocin, and complications were tabulated and the Student's T test was used to examine for statistical significance.

## Results

Table I details the clinical characteristics of the two study groups. Although randomization was not used to select the patients, the two groups were comparable by these criteria and did not show any statistical differences.

Table II lists the clinical outcome following urea plus $PGF_2a$ (5 mg) injection. Complete abortion was defined as non-instrumented passage of fetus and placenta within two hours of each other. Incomplete abortion was defined as the need for instrumental removal of the placenta two hours after fetal abortion or in the presence of hemorrhage or infection. Failed procedures were defined as the need for instrumental removal of all products of conception in the absence of abortion about 48 hours after initial urea injection or in the presence of hemorrhage or infection. No significant difference in clinical outcome was observed between the two treatment groups.

Table III lists the injection-abortion intervals for nulliparous and multiparous pateints within each study group. The mean injection-abortion interval was significantly different between the two study groups when the results of the nulliparous and parous patients were combined. The percent of patients with injection-abortion intervals of more than 24 hours for the standard oxytocin group and reduced oxytocin group were 18.0% and 35.8% respectively.

Table IV details the requirement for oxytocin for both study groups. No major differences existed between nulliparous and parous patients except that parous patients in the reduced dosage group required no oxytocin only 21% of the time. As shown, although both study groups had about equal frequency of use of oxytocin, the time of infusion was less for the reduced oxytocin group.

Figure 1 examines the relationship between time of spontaneous membrane rupture and injection-abortion interval. There was no significant difference between nulliparous and parous patients in the reduced oxytocin dosage study group so that this data is presented in a combined manner. As shown, the curves of patients in the reduced oxytocin group and nulliparous patients in the standard oxytocin group were similar. However, the curve of parous patients in the standard oxytocin group shows that when membrane rupture occurred within the first eight hours after initial injection, injection-abortion intervals were shorter. This difference was significant ($p < 0.01$). When patients not receiving oxytocin four hours after membrane rupture were eliminated, the curves remained similar in shape. However, statistical significance ($p < 0.01$) between the multiparous standard oxytocin group and the other groups was only obtained when membrane rupture occurred within the first four hours.

Table V lists the complications encountered in both study groups. No patient in either group received a blood transfusion, and there were no instances of exploratory laparotomy, hysterotomy, or hysterectomy.

The complication of endometritis was examined for possible relationship to injection-abortion interval, time of membrane rupture, parity, and use of curettage for management of incomplete abortion. The only difference noted in comparison of the two study groups was that patients with endometritis underwent curettage for incomplete abortion slightly less frequently (27.2%). However, this difference was not statistically significant.

## Discussion

An ideal augmenting agent for use in conjunction with intra-amniotic hyperosmolar urea has not been found. Although success has been achieved with intra-amniotic $PGF_2a$ (5 mg) as the primary augmenting agent[2], studies with a further reduced dose of $PGF_2a$ have yet to be completed.

Intra-amniotic $PGF_2a$ as an augmenting agent has the advantages of simplicity of administration, a reduced requirement for intravenous administration of medication, and decreased concern for fluid-electrolyte derangement. However, in order to maintain injection-abortion intervals of 24 hours or less, a secondary agent such as intravenous oxytocin is often necessary.

This report demonstrates that mean injection-abortion intervals of less than 24 hours can be achieved if one restricts the use of intravenous oxytocin to patients not in labor 24 hours after urea injection and in instances of incomplete abortion. However, about one-third of patients will have intervals longer than 24 hours. Although the total number of patients receiving oxytocin is not significantly changed by this reduced dose schema, the total

time of infusion of oxytocin is decreased. In addition, the complication rate does not appear to be significantly different when patients in the standard and reduced oxytocin groups are compared. For example, the rate of endometritis is slightly higher in the reduced oxytocin group, but this rate is quite similar to that reported for patients in other studies utilizing hyperosmolar urea and $PGF_2a$ with our standard oxytocin method.[1]

The data relating time of membrane rupture to injection-abortion intervals indicates that parous patients may benefit from the standard method starting intravenous oxytocin within four hours of membrane rupture. However, this benefit may only be realized if membrane rupture occurs within the first four hours after initial injection.

Finally, it is important to note that there were only two instances of cervical injury for the total group of 300 patients. One patient had a small vertical laceration necessitating one or two interrupted sutures for repair while the other patient had a partial detachment of a small portion of the anterior distal lip of the uterine cervix. The etiology for this second type of cervical injury is unknown but this case represents the second instance of such an injury reported in association with a urea plus $PGF_2a$ amnioinfusion.[6]

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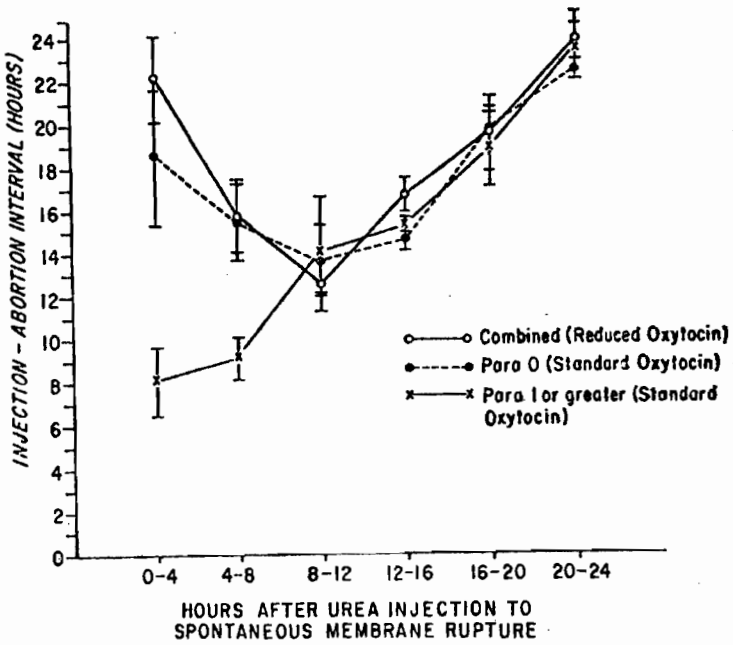


Fig. 1 A comparison of the injection-abortion intervals related to time of membrane rupture for the combined reduced oxytocin group and parous and nulliparous standard oxytocin group. Brackets around each value represent the standard error of the mean.

TABLE I

CLINICAL CHARACTERISTICS OF STUDY GROUPS*

| | No. | Mean Age | Para 0 | Para 1 or Greater | Mean Duration of Gestation (wk) | Fluid Removed at Amniocentesis (cc) |
|---|---|---|---|---|---|---|
| Urea + $PGF_2a$ (5 mg) (Standard Oxytocin) | 150 | 20.59 (13-40) | 82 | 68 | 19.48 (14-24) | 210.24 (100-315) |
| Urea + $PGF_2a$ (5 mg) (Reduced Oxytocin) | 150 | 21.34 (14-43) | 80 | 70 | 19.24 (15-24) | 216.15 (45-530) |

*Ranges in parentheses

TABLE II

OUTCOME

| | | Complete Abortion | | Incomplete Abortion | | Failure | |
|---|---|---|---|---|---|---|---|
| | No. | No. | % | No. | % | No. | % |
| Urea + $PGF_2a$ (5 mg) (Standard Oxytocin) | 150 | 95 | 63.3 | 54 | 36.0 | 1 | 0.7 |
| Urea + $PGF_2a$ (5 mg) (Reduced Oxytocin) | 150 | 87 | 58.0 | 61 | 40.7 | 2 | 1.3 |

TABLE III

INJECTION-ABORTION INTERVALS

| | No. | Injection-Abortion Interval (hr)* | | | |
|---|---|---|---|---|---|
| | | Range | Mean | S.E.M.** | p value |
| **Parity: 0** | | | | | |
| Urea + $PGF_2a$ (5 mg) (Standard Oxytocin) | 81 | 6-45 | 18.37 | 0.96 | N.S. |
| Urea + $PGF_2a$ (5 mg) (Reduced Oxytocin) | 78 | 4-50 | 20.54 | 1.09 | |
| **Parity: 1 or more** | | | | | |
| Urea + $PGF_2a$ (5 mg) (Standard Oxytocin) | 68 | 3-46 | 16.43 | 1.11 | N.S. |
| Urea + $PGF_2a$ (5 mg) (Reduced Oxytocin) | 70 | 4-41 | 19.49 | 1.18 | |
| **Total Groups:** | | | | | |
| Urea + $PGF_2a$ (5 mg) (Standard Oxytocin) | 149 | 3-46 | 17.48 | 0.73 | 0.02 |
| Urea + $PGF_2a$ (5 mg) (Reduced Oxytocin) | 148 | 4-50 | 20.04 | 0.80 | |

* From intra-amniotic injection of medication to abortion of fetus

** Standard error of the mean

TABLE IV

REQUIREMENT FOR OXYTOCIN

| | Urea + $PGF_2a$ (5 mg) (Standard Oxytocin) | Urea + $PGF_2a$ (5 mg) (Reduced Oxytocin) |
|---|---|---|
| No Oxytocin | 28.0% | 26.0% |
| At 24 hours | 13.3 | 40.7 |
| Four hours after membrane rupture | 34.0 | — |
| With incomplete abortion | 24.7 | 33.3 |

TABLE V

COMPLICATIONS

| | Urea + $PGF_2a$ (5 mg) (Standard Oxytocin) | | Urea + $PGF_2a$ (5 mg) (Reduced Oxytocin) | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Hemorrhage (500 cc or need for curettage) | 15 | 10.0 | 13 | 8.7 |
| Endometritis | | | | |
| Hospitalization required | 0 | — | 3 | 2.0 |
| Outpatient treatment | 3 | 2.0 | 5 | 3.3 |
| Cervical Laceration | | | | |
| Transverse | 0 | — | 0 | — |
| Vertical | 1 | 0.7 | 0 | — |
| Other* | 0 | — | 1 | 0.7 |
| Gastrointestinal | | | | |
| Nausea-vomiting | 95 | 63.0 | 91 | 60.7 |
| Diarrhea | 0 | — | 0 | — |

*Partial detachment of the anterior lip of the cervix

# RANDOMIZED TRIAL OF HYPEROSMOLAR UREA PLUS PROSTAGLANDIN $F_{2}a$ IN DOSAGES OF 1 AND 5 MILLIGRAMS INCLUDING THE USE OF LAMINARIA TENT

## Introduction

The Fertility Control Center of the Johns Hopkins Hospital has completed a number of studies utilizing hyperosmolar urea in combination with either intravenous oxytocin or intra-amniotic prostaglandin $F_2a$ for augmentation. During this time period several protocols and procedures have evolved and persisting problems have been identified. Of major importance among the persisting problems yet to be solved with this approach is the need to develop a minimum effective dose of prostaglandin for augmentation. Also reduction in complications particularly cervical laceration is identified as of great importance.

This report details the use of 1 mg of prostaglandin $F_2a$ versus a standardized dose of 5 mg in a nulliparous and multiparous population. In addition, the use of laminaria tents was uniform for all nulliparous patients. Therefore, this gave us a chance to evaluate both the effectiveness of the reduced dose of prostaglandin $F_2a$ as well as the effectiveness of laminaria tents.

## Materials and Methods

All patients underwent evaluation in the usual fashion utilized at the Fertility Control Center for the past 5 years. Following amniocentesis and after obtainment of free-flow of clear fluid approximately 200 cc of amniotic fluid was removed and the urea was then slowly infused. At this point the patients were randomized into the 1 mg or 5 mg group. If placed in the 1 mg group, the prostaglandin was diluted to a solution of 10 cc and slowly infused over approximately 1 minute. If within the 5 mg group, 2.5 cc of prostaglandin was injected over 1 minute then if no untoward reactions occurred, the remaining dose was injected within 5 minutes.

For patients receiving laminaria tents, they underwent speculum and bimanual examination approximately 20 hours prior to the injection. At this point, after first prepping the vagina with betadine, a large sized Berkley laminaria tent (5 cm) was inserted into the internal os of the cervix and packed in place. These tents were left in place until spontaneously expelled or until abortion occurred.

Oxytocin was utilized at 332 mu/min for patients who were unaborted or not in active labor by 24 hours, or in instances of incomplete abortion.

For patients who were incomplete or who had undergone failure of their primary procedure, ring forceps curettage utilizing intravenous meperidene and diazepam was carried out. In instances of incomplete abortion, this procedure was carried out in 2 hours if the placenta had not been passed. In instances of failure, it was carried out at approximately 48 hours.

## Results

Table I lists the clinical characteristics of the various study groups. As shown, only nulliparous patients within the series received laminaria tents. The characteristics were not dissimilar among all groups except, as one would expect, the multiparous patients were older.

## Injection-Abortion Interval

Table II details the injection-abortion intervals of the various groups. As shown, nulliparous patients with laminaria tents tended to have injection-abortion intervals 5 hours less in duration than did patients receiving the 1 mg dose. This difference was significant ($p < .05$). However, the situation was essentially reversed for multiparous patients not receiving laminaria tents in that the 5 mg dose

patients tended to have slightly longer injection-abortion intervals. However, this difference was not significant. In none of the groups was a live fetal abortion experienced.

Clinical Outcome

Table III lists for the groups the number and percent of complete and incomplete abortion and failure of the initiating procedure. No significant differences were noted between the nulliparous groups undergoing laminaria tent placement. However, when nulliparous patients were compared with the multiparous patients, it appears that the frequency of incomplete abortion was reduced. Also 1 failure was experienced by 1 patient in the nulliparous 5 mg group.

Major Complications

Table IV describes the significant complications which occurred within the various study groups. As shown, hemorrhage of greater than 500 cc was an occasional problem and one patient underwent transfusion in the urea $PGF_2a$ 5 mg group. Endometritis also was an occasional problem with 2 patients requiring hospitalization. No other major complications were experienced by any patient within the study groups.

Discussion

It is apparent that the use of 1 mg of prostaglandin $F_2a$ in conjunction with urea can be effective as an augmenting agent. Although in nulliparous patients who underwent prior laminaria tent placement, the 1 mg dose was slightly less effective, injection-abortion intervals were achieved within 24 hours. Among multiparous patients, no significant difference was noted between the 1 mg and 5 mg dosage.

The use of laminaria tents most strikingly reduced the injection-abortion interval in the nulliparous patients. The differences between nulliparous patients and parous patients was significant. As a result of this experience, laminaria tents are now being utilized on all patients and our protocol has been altered. In this present study, patients had laminaria tents placed the afternoon prior to their injection the following morning. This requires approximately a 36 hour hospitalization. With our new protocol, laminaria tents are being placed during the morning of admission and the injection procedure is being carried out 4 to 6 hours later. With this methodology, it appears, after an experience approaching 100 cases, that most patients will realize a 24 hour admission period.

TABLE I

CLINICAL CHARACTERISTICS OF STUDY GROUPS*

| | No. | Mean Age | Para 0 | Para 1 or Greater | Mean Duration of Gestation (wk) | Fluid Removed at Amniocentesis (cc) |
|---|---|---|---|---|---|---|
| Urea, $PGF_2a$ (1 mg), laminaria | 25 | 18.8 (14-26) | 25 | 0 | 20.3 (18-22) | 192 (100-255) |
| Urea, $PGF_2a$ (5 mg), laminaria | 22 | 18.7 (13-30) | 22 | 0 | 19.1 (16-22) | 178 (120-250) |
| Urea, $PGF_2a$ (1 mg) | 25 | 24.2 (17-40) | 0 | 25 | 19.2 (16-22) | 181 (130-240) |
| Urea, $PGF_2a$ (5 mg) | 24 | 26.6 (17-41) | 0 | 24 | 19.2 (17-22) | 171 (120-240) |

* Ranges in parentheses

TABLE II

INJECTION-ABORTION INTERVALS*

| | | Injection-Abortion Intervals (Hours) | |
|---|---|---|---|
| | No. | Range | Mean |
| Parity: 0 | | | |
| Urea, $PGF_2a$ (1 mg), laminaria | 25 | 3.2-38.7 | 15.3 |
| Urea, $PGF_2a$ (5 mg), laminaria | 21** | 2.2-27.5 | 10.2 |
| Parity: 1 or more | | | |
| Urea, $PGF_2a$ (1 mg) | 25 | 2.5-45.3 | 19.6 |
| Urea, $PGF_2a$ (5 mg) | 24 | 5.3-44.3 | 21.5 |

* From intra-amniotic injection of medication to abortion of fetus
** 1 Failure in this group is not listed

TABLE III

OUTCOME

| | | Complete Abortion | | Incomplete Abortion | | Failure | |
|---|---|---|---|---|---|---|---|
| | No. | No. | % | No. | % | No. | % |
| Urea, $PGF_2a$ (1 mg), laminaria | 25 | 18 | 72.0 | 7 | 28.0 | 0 | — |
| Urea, $PGF_2a$ (5 mg), laminaria | 22 | 16 | 72.8 | 5 | 22.7 | 1 | 4.5 |
| Urea, $PGF_2a$ (1 mg) | 25 | 16 | 64.0 | 9 | 36.0 | 0 | — |
| Urea, $PGF_2a$ (5 mg) | 24 | 14 | 58.3 | 10 | 41.7 | 0 | — |

TABLE IV

MAJOR COMPLICATIONS

| | Urea, $PGF_2a$ (1 mg), Laminaria (25 cases) | | Urea, $PGF_2a$ (1 mg), Laminaria (22 cases) | | Urea, $PGF_2a$ (5 mg) (25 cases) | | Urea, $PGF_2a$ (5 m (24 cases) | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| Hemorrhage (500 cc or greater) | 5 | 20.0 | 0 | — | 2 | 8.0 | 5 | 20.8 |
| Endometritis | | | | | | | | |
| Hospitalization required | 0 | — | 0 | — | 2 | 8.0 | 0 | — |
| Outpatient treatment | 2 | 8.0 | 1 | 4.5 | 1 | 4.0 | 1 | 4.2 |

# THE USE OF HYPEROSMOLAR UREA FOR THE ELECTIVE ABORTION OF MIDTRIMESTER ABORTION

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## Introduction

With the liberalization of elective abortion within the United States during the past five years, a number of investigators have attempted to improve approaches to the termination of mid-trimester pregnancies. This need has persisted despite a gradual decline from approximately 20% of procedures being in the mid-trimester in 1972, to about 12% being in the midtrimester in 1975.[1] A number of techniques, particularly intra-amniotic hypertonic sodium chloride, hyperosmolar urea, and prostaglandin $F_2a$ had been utilized during this time interval, but all the methods have disadvantages and advantages and to date none has evolved as the ideal approach.

Since 1973, the Fertility Control Center of the Johns Hopkins Hospital has completed a number of studies utilizing hyperosmolar urea in combination with either intravenous oxytocin or intra-amniotic prostaglandin $F_2a$ for augmentation. During this time period, several protocols and procedures have evolved and this report will summarize the experience.

## Materials and Methods

All patients for midtrimester amnioinfusion undergo a careful history and physical examination, discussion of the procedure and its inherent risks, and proper execution of an informed consent. Sonography is utilized extensively to determine gestational age particularly in borderline cases. Routinely procedures are done between 16 and 22 weeks duration of gestation from the last menstrual period.

Prior to the injection procedure, hyperosmolar urea is prepared by mixing 40 cc of 5% dextrose and water with a 40 gram bottle of urea salt. Two such bottles are prepared to give a final amount for infusion of 80 grams which constitutes a volume of approximately 135 cc or a 59.7% concentration.

Pre-operative abdominal and pelvic examinations and confirmation of the presence of a fetal heart are carried out after the patient has emptied her bladder. The abdomen is prepped with antiseptic and then draped with towels. A site for amniocentesis is then selected in the midline between the umbilicus and symphysis pubis. Utilizing about 5 to 10 cc of 1% Lidocaine, local anesthetic is infiltrated around the injection site, and amniocentesis is then carried out using a 3.5 inch number 16 Tuohy epidural needle.

After obtainment of free-flow of clear fluid, approximately 200 cc of amniotic fluid is removed using disposable extension tubing attached to a three-way stop cock. The prepared urea is then slowly infused via gravity. During the past five years, studies have been completed using intravenous oxytocin at 332 mu/min[2], intra-amniotic prostaglandin $F_2a$ at doses of 20 mg[3], 10 mg[4], and 5 mg[5] for augmentation. When utilizing prostaglandin $F_2a$, a 2.5 mg test dose is administered intra-amniotically and if no untoward reactions occur, the remaining dose is given within five minutes. Prostaglandin $F_2a$ is not utilized for patients with a history of asthma or a history of previous sensitivity to prostaglandin.

Post-procedure care includes monitoring vital signs frequently, careful recording of intake and output and observation for ruptured membranes, vaginal bleeding, labor and abortion.

The following constitute indications for oxytocin: In the absence of labor, approximately four hours after rupture of membranes; 24 hours after injection if labor has not occurred; and after fetal abortion to control bleeding or to assist in placental expulsion. When oxytocin is used, it is administered at a 332 mu/min. This rate is achieved by dissolving 200 units of oxytocin in 500 cc of 5% dextrose plus 0.2% sodium chloride and infusing the solution at a rate of 50 cc per hour with a mechanical or electronic pump. When using oxytocin at such high doses, electrolytes are monitored every 12 hours and a careful record of intake and output is maintained. Oxytocin is stopped for at least two to four hours for the following indications: A clinical suggestion of water intoxication; a serum sodium value of less than 132 meq/liter; intake exceeding output by more than 1500 cc over a 24 hour period; poor urinary output; and suspected intravascular injection of urea.

Following fetal abortion, ring forcep curettage utilizing intravenous meperidene and diazepam is carried out if the placenta is not passed within two hours. Such cases are termed incomplete procedures.

If fetal abortion has not occurred within 48 hours and is not felt to be immiment, such cases are termed failure of the primary procedure and vaginal evacuation of the uterus utilizing intravenous analgesia is carried out. After observation for at least four hours after completion of abortion, patients are given detailed instructions regarding potential complications, are provided with contraception or contraceptive advice, and are given follow-up appointments for about four weeks later. Approximately 80% of our patients either return for a follow-up visit or give follow-up information via the telephone.

## Results

The results in this section include cases contained within a recent report which summarized our experience in 1913 cases.[6] In addition an additional 199 cases utilizing 5 mg of prostaglandin $F_2a$ for augmentation are included.

## Clinical Characteristics

Table I lists the clinical characteristics of the various study groups. As shown the groups were for the most part comparable. The earlier mean duration of gestation for the urea-oxytocin group probably reflects the use of menstrual data and clinical examination for calculating such durations during that time period. For the past four years, fetal foot length measurements according to the tables of Streeter[7], have been utilized and we have subsequently noted an increase in the mean duration of gestation. Although the mean age of patients was approximately 20 years, it should be noted that 60% of the patients undergoing urea infusion were teenagers with approximately one-third being aged 17 or less.

## Injection-Abortion Interval

Table II details the injection-abortion intervals of the various groups. For the total groups, the differences in interval between urea-$PGF_2a$ (5 mg) versus urea-oxytocin and urea-$PGF_2$ (10 mg) was significant ($p < .001$ and $p < .01$ respectively). In addition, the difference between urea-oxytocin and the urea-$PGF_2a$ (10 mg) groups was also significant ($p < .001$).

Finally, no live fetal abortions occurred within the series.

## Clinical Outcome

Table III lists the groups in terms of number and percent of complete and incomplete abortions, and failure of the initiating procedure. As shown, with evolution of reduced doses of

prostaglandin $F_2a$, the failure rate has shown a gradual decline, while the rates for incomplete abortion have not undergone much change. In addition, the requirement for oxytocin was analyzed for both the urea-$PGF_2a$ (10 mg) and urea-$PGF_2a$ (5 mg) groups and is shown in Table IV.

Complications

Table V describes the significant complications which occurred within the various study groups. Other complications include the following: one patient within the urea-oxytocin group and one within the urea-$PGF_2a$ (10 mg) group experienced clinical coagulopathy; intravascular injection or spill was experienced by one patient in the urea-oxytocin group, two patients in the urea-$PGF_2a$ (10 mg) group, and one patient within the urea-$PGF_2a$ (5 mg) group. Intravascular injection was characterized by flushing, dizziness, and intense abdominal cramping of up to 30 minutes duration which appeared to be self-limiting and was not associated with any long-term effect. One patient in the urea-$PGF_2a$ (5 mg) group was admitted approximately six weeks after her procedure for treatment of deep leg venous thrombophlebitis. One patient in the urea-oxytocin group developed Staphylococcus aureus, subacute bacterial endocarditis, and one patient in the urea-$PGF_2a$ (10 mg) group required hysterectomy for Clostridia perfringens endometritis and sepsis. This latter case was the only incident of hysterectomy or exploratory laparotomy within the entire series.

Discussion

The advantages of hyperosmolar urea in comparison with either intra-amniotic hypertonic sodium chloride or prostaglandin $F_2a$ are several-fold. Urea readily crosses cell membranes and is an osmotic diuretic so that one has less concern in instances of inadvertent

intravascular injection.[8] Although changes in clotting factors have been reported, the occurrence of clinical coagulopathy is infrequent.[9] With the use of augmentation, injection-abortion intervals are consistently less than 24 hours and failures are uncommon. Finally, since hyperosmolar urea leads to fetal death within two hours,[6] the abortion of live fetuses is not a problem.

However, there are several problems or disadvantages which persist with the use of hyperosmolar urea. Augmentation is required in order to achieve injection-abortion intervals of less than 24 hours.[6] For this purpose, the use of intra-amniotic prostaglandin $F_2a$ instead of high dose intravenous oxytocin primarily reduces one's concern for problems related to fluid retention and electrolyte abnormality. However, a significant proportion of patients still receive oxytocin although for shorter intervals. The rate of incomplete abortion requires that physicians adept at removal of retained placentae with intravenous analgesia be readily available. Complications such as hemorrhage, infection and gastrointestinal problems are, in general, readily managed from a clinical standpoint.

The most troublesome complication in our opinion is the occurrence of cervical laceration. This complication is most frequent among young nulliparous patients. Although reduction of the dose of prostaglandin $F_2a$ used for augmentation has led to a decreased frequency of this complication, the rates still are too high. Unfortunately, many of these injuries are difficult to repair primarily, and subsequent breakdown is quite common. Furthermore, the future obstetrical outcome of such patients is unknown.

In an effort to eliminate or reduce this complication, we are utilizing laminarias for nulliparous patients inserted intracervically 12 to 18 hours prior to intra-amniotic injection of urea. Preliminary experience in almost 100 cases has demonstrated no cervical injuries to date, and an apparent reduction in injection-abortion intervals. In addition to the use of laminarias, evaluation of intra-amniotic prostaglandin $F_2a$ (1 mg) for augmentation is also being undertaken.

In summary, the use of intra-amniotic hyperosmolar urea for the induction of midtrimester abortion appears safe and efficacious. Although urea has several advantages when compared with either intra-amniotic hypertonic sodium chloride or prostaglandin $F_2a$, a number of problems still persist. Such problems require further research in order to develop more ideal approaches to the termination of late midtrimester pregnancies.

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TABLE I

CLINICAL CHARACTERISTICS OF STUDY GROUPS

| | No. | Mean Age | Para 0 | Para 1 or Greater | Mean Duration of Gestation (wk) | Fluid Removed a Amniocentesis |
|---|---|---|---|---|---|---|
| Urea + Oxytocin (332 mu/min) | 650 | 20.73 (11-48) | 355 | 295 | 16.96 (13-22) | 207.34 (40-350) |
| Urea + $PGF_2a$ (20 mg) | 30 | 20.40 (13-40) | 17 | 13 | 19.27 (13-22) | 221.60 (85-350) |
| Urea + $PGF_2a$ (10 mg) | 1083 | 20.34 (12-42) | 624 | 459 | 19.90 (13-24) | 204.44 (60-340) |
| Urea + $PGF_2a$ ( 5 mg) | 349 | 20.04 (13-40) | 216 | 133 | 19.56 (14-24) | 203.20 (90-315) |

* Ranges in parentheses

TABLE II

INJECTION-ABORTION INTERVALS

| | | Injection-Abortion Interval (hr)* | | |
|---|---|---|---|---|
| | No. | Range | Mean | S.E.M. + |
| Parity: 0 | | | | |
| Urea + Oxytocin | 341 | 3-61 | 21.49 | 0.58 |
| Urea + $PGF_2a$ (20 mg) | 16 | 4-36 | 18.23 | 2.48 |
| Urea + $PGF_2a$ (10 mg) | 632 | 2-60 | 15.92 | 0.32 |
| Urea + $PGF_2a$ ( 5 mg) | 213 | 6-45 | 17.00 | 0.56 |
| Parity: 1 or more | | | | |
| Urea + Oxytocin | 289 | 4-54 | 16.58 | 0.56 |
| Urea + $PGF_2a$ (20 mg) | 12 | 3-30 | 13.70 | 2.46 |
| Urea + $PGF_2a$ (10 mg) | 434 | 1-47 | 13.93 | 0.38 |
| Urea + $PGF_2a$ ( 5 mg) | 133 | 3-46 | 16.25 | 0.78 |
| Total Groups: | | | | |
| Urea + Oxytocin | 630 | 3-61 | 19.24 | 0.42 |
| Urea + $PGF_2a$ (20 mg) | 28 | 3-36 | 16.29 | 1.79 |
| Urea + $PGF_2a$ (10 mg) | 1066 | 1-60 | 15.11 | 0.25 |
| Urea + $PGF_2a$ ( 5 mg) | 346 | 3-46 | 16.71 | 0.46 |

* From intra-amniotic injection of medication to abortion of fetus

\+ Standard error of the mean

TABLE III

OUTCOME

| | No. | Complete Abortion No. | Complete Abortion % | Incomplete Abortion No. | Incomplete Abortion % | Failure No. | Failure % |
|---|---|---|---|---|---|---|---|
| Urea + Oxytocin | 650 | 385 | 59.2 | 245 | 37.7 | 20 | 3.1 |
| Urea + $PGF_2a$ (20 mg) | 30 | 16 | 53.3 | 12 | 40.0 | 2 | 6.7 |
| Urea + $PGF_2a$ (10 mg) | 1083 | 566 | 52.2 | 500 | 46.2 | 17 | 1.6 |
| Urea + $PGF_2a$ ( 5 mg) | 349 | 222 | 63.6 | 124 | 35.5 | 3 | 0.9 |

TABLE IV

REQUIREMENT FOR OXYTOCIN

| | Urea-$PGF_2a$ (10 mg) | Urea-$PGF_2a$ (5 mg) |
|---|---|---|
| No oxytocin | 31.8% | 30.7 |
| At 24 hours | 9.5 | 16.3 |
| Four hours after membrane rupture | 27.5 | 30.4 |
| With incomplete abortion | 31.2 | 22.6 |

## TABLE V

## COMPLICATIONS

| | Urea + Oxytocin (650 Cases) | | Urea + $PGF_2a$ (20 mg) (30 Cases) | | Urea + $PGF_2a$ (10 mg) (1083 Cases) | | $PGF_2a$ (5 mg) (349 Cases) | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| Hemorrhage (500 cc or* need for curettage) | 35 | 5.4 | 3 | 10.0 | 102 | 9.4 | 31 | 8.9 |
| Endometritis | | | | | | | | |
| Hospitalization required | 29 | 4.5 | 1 | 3.3 | 54 | 5.0 | 6 | 1.7 |
| Outpatient treatment | 30 | 4.6 | 1 | 3.3 | 66 | 6.1 | 11 | 3.2 |
| Cervical Laceration | | | | | | | | |
| Transverse | 6** | 0.9 | 1 | 3.3 | 4 | 0.4 | 3 | 0.9 |
| Vertical | 0 | — | 0 | — | 31 | 2.9 | 5 | 1.4 |
| Gastrointestinal | | | | | | | | |
| Nausea-vomiting | 347 | 53.4 | 22 | 73.3 | 788 | 72.8 | 213 | 61.0 |
| Diarrhea | 5 | 0.8 | 1 | 3.3 | 51 | 4.7 | 9 | 2.6 |

* Four patients required blood transfusion

** Post-abortal examinations not routine for this entire group

# INJECTION-ABORTION INTERVAL AND THE HISTOPATHOLOGY OF THE PLACENTA FOLLOWING SECOND TRIMESTER PREGNANCY TERMINATION

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ABSTRACT

Placentae spontaneously passed after second trimester terminations of pregnancy using either hypertonic sodium chloride or hyperosmolar urea plus prostaglandin $F_2a$ ($PGF_2a$) were examined to determine histological characteristics. The placentae of hypertonic sodium chloride terminations demonstrated a type of "coagulation necrosis" that has been described previously, while placentae of pregnancies terminated by hyperosmolar urea plus $PGF_2a$ showed a similar pattern in about one-half the cases but a histologic pattern of less severe damage in the remaining cases. The two groups showed no significant differences when characteristics such as injection-abortion interval or estimated hypertonicity of the fluid were examined.

## Introduction

The histopathologic changes in the placentae from second trimester pregnancy terminations induced by hypertonic sodium chloride have been described in several publications.[1-6] Characteristically, there is a zone of "coagulation necrosis" extending from the amniotic membrane for several millimeters into the substance of the placenta. Within this zone most of the vessels are thrombosed, most cell structure is lost, and at the junction of this zone with the normal placenta there is an intense inflammatory infiltrate (Fig. 1). These changes have been attributed to the hypertonicity of the sodium chloride solution.[1-6] In two cases, hypertonic glucose produced similar patterns.[4] In contrast, when placentae following intra-amniotic injection of prostaglandin $F_2a$ ($PGF_2a$) were examined, the only finding was apparent constriction of the chorionic plate vessels without necrosis of the substance of the placenta.[1]

In this study we have examined the placentae obtained from both hypertonic sodium chloride and hyperosmolar urea plus $PGF_2a$ induced abortions in an effort to detect any histologic variations.

## Materials and Methods

Our methods of pregnancy termination utilizing 20% sodium chloride[7] and urea plus $PGF_2a$[8] have been previously described. Briefly, 20% sodium chloride is placed into the amniotic cavity utilizing an exchange technique. Fifty cubic centimeters of amniotic fluid is first withdrawn, then 50 cc aliquots of sodium chloride are exchanged with amniotic fluid until a total of 350

cc has been exchanged. Intravenous oxytocin at a rate of 332 mu/min is then started and continued until completion of the abortion process.

In the case of hyperosmolar urea plus $PGF_2a$, about 200 cc of amniotic fluid is withdrawn followed by an infusion of 135 cc of a 59.7% concentration of urea. A test dose of 2.5 mg of $PGF_2a$ is then injected followed by an additional 2.5 mg in 5 minutes if no untoward reactions occur.

With both methods, all products are allowed to pass spontaneously except for the following circumstances. If the placenta has not passed spontaneously two hours after fetal abortion, instrumental removal is carried out under local anesthesia. In addition, if no products are passed by 48 hours from initial injection, evacuation of the uterine contents is performed. In the current study, only placentae spontaneously passed as the result of second trimester pregnancy terminations were utilized.

After the placentae were obtained, they were spread out, fixed overnight and then full thickness blocks were prepared from the central and peripheral portion of the placenta. Routine hematoxylin and eosin sections were prepared so that one could evaluate the full thickness of the placenta from the decidua basalis to the chorionic plate. The sections were then examined without knowledge of which abortifacient had been used to terminate the pregnancy.

## Results

Seventeen placentae from hypertonic sodium chloride induced abortions and 45 from terminations induced with hyperosmolar

urea plus $PGF_2a$ underwent study. It became immediately apparent that in addition to the pattern previously described for hypertonic sodium chloride, there was an additional pattern showing less severe damage and accordingly the cases were classified as to whether or not they displayed the type of damage previously described for sodium chloride abortions (Type A) or the less severe pattern (Type B).

All but one of the 17 placentae obtained from hypertonic sodium chloride induced abortions showed the severe type of zonal necrosis, "Type A," that had been previously described (Figure 1).[1-6] However, only approximately one-half of the hyperosmolar urea cases displayed the same type of damage. The remainder of the urea cases showed a less severe type of damage that was characterized by a more diffuse inflammatory infiltrate in the upper layers of the placenta, the absence of thrombosis of the chorionic plate vessels, and less severe cellular destruction in the tissues immediately beneath the amniotic surface of the placenta (Figure 2).

The cases of hyperosmolar urea were divided into two groups on the basis of this difference in placental pathology and an attempt was made to correlate the pathologic change observed in the placenta with the clinical characteristics (Table I). Although no statistical differences could be shown between the two groups, the injection-abortion interval was longer and the amount of fluid removed less in patients showing the more severe damage (Type A). Therefore a scattergram was used to plot injection-abortion intervals versus an estimate of relative urea concentration (Figure 3). For the estimate of intra-amniotic

relative concentration of urea, the ratio of fluid removed at amniocentesis over gestational age was utilized, assuming that if a large volume of fluid was removed from an early gestation it would result in the least volume of amniotic fluid left to dilute the urea. The scattergram was then divided into four quadrants utilizing the mean value of the injection-abortion interval and the mean value of the ratio of the amniotic fluid removed over gestational age. As shown, when one examines patients receiving a high estimated concentration of urea, those with longer injection-abortion intervals showed more severe damage ( Type A) while those with shorter injection-abortion intervals demonstrated less damage (Type B). However, when the estimated concentration of urea fell below the mean value, the injection-abortion interval did not appear to make a major difference in the outcome of the placenta pathology. It should be noted that these differences were not statistically significant.

Discussion

It has been postulated that the pathologic features seen in the placentae of pregnancies terminated with intra-amniotic hypertonic sodium chloride are due to the hypertonicity of the infused agent. Certainly the "coagulation necrosis" seen is most compatible with this explanation. The occurence of less severe damage suggests that hyperosmolar urea plus $PGF_2a$ in some instances produces less destruction of tissues than does hypertonic sodium chloride. It was our impression that differences in the rate or amount of tissue destruction might be associated with differences in the injection-abortion interval, but we were unable to

statistically correlate injection-abortion intervals with the amount of tissue destruction as indicated by the placental pathology, Figure 3 appears to suggest that a high concentration of urea in association with a long injection-abortion interval predisposes to the more severe type of damage (Type A), but if the interval is of shorter duration, there is a tendency to achieve less severe placental damage (Type B). Again however, these are not significant differences.

Previous studies have shown that the use of urea when compared with hypertonic sodium chloride is associated with a decreased frequency and magnitude of clotting factor change and a decreased frequency of clinical coagulopathy[8,9]. This difference may also be a reflection of the less severe tissue destruction associated with the use of hyperosmolar urea.

## LEGENDS

Fig. 1. Type "A" zonal necrosis. Cell detail in the amnion and chorion is lost. The zone of hemorrhage beneath them defines the zone of necrotic villi. This zone extends across most of the plate.

Fig. 2. Type "B" damage. In contrast to type "A", this placenta shows inflammation but no loss of cell detail in the amnion and chorion. Within the intervillous space there is no necrosis.

Fig. 3. Type "A" zonal necrosis is indicated by the solid dots (●) and type "B" damage by the open ones (○). The highest estimated concentrations of urea and the longest injection-abortion are questionably related to the most severe type of placental damage.

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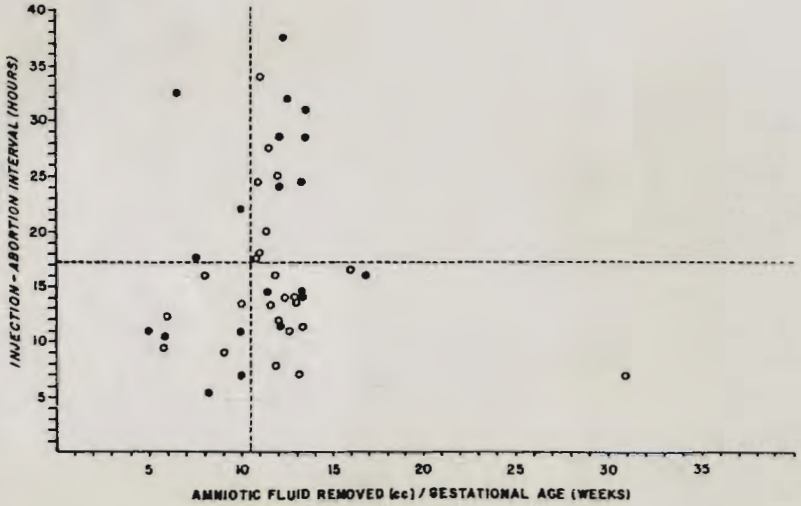
TABLE I

CLINICAL CHARACTERISTICS OF PATIENTS RECEIVING HYPEROSMOLAR UREA PLUS $PGF_{2}a$*

| athologic Class | Number | Age (Years) | Parity** | Duration of Gestation (Weeks) | Injection Abortion Interval (hrs) | Amniotic Fluid Removed (cc's) |
|---|---|---|---|---|---|---|
| Type A | 20 | 19.5(15-25) | 1.3(0-4) | 18.6(15-21) | 19.7(5.5-37.5) | 202(115-309) |
| Type B | 24 | 20.5(16-40) | 1.3(0-2) | 19.4(17-22) | 15.1(6-34) | 236(105-530) |

* The first number is the mean and the ranges are in parentheses.

** 5 primigravidas were contained within both the Type A and Type B groups.


INJECTION-ABORTION INTERVAL (HOURS)
40
35
30
25
20
15
10
5
5
10
15
20
25
30
35
AMNIOTIC FLUID REMOVED (cc) / GESTATIONAL AGE (WEEKS)

Project III

Study 2: In Vitro Studies.

Studies concerning the effect of oxytocin have been completed. Results have been abstracted in the Proceedings of the Endocrine Society and in a manuscript in preparation, an early draft of which is included. Another *in vitro* study involving drugs which affect calcium has also been completed. A manuscript entitled "Effect of EGTA and Sodium Azide on Contractile Activity and Prostaglandin Production of In Vitro Pregnant Rat Uteri" has been included in this report and submitted to a Journal for publication.

THE ROLE OF PROSTAGLANDINS IN SPONTANEOUS AND OXYTOCIN-INDUCED UTERINE CONTRACTIONS.

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The role of prostaglandins (PG) on spontaneous and oxytocin (OXY)-induced uterine contractions was studied. Uterine segments from pregnant rats under 2 gm tension were monitored for isometric contractile activity in a 25 ml Krebs ringer medium (95% $O_2$, 5% $CO_2$; 37° C). The medium was sampled periodically and assayed for PGE and F by radioimmunoassay. In spontaneously contracting 21 Day pregnant rat uteri (n = 26), PG production correlated with frequency of contraction (r = 0.69 and 0.62 for PGF and PGE respectively; P<.001) and integrated contractile force (ICF) (r = 0.48 and 0.47 for PGF and PGE respectively; P<.05) but not with average amplitude or duration of the contractile wave. Using 18 Day pregnant rat uteri, OXY (20 mUnits) increased ICF (92.8 ± 32.9 SEM gm-min/gm tissue for a 45 minute experimental period) compared to untreated uteri (22.6 ± 10.4 gm-min/gm) but did not affect PGE or PGF concentrations in the medium. Preincubation of uterine tissue with indomethacin (INDO, 11.2 μM) reduced PGF from 40.4 ± 11.7 to 5.8 ± 1.7 ng/gm tissue and PGE from 5.0 ± 2.0 ng/gm to undetectable levels. ICF decreased to 3.4 ± 1.4 gm-min/gm. Despite suppressed production of PG's, OXY also caused an increase in ICF in tissues pretreated with INDO for 15 minutes (45.1 ± 12.1 gm-min/gm) or 30 minutes (30.1 ± 8.8 gm-min/gm) compared to uteri treated with indomethacin alone (3.4 ± 1.4 gm min/gm). These data demonstrate that OXY can increase uterine ICF even when PG production is inhibited, but suggest that some minimal level of PG is required for the maximal response.

(Supported by Contract AID/csd-3627)

Endocrine Society 60th Annual Meeting-Abstract #896, 1978.

Early Draft
Revised

# ROLE OF PROSTAGLANDIN PRODUCTION IN SPONTANEOUS AND OXYTOCIN-INDUCED UTERINE CONTRACTILE ACTIVITY IN *IN VITRO* PREGNANT RAT UTERI

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King, T.M.

## INTRODUCTION

Oxytocin induced prostaglandin production by uterine tissue has been demonstrated in several species including rats (Roberts et al., 1976) and sheep (Roberts and McCracken, 1976). Inhibition of uterine contractions induced by oxytocin by prostaglandin systhetase inhibitors is reported by some investigators (Laudanski et al., 1977), however, others report no effect (Fuchs et al., 1976; Roberts et al., 1976). Differences in species and reproductive states complicate interpretation. Only a few studies have been done using pregnant uteri, an appropriate model if one is interested in labor induction. Fuchs et al. (1976) reported oxytocin induced uterine contractions in the presence of indomethacin treatment in pregnant rats. Chan (1977) has reported increased prostaglandin production in pregnant rat uteri *in vitro* and hypothesizes that the marked increase in sensitivity of the parturient uterus to oxytocin is related to an enhanced prostaglandin synthesis and release.

The present study explores the relationship between prostaglandin and uterine activity by simultaneously measuring uterine contractile activity and prostaglandin production in isolated pregnant rat tissues. While spontaneous contractions are correlated with prostaglandin production in these tissues evidence is presented showing uterine activity can be induced by oxytocin in the presence of indomethacin despite documented reductions in prostaglandin production.

## MATERIALS AND METHODS

### Tissue Preparations

Sprague-Dawley rats, purchased timed pregnant (day after cohabitation = day 1) from Charles River, Wilmington, Massachusetts, were killed by cervical dislocation. The uterine horn containing the most implantations was slit

longitudinally and fetal tissues, placenta and membranes were removed. A uterine segment containing both endometrial and myometrial tissue measuring approximately 1 x 2 cm and weighing approximately 1 gm (wet weight) was suspended in a 25 ml buffer bath (Krebs bicarbonate Ringer pH 7), maintained at 37°C, and continuously gassed with 95% $O_2$, 5% $CO_2$. Two segments from each animal were studied concurrently in separate chambers. A 2 gm tension was applied to the uterine segment and isometric contractions were monitored via a Statham strain gauge transducer (Model UC2) and recorded on a Gould Brush Multichannel polygraph (Model 260). An integrator Circuit (Integrator Coupler Model 11-4307-15) permitted continuous monitoring of the area under the contraction curve. The integrator was standardized against a 1 gm tension (Figure 1) and integrated contractile force (ICF) was expressed as gm-min (ICF; see Figure 1). The portion of the curve representing basal tone was continuously readjusted to zero on the polygraph. Thus, the integrated contractile force represented area under contractile waves and did not include changes due to tone.

Study 1:

Uteri from 19 individual 18 day pregnant rats and 26 individual 21 day pregnant rats were studied for spontaneous activity. Following a 15 minute equilibration period, the bath chamber was emptied and refilled with fresh oxygenated, buffer, 37°C, a process taking approximately 5 seconds. Contractile activity was then monitored for 15 minutes. Frequency of contractions, average amplitude and average duration of contractile waves, and/or integrated contractile force was determined for each tissue. At the end of the incubation period, an aliquot of the bathing medium was analyzed for prostaglandin $E_2$ (PGE) and prostaglandin $F_{2\alpha}$(PGF) concentration. Comparisons between gestational ages were made by students t test. Correlation coefficients were determined between each of the measurements in the 21 day pregnant uteri.

Study 2:

In a preliminary experiment, increasing doses of oxytocin (Pitocin[R], Parke-Davis) were added in a stepwide fashion to an 18 day pregnant rat uterine segment. Isometric tension increased linearly with logarithmic increases in dose. The minimal amount added to the bath which stimulated contraction was 2.5 mU while maximal response occurred when 160 mU was added. Half maximal response was achieved with 20 mU and this dose was chosen for the following experiment.

To the bath buffer was added oxytocin (20 mU), indomethacin (11.2 μM), both of these drugs, or neither. On each day of experiment, each of the four treatment groups was represented. The experiment was replicated on six different days. All tissues were preincubated in the bath chambers 15 minutes prior to the experimental period. Those tissues receiving indomethacin had this drug added to the bath during the preincubation as well as the experimental period. At the beginning of the experimental period the bath buffer was emptied from the chamber and refilled. An initial 1 ml sample of buffer was taken immediately and replaced with fresh buffer. Treatment drugs were added at this time. At 15 minute intervals additional 1 ml samples were taken and replaced by fresh buffer for a 45 minute experimental period.

PGE, PGF and PGFM production as well as ICF and frequency of contractions for each 15 minute interval were determined. Only those contractions having an amplitude greated or equal to 1 gm tension were counted for the frequency determination.

Data was subjected to analysis of variance with F ratios used to test for significance between treatments.

PGF Assay

Antibody to prostaglandin $F_{2\alpha}$ was made in rabbit following the carbodiimide reaction to link the antigen to bovine serum albumin (Caldwell et al., 1971). PGF was analyzed by radioimmunoassay by the method of Salmon and Amy . Samples of incubation medium (0.1 or 0.2 ml) were incubated at 4°C overnight with 0.5 ml antiserum (titre = 1:20,000) and 5,6,8,9,11,12,14,15-$^3$H(N)-Prostaglandin $F_{2\alpha}$ (specific activity; 120 Ci/mmol; New England Nuclear, Boston, Mass.) in a final volume of 0.8 ml of gel phosphate buffer. Dextran coated charcoal was used to precipitate free antigen. The antibody bound antigen was decanted into a toluene based scintillation fluid containing 33% Triton X and counted in a Beckman liquid scintillation counter (Model LS-233) with a counting efficiency of 55.3% for tritium. The limit of detection of the standard curve was 12 pg. Cross reactions with related prostaglandins are as follows: $PGF_{1\alpha}$, 56%; 13,14-dihydro-prostaglandin $F_{2\alpha}$, 2%; 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, < 1%; $PGA_2$, < 1%, $PGB_2$, < 1%; $PGE_2$, < 1%.

PGE Assay

Antibody to prostaglandin $E_2$ was made in rabbit by coupling the antigen to bovine serum albumin using tributylamine and isobutyl chloroformate (Goldyne et al., 1973). The radioimmunoassay for PGE was analogous to that described for PGF. The limit of detection of the standard curve was 12 pg. Cross reactions were as follows: $PGE_1$, 130%; $PGF_{2\alpha}$, < 1%; $PGA_2$, < 1%; $PGB_2$, < 1%.

Within assay coefficient of variation was less than 10% and between assay less than 15% for both of these assays.

PGFM Assay

A standard curve was prepared in tricine buffered saline (TBS) containing gelatin (1 mg/ml) for the range of 0 to 320 pg PGFM. Antiserum prepared by the

carbodiimide method (Caldwell et el., ) was diluted with buffer (1:20,000) and 0.1 ml of this dilution was added to a tube containing 0.1 ml unextracted sample, 0.1 ml standard and was incubated for 30 minutes at room temperature. (5,6,8,11,12,14(n) -$^3$H) 13,14-dihydro-15-keto-PGF (specific activity; 85 Ci/mM) was obtained from Amersham Searle Corporation, Chicago, Illinois. A 0.1 ml volume containing approximately 2500 counts per minute was added to each tube and incubated overnight at 4°C. The next day the antibody-bound complex was precipitated by addition of 1.0 ml of 68% saturated ammonium sulfate suspension containing 4% calcium sulfate (Hennam, 1974). The contents were mixed and allowed to stand at 4°C for 10 minutes. The tubes were centrifuged for 30 minutes at 2000 r.p.m. at 4°C. 0.8 ml of supernatant (free phase) was added to 10 ml of toluene based scintillation fluid containing 33% Triton X and counted for 10 minutes. The corresponding values were read from the standard curve and expressed in terms of PGFM concentration (pg/ml) of sample.

The minimum mass of PGFM necessary to cause a displacement of $^3$H-PGFM from the antibody which was significantly different from the zero tube was 1.25 pg and the working range of the standard curve extended to 100 pg. The within assay coefficient of variation was 5% and the between assay coefficient of variation was 16%. The cross reactions of structurally related prostaglandins were as follows: 13,14-dihydro-prostaglandin $F_{2\alpha}$, 3%; 15-keto-prostaglandin $F_{2\alpha}$, .01%; 13,14-dihydro-15-keto-prostaglandin $E_2$, 1%; 15-keto-prostaglandin $E_2$, $<$.01%; prostaglandin $F_{2\alpha}$, .01%.

For all prostaglandin analyses, a correction factor (see appendix, Dubin et al., 1977) was used in determining the concentration of samples from each time period since each subsequent sample was, in effect, diluted by the addition of fresh buffer. Prostaglandin production was calculated by

determining the difference in corrected concentrations between sequential samples. All samples were analyzed in duplicate.

## RESULTS

### Study 1

Spontaneous ICF and prostaglandin E and F production was greater in uteri from 21 day pregnant rats compared to uteri from 18 day pregnant rats (Table 1).

Table 2 illustrates correlation between prostaglandin concentrations and uterine contractile activity. Uterine frequency ($P < .001$) and ICF ($P < .05$) both show significant positive correlation with PGE and PGF concnetration in the bathing medium. The prostaglandin concentrations are not correlated with average duration or average amplitude during the incubation period. Other significant correlations include a positive relationship between PGE and PGF concentration in the bath ($P < .001$): ICF and average amplitude ($P < .01$); ICF and average duration ($P < .05$); average duration and average amplitude ($P < .05$).

### Study 2

Representitive tracings of each study group are presented in Figure 2. Quantitative evaluation of the contractile activity is presented below.

PGE production by the uterus was essentially blocked while PGF and PGFM production were greatly reduced ($P < .001$) following indomethacin treatment (Figure 3). Addition of oxytocin to the bath did not affect production of any of the prostaglandins measured.

Indomethacin caused a decreased frequency, while oxytocin increased frequency, primarily during the first 15 minutes incubation (Figure 4). The oxytocin effect on frequency was not blocked by indomethacin. ICF decreased following addition of indomethacin to the bath. Oxytocin greatly enhanced ICF during the entire incubation period. Indomethacin had little effect on

oxytocin-induced increase in ICF during the first 15 minute period of incubation, but thereafter oxytocin-induced ICF was reduced by the presence of indomethacin.

## DISCUSSION

Vane and Williams (1973) originally showed an increase in bioassayable prostaglandin production by *in vitro* rat uteri with advancing gestational age. The present study shows higher production of PGE and F and 21 days of pregnancy compared to 18 days. This increase in prostaglandin production is associated with increased uterine contractility as expressed as ICF. Prior studies indicated what ICF was significantly correlated with PGF or PGE production in tissues throughout the gestational range of 16 to 21 days (Dubin, 1977b). The present study shows a correlation between prostaglandin production and uterine activity still exists when only one gestational age is analyzed. Indomethacin inhibits PG production on both 18 and 21 days of pregnancy and this is correlated with an inhibition of ICF. Also PG's are known to stimulate contractions of *in vitro* rat uteri (Paton and Daniel, 1967; Chan et al., 1974). The preceding findings provide evidence that the spontaneous contractions of *in vitro* uteri are mediated by prostaglandin production. This conclusion should be tempered, however, since it is possible that there may be other smooth muscle-stimulating products of the arachadonic cascade (Ramwell et al., 1977) which also are inhibited by indomethacin.

Does oxytocin exert its well known effects on uterine contractions through prostaglandin release? The major source of prostaglandin production appears to be the endometrium (Anteby, 1975; Dowing and Williams, 1977) and this tissue appears to have specific high affinity receptors for oxytocin (Roberts et al., 1976; Soloff et al., 1977). Oxytocin reportedly stimulates

prostaglandin production by uterine tissue in the rat (Roberts et al., 1976; Chan, 1977) sheep (Roberts and McCracken, 1976), and rabbit (Small et al., 1973). In contrast oxytocin was not found to have an effect on prostaglandin production in non-pregnant in vitro rat uteri (Ishikawa and Fuchs, 1978). Our studies fail to demonstrate increased PGE or PGF production following addition of oxytocin to the bath at a dose which greatly enhances uterine contractility. It is possible that PGF is being produced and being rapidly metabolized so that no increase in concentration is seen; however, PGFM one major metabolite of PGF was also measured and no increased production was found.

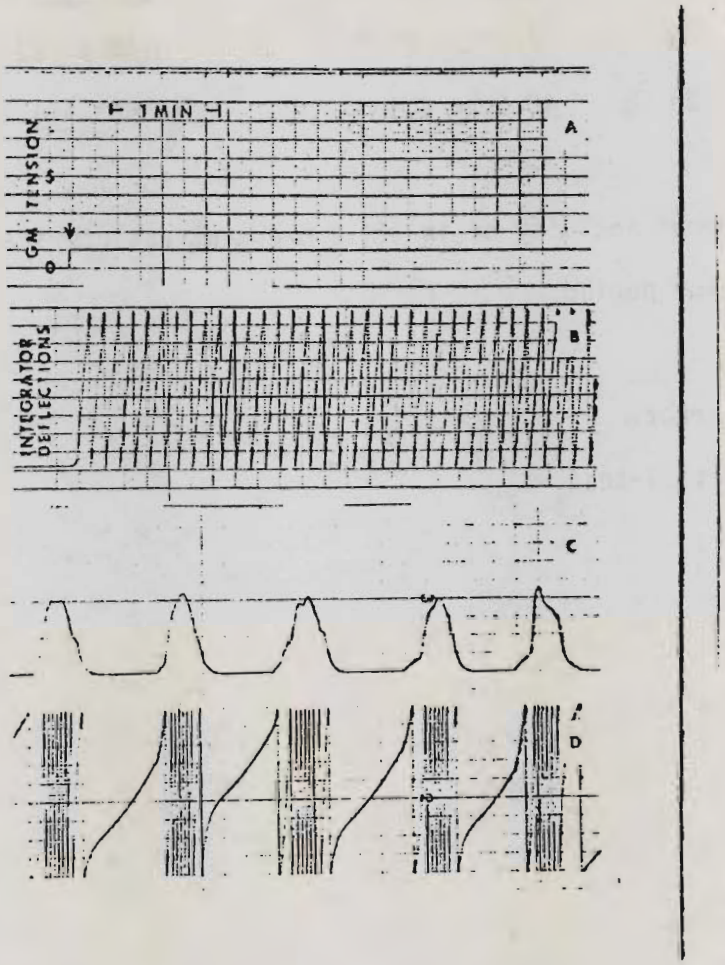
Chan (1977) who also studied in vitro rat uteri reports increased PG production. In those studies pooled uteri from rats at 19-22 days gestation were used for prostaglandin determinations by bioassay. Qualitative determinations of uterine contractions were made on other tissues. In our studies, uteri from 18 day pregnant rats were studied, prostaglandins were determined by radioimmunoassay and quantitative determinations of uterine contractile activity were made that could be related to prostaglandin levels in the media from the same tissues.

Oxytocin not only caused uterine contraction without concommitant increases in PG, it was also able to do so vis a vis documented reductions in prostaglandin. Following addition of indomethacin to the bathing media PGF production was reduced to approximately half the control value for the first 15 minutes of the experimental period; PGE production was essentially blocked. Despite the inhibition of PG production oxytocin still caused increased frequency of contractions and ICF to levels near that of the oxytocin treated tissue without indomethacin. During the next 30 minute period, the frequency of contractions remained the same in oxytocin treated tissues regardless of indomethacin treatment. The strength of those contractions, however, was

diminished in indomethacin treated tissues as reflected by decreased ICF. Thus, while oxytocin can cause increased contractions without increased PG levels, the maximal contractile response of uterine tissue to oxytocin may depend on some threshold level of prostaglandin. Synergistic effects of PG's and oxytocin have been previously reported (Brunner, 1971).

Figure 1. (A) Tracing following placement of 1 gm wt on transducer at the time indicated by the arrow. (B) Tracing of the deflections of the integrator circuit corresponding to tracing A. Thus, 1 gm = x deflections/min and each deflection = gm-min/x. (C) Spontaneous activity in a 21 day pregnant rat uterus. (D) Tracing of deflections in the integrator circuit corresponding to tracing C. Deflections are counted and the integrated contractile force is computed. ICF = (number of deflections)(gm-min/x).

Figure 1



SPONTANEOUS ACTIVITY IN VITRO UTERI FOR 15 MINUTE INCUBATION

| Pregnancy Day | n | ICF gm-min/gm-tissues | PGE ng/gm-tissues | PGF ng/gm-tissues |
|---|---|---|---|---|
| 18 | 19 | 7.24[a] ± 2.10 | 3.80 ± .87 | 29.14 ± 4.0 |
| 21 | 26 | 40.6* ± 4.1 | 16.66* ± 1.64 | 88.34* ± 7.61 |

Table 1: Spontaneous activity of *in vitro* pregnant rat uteri after a 15 minute incubation period.

[a]Mean ± standard error.

*P<.001 by Students' T-test.

| | PGE | PGF | Average Duration | Average Amplitude | Frequency | ICF |
|---|---|---|---|---|---|---|
| PGE (4.5-34.2 ng/gm tissue) | - | 0.63*** | -0.25 | 0.05 | 0.62*** | 0.48* |
| PGF (29.2-175.6 ng/gm tissue) | 0.63*** | - | -0.10 | 0.05 | 0.69*** | 0.47* |
| Average Duration (22.4-49.3 + sec.) | -0.25 | -0.10 | - | 0.47* | -0.25 | 0.14 |
| Average Amplitude (2.5-10.3 + gms) | 0.05 | 0.05 | 0.47* | - | 0.08 | 0.55** |
| Frequency (4-15 contractions/ 15 min.) | 0.62*** | 0.69*** | -0.25 | 0.08 | - | 0.57** |
| ICF (7.2-100.0 gm min/gm tissue) | 0.48* | 0.47* | 0.14 | 0.55** | 0.57* | - |

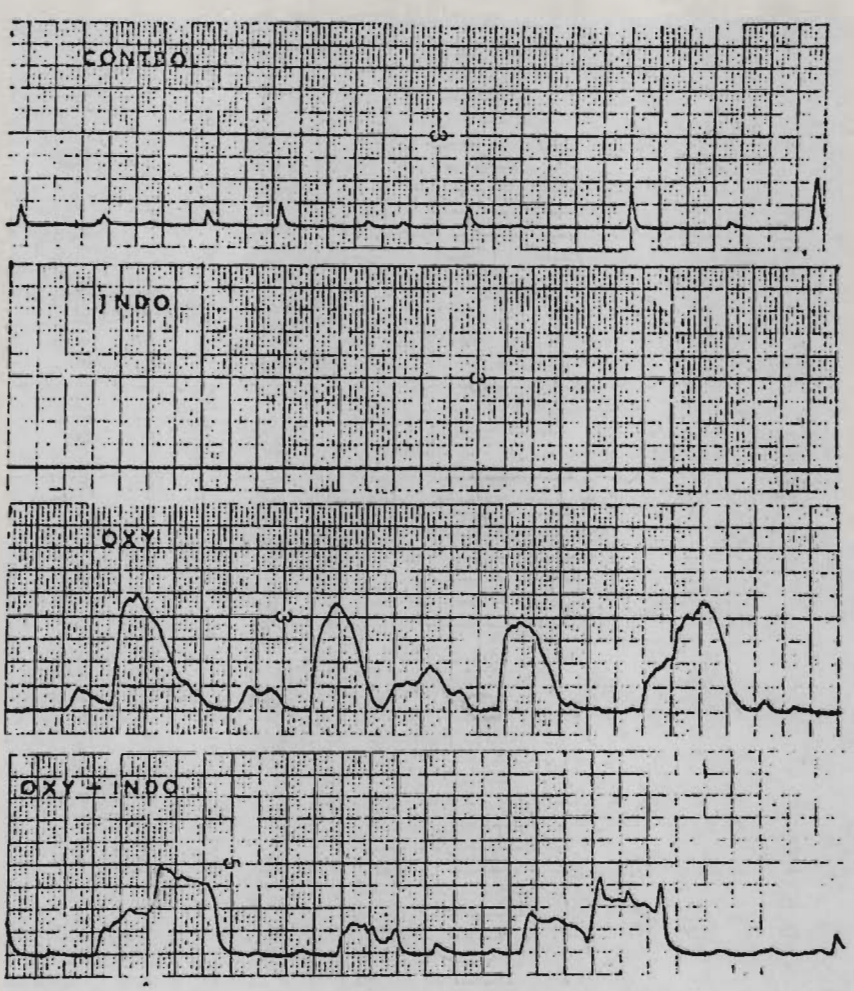
Table 2: Correlation coefficients between various uterine contractile activities and prostaglandin concentrations in spontaneously contracting 21-day pregnant rat uterine segments in vitro. Numbers in parentheses are the range for each measurement. Number at each intersection is the product-moment correlation coefficient as determined in 26 uterine segments.

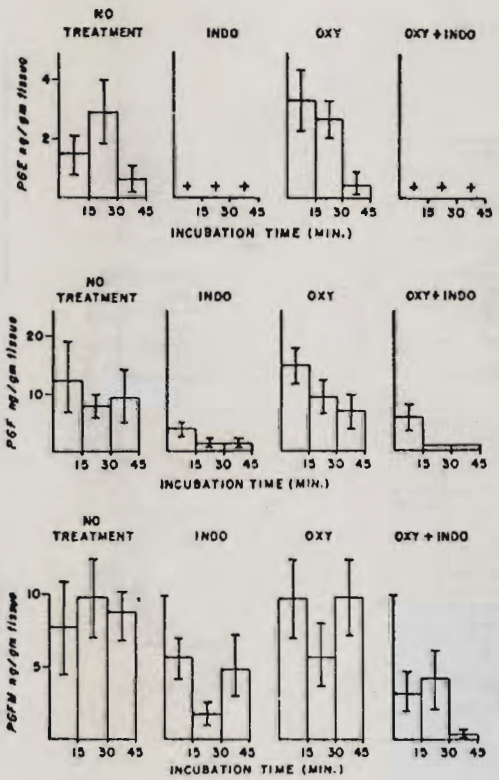
*P<.05

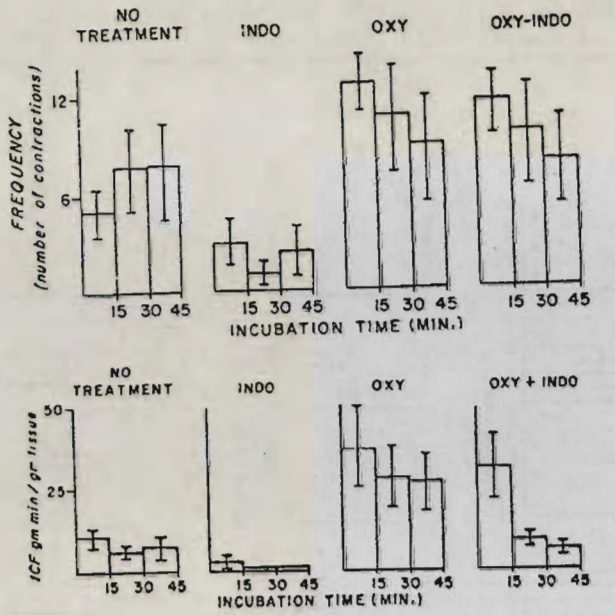
**P<.01

***P<.001

Figure 2




NO TREATMENT
INDO
OXY
OXY + INDO
PGE ng/gm tissue
4
2
15 30 45
INCUBATION TIME (MIN.)
PGF ng/gm tissue
20
10
PGFM ng/gm tissue
10
5


NO TREATMENT
INDO
OXY
OXY-INDO
FREQUENCY (number of contractions)
12
6
15 30 45
INCUBATION TIME (MIN.)
NO TREATMENT
INDO
OXY
OXY + INDO
ICF gm min/gr tissue
50
25
15 30 45
INCUBATION TIME (MIN.)

# EFFECT OF EGTA AND SODIUM AZIDE ON CONTRACTILE ACTIVITY AND PROSTAGLANDIN PRODUCTION OF *IN VITRO* PREGNANT RAT UTERI

RUNNING TITLE: Uterine Prostaglandins and Contractions

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## ABSTRACT

Two drugs which are reported to affect calcium movement, namely ethylene glyco bis (β-aminoethylether)N, N-tetraacetic acid (EGTA) and sodium azide ($NaN_3$) were studied for their effects on uterine contraction and prostaglandin production. Uteri from rats 21 days pregnant were examined for isometric contractile activity by determining the frequency of contractions and/or integrated contractile force (ICF). In addition the bath medium was sampled at 15 minute intervals for subsequent prostaglandin $F_{2\alpha}$ (PGF), prostaglandin $E_2$ (PGE) and 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) determinations by radioimmunoassay. Addition of EGTA to the bath virtually eliminated contractile activity. Prostaglandin production was not greatly affected by EGTA indicating that contractions *per se* play a relatively minor role in prostaglandin production by this *in vitro* system. In a separate study, $NaN_3$ added to the bath caused an initial increase in frequency, but a decrease in ICF. There was a dramatic increase in PGE, PGF and PGFM production following addition of $NaN_3$ to the bath. This increase could be prevented by preincubation with indomethacin.

## INTRODUCTION

The role of calcium in uterine contractions has been widely investigated (Abe, 1971; Carsten, 1973a;Malmstrom and Carafoli, 1977; Wanner et al., 1977). There is evidence that prostaglandins may cause myometrial contraction by facilitatin calcium movement into the cell (Torok and Csapo, 1976) or by affecting intracellular calcium movements (Carsten, 1972). The ionophoric activity of prostaglandins may be due to aggregation of prostaglandins with the calcium ion, producing a lipid soluble complex (Bikhazi et al., 1977). On the other hand, recent studies have also implicated a role for calcium in prostaglandin production (Victoria et al., 1971; Lands and Rome, 1976; Rittenhouse-Simmons et al., 1977). This study reports on two drugs which affect calcium movement, namely ethylene glycol bis (β-aminoethylether) N,N-tetraacetic acid (EGTA) and sodium azide ($NaN_3$), and examines their effect on in vitro uterine contractile activity while simultaneously measuring prostaglandin release by this tissue.

## MATERIALS AND METHODS

### Tissue Preparations

Sprague-Dawley rats, purchased timed pregnant from Charles River, Wilmington, Masachusetts, were killed on day 21 of pregnancy (day after cohabitation = day 1) by cervical dislocation. The uterine horn containing the most implantations was slit longitudinally and fetal tissues, placenta and membranes were removed. A uterine segment containing both endometrial and myometrial tissue measuring approximately 1 x 2 cm and weighing approximately 1 gm (wet weight) was suspended in a 25 ml buffer bath (Krebs bicarbonate Ringer pH 7), maintained at $37^{0}$ C, and continuously gassed with 95% $O_2$, 5% $CO_2$. Two segments from

each animal were studied concurrently in separate chambers. A 2 gm tention was applied to the uterine segment and isometric contractions were monitored via a Statham strain gauge transducer (Model UC2) and recorded on a Gould Brush Multichannel polygraph (Model 260). An integrator circuit (Integrator Coupler Model 11-4307-15) permitted continuous monitoring of the area under the contraction curve. The integrator was standardized against a 1 gm tension monitored over 1 minute and integrated contractile force (ICF) was expressed as gm - minutes per gm tissue per unit time. The portion of the curve representing basal tone was continuously readjusted to zero on the polygraph. Thus, all the integrated contractile force represented area under contractile waves and did not include changes due to tone.

Study 1

For each pair of uterine segments, one served as a control while the other received EGTA (final concentration = 10 mM). Pairs from six rats were studied. Following a fifteen minute equilibration period the buffer was completely removed from the chamber and replaced with fresh oxygenated $37^{o}$ C buffer. Immediately a 1 ml sample was removed for subsequent prostaglandin analysis and replaced with 1 ml fresh buffer. This was considered the beginning (time = 0) of the experimental period. If EGTA was to be added it was done at this time. Other 1 ml samples were taken and replaced at 15 minute intervals for a 45 minute experimental period. Prostaglandin $F_{2\alpha}$ (PGF), prostaglandin $E_2$ (PGE), and 13,14-dihydro-keto-prostaglandin $F_{2\alpha}$ (PGFM) production and ICF for each 15 minute interval were determined.

Study 2

To the bath buffer was added either $NaN_3$ (20 mM), indomethacin (11.2 μM),

both of these drugs, or neither. On each of the 6 days of experiment, each of the 4 treatment groups was represented. Treatments were assigned to uterine segments so as to average differences among uteri to zero. All tissues were preincubated in the bath chambers 15 minutes prior to the experimental period. Those tissues receiving indomethacin had this drug added to the bath during the preincubation as well as the experimental period. At the beginning of the experimental period the bath buffer was emptied from the chamber and refilled. An initial 1 ml sample of buffer was taken immediately and replaced with fresh buffer. Treatment drugs were added at this time. At 15 minute intervals additional 1 ml samples were taken and replaced by fresh buffer during a 45 minute experimental period.

PGF, PGE and PGFM production as well as ICF and frequency of contractions for each 15 minute interval were determined. Only those contractions having an amplitude greater than or equal to 1 gm tension were counted for the frequency determination.

PGFM Assay

A standard curve was prepared in tricine buffered saline (TBS) containing gelatin (1 mg/ml) for the range of 0 to 320.0 PGFM. Antiserum prepared by the carbodiimide method (Caldwell *et al.*, 1971) was diluted with buffer (1:20,000) and 0.1 ml of this dilution was added to a tube containing 0.1 ml unextracted sample, 0.1 ml standard or 0.1 ml TBS (zero tube) and was incubated for 30 minutes at room temperature. (5,6,8,11,12,14 (n) -$^3$H) 13,14-dihydro-15-keto-PGF (specific activity; 85 Ci/mM) was obtained from Amersham Searle Corporation, Chicago, Illinois. A stock solution containing 10 μCi/ml in ethanol:water (7:3) was stored at -20$^{o}$ C. On the day of assay, 25 μl were removed, dried under nitrogen and redissolved in 106 ml of TBS. A 0.1 ml volume

(approximately 2500 c.p.m. with a mass of 10 pg) was added to each tube and incubated overnight at $4^{o}$ C.

The next day the antibody-bound complex was precipitated by addition of 1.0 ml of 68% saturated ammonium sulfate suspension containing 4% calcium sulfate as described previously (Hennam et al., 1974). The contents were mixed and allowed to stand at $4^{o}$ C for 10 minutes. The tubes were centrifuged for 30 minutes at 2000 r.p.m. at $4^{o}$ C. 0.8 ml of supernatant (free phase) was added to 10 ml of Toluene based scintillation fluid containing 33% Triton X and counted on a Beckman LS-233 scintillation counter for 10 minutes. The corresponding values were read from the standard curve and expressed in terms of PGFM concentration (pg/ml) of sample. All samples were analyzed in duplicate.

The minimum mass of PGFM necessary to cause a displacement of $^{3}$H-PGFM from the antibody which was significantly different from the zero tube was 1.25 pg and the working range of the standard curve extended to 100 pg. The within assay coefficient of variation was 5% and the between assay coefficient of variation was 16%. The cross reaction of structurally related prostaglandins was tested for their relative ability to displace the $^{3}$H-PGFM from the antibody relative to PGFM. The results found were as follows: 13,14-dihydro-prostaglandin $F_{2\alpha}$, 3%; 15-keto-prostaglandin $F_{2\alpha}$, .01%; 13,14-dihydro-15-keto-prostaglandin $E_2$, 1%; 15-keto-prostaglandin $E_2$, <.01%; prostaglandin $F_{2\alpha}$, .01%.

PGF and PGE Assay

PGF was analyzed by radioimmunoassay by the method of Salmon and Karim (Salmon and Karim, 1975). The antiserum was provided by Dr. S. M. M. Karim, Singapore. PGE was assayed in an analogous manner to PGF. Within assay coefficient of variation was less than 10% and between assay less than 15%

for both of these assays. For all prostaglandin analyses, a correction factor (see appendix, reference Dubin et al., 1977) was used in determining the concentration of samples from each time period since each subsequent sample was, in effect, diluted by the addition of fresh buffer. Prostaglandin production was calculated by determining the difference in corrected concentrations between sequential samples.

Analysis of Data

In Study 1, the data was analyzed by 3 factor analysis of variance. Thus the relative effect of each of three factors (EGTA, time period of incubation, uteri) on each of the dependent variables (ICF, PGF, PGE, PGFM) could be assessed and any interaction determined. As a check, the data were also analyzed after airing data for each uterus.

In Study 2, the data was analyzed by 4 factor ($NaN_3$, indomethacin, time period of incubation, day of experimental replication) analysis of variance. The computations for the above factorial designs are fully described (Snedecor and Cochran, 1974) and were programmed for a Wang desk top computer. The programs were checked against previously worked examples prior to use.

RESULTS

Study 1

EGTA (10 mM) substantially reduced ICF ($P<.001$) with contractions virtually eliminated during the last 30 minutes of the experimental period (Figure 1). Small reductions in PGE ($P<.01$) and PGF ($P<.05$) were also observed (Figure 2). No change in PGFM production was observed following addition of EGTA to the media.

Time of incubation was an important determinant in PGE and PGF production, the greatest production occurring during the first 15 minutes of the

experimental period, and also PGFM where production was lowest during the final 15 minute period. ICF was similarly influenced by time of incubation; however, the effect was only significant in control tissues (compare F ratio of EGTA x time interaction with time main effect in Figure 1).

Variation among uteri was small compared to the other factors and, except for PGE, was not statistically significant (Figures 1, 2).

Study 2

Following addition of $NaN_3$ to the bath, there was an initial increase in frequency of contractions (Figure 3); however, there was an overall reduction in ICF. $NaN_3$ caused an increase in PGE for the first 15 minutes (Figure 4) and an increase in PGF and PGFM for the entire experimental period.

Indomethacin caused a reduction in frequency of contractions and ICF, to the greater extend in uteri not treated with $NaN_3$. Indomethacin reduced PGE, PGF and PGFM production regardless of azide treatment. Note that large interactions involving indomethacin are present on the analysis for each dependent variable studied. Statistically, these interactions imply that the effect of indomethacin is not additive with other mean effects; biologically, they result from the virtually total inhibition of response by indomethacin treatment, regardless of control levels of response.

Time period of incubation was a significant factor in PGF production, PGE production, frequency of contraction, and ICF, but not for PGFM. For frequency and PGE, the influence of time period of incubation depended on which other factors were present (see interactions in Figures 3 and 4).

Day of experiment was a relatively minor source of variation for all of the measurements.

## DISCUSSION

In the present study EGTA, a chelating agent with high specificity for the calcium ion, essentially blocked the spontaneous uterine contractions of the 21 day isolated pregnant rat uterus. PGF and PGE production by this tissue decreased, but only slightly. Thus, for this *in vitro* system, spontaneous contractions *per se* play a relatively minor role in prostaglandin production.

Sodium azide inhibits calcium uptake by mitochondrial fractions but not fractions containing sarcoplasmic reticulum in the pregnant human uterus (Carsten, 1973b). While sarcoplasmic reticulum is believed to play a major role in sequestering and release of intracellular calcium during smooth muscle contraction, the role of mitochondria is not clear (Devine *et al.*, 1972; Huddart and Price, 1976), but there is evidence that a high intracellular calcium concentrations, mitochondria is an important calcium control system in the rat myometrium (Janis *et al.*, 1977). In the present study, addition of sodium azide to the bathing medium initially increased frequency of contractions, but the size of contractions was smaller than in spontaneously contracting control uteri (Figure 5) and the overall ICF was reduced.

While *in vitro* spontaneous prostaglandin production and contractile activity are positively associated (Vane and Williams, 1973; Dubin *et al.*, 1976), the decreased ICF in $NaN_3$ treated tissue was associated with a dramatic increase in PGF and PGE concentration in the bath. The increased levels of PGFM suggest that the high concentration is due to increased production rather than decreased metabolism.

$NaN_3$ may block calcium uptake by virtue of inhibition of calcium-dependent ATPase activity (Fanburg and Gergely, 1965). Ouabain, which blocks Na-K dependent ATPase activity also is reported to cause increased prostaglandin

synthesis (Coburn and Soltoff, 1977). If $NaN_3$ is blocking the reuptake of calcium by subcellular structures, high levels of free calcium may stimulate prostaglandin production since calcium has been shown to enhance phsopholipase activity (Victoria et al., 1971; Lands and Rome, 1976) and mobilization of arachadonic acid (Rittenhouse-Simmons, 1977). Alternatively azide is a known inhibitor of respiratory enzymes (Dixon and Webb, 1964) and energy from oxidative metabolism has been reported necessary for maintenance of intact lysosomal membranes (Lefer, 1976). Azide may simulate a hypoxic state in the tissue resulting in phospholipase release from lysosomes and consequently increasing prostaglandin synthesis; thus the $NaN_3$ effect may mimic a physiologic condition where the uterus is subjected to periodic hypoxic states during contractions. The hypothesis that hypoxia causes uterine release of prostaglandin is currently being tested in this system. Prostaglandin action on arterial smooth muscle is dependent upon energy derived from an oxidative metabolic pathway (Altura and Altura, 1976). The paradoxical reduction in ICF in the presence of increased prostaglandin, as seen in the present study, might also be explained by blocked oxidative metabolism.

## ACKNOWLEDGEMENTS

Thanks to Dr. J. E. Pike, Upjohn, Kalamazoo, Michigan for the generous supply of prostaglandins and Dr. J. H. Robinson of Merck, Sharp and Dohme, Rahway, New Jersey for the indomethacin. The secretarial assistance of Ms. Patricia Gottal is sincerely appreciated. These studies were supported by contract AID/csd-3627 and NICHD grant HD-06268. Part of this work was presented at the 10th Annual Meeting of the Society for the Study of Reproduction, 1977, Abstract No. 469.

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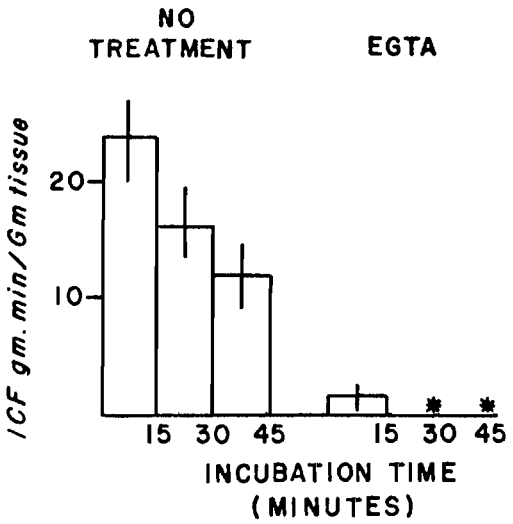
Figure 1. Effect of EGTA (10 mM) on ICF. Vertical lines represent $\pm$ standard error of the mean. * indicates undetectable contractions. For analysis of variance in this and following figures: n.s. = not significant ($P \geq .05$); S = significant, but F ratio is accounted for by an interaction of near equal or greater magnitude. All main effects of sources of variation are indicated above dotted line. Significant interactions are indicated below dotted line. Some significant but unimportant interactions may be omitted.

For this and next figure, degrees of freedom for the sources of variation are as follows: Uteri, 1; EGTA, 1; Time (i.e. time period of incubation, 2; residual, 10.

FIGURE 1

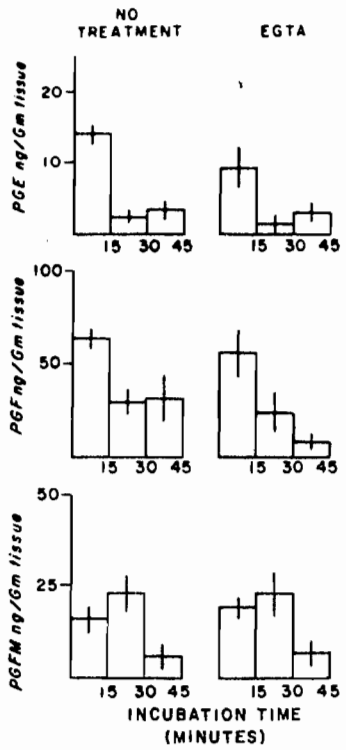



ANALYSIS OF VARIANCE

| Source | F ratio | |
|---|---|---|
| Uteri | 2.9 | n.s. |
| EGTA | 187.7 | P<.001 |
| Time | 16.8 | S |
| EGTA x Time | 11.1 | P<.001 |

Figure 2. Effect of EGTA (10 mM) on prostaglandin $E_2$ (PGE), prostaglandin $F_{2\alpha}$ (PGF) and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM). See Figure 1 for degrees of freedom in anaylsis of variance.

FIGURE 2




ANALYSIS OF VARIANCE

| Source | F ratio | |
|---|---|---|
| Uteri | 4.9 | P<.05 |
| EGTA | 11.8 | P<.01 |
| Time | 76.7 | P<.001 |

ANALYSIS OF VARIANCE

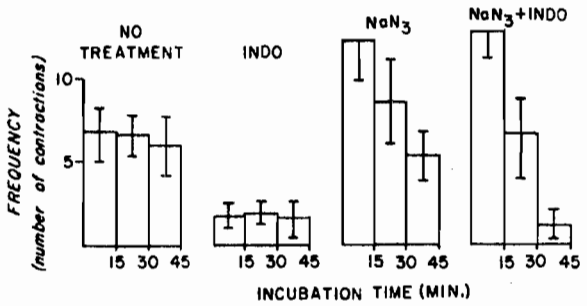
| Source | F ratio | |
|---|---|---|
| Uteri | 3.0 | n.s. |
| EGTA | 8.6 | P<.05 |
| Time | 136.6 | P<.001 |

ANALYSIS OF VARIANCE

| Source | F ratio | |
|---|---|---|
| Uteri | 0.2 | n.s. |
| EGTA | 3.8 | n.s. |
| Time | 5.5 | P<.05 |

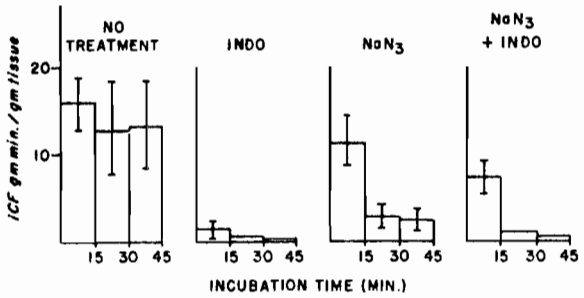
Figure 3. Effect of sodium azide ($NaN_3$, 20 mM) and Indomethacin (Indo, 11.2 μM) on frequency of contractions and integrated contractile force (ICF). For this and Figure 4, degrees of freedom for the sources of variation are as follows: Day (i.e. day of experimental replication), 5; $NaN_3$, 1; Indo, 1; Time (i.e. time period of incubation, 2; residual, 10.

FIGURE 3




ANALYSIS OF VARIANCE

| Source | F ratio | |
|---|---|---|
| Day | 3.5 | S |
| $NaN_3$ | 30.3 | P<.001 |
| Indo | 25.0 | P<.001 |
| Time | 18.4 | S |
| $NaN_3$ x Indo | 5.0 | P<.05 |
| $NaN_3$ x Time | 14.3 | P<.01 |

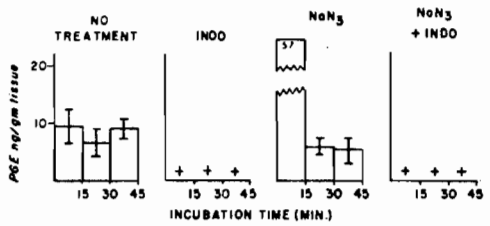



ANALYSIS OF VARIANCE

| Source | F ratio | |
|---|---|---|
| Day | 1.7 | n.s. |
| $NaN_3$ | 11.1 | S |
| Indo | 78.8 | P<.001 |
| Time | 11.9 | P<.01 |
| $NaN_3$ x Indo | 27.7 | P<.001 |

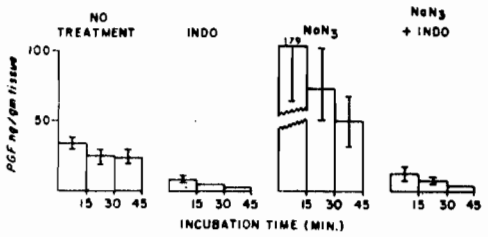
Figure 4. Effect of sodium azide ($NaN_3$, 20 mM) and indomethacin (Indo, 11.2 μM) on prostaglandin $E_2$ (PGE), prostaglandin $F_{2\alpha}$ (PGF) and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM). See Figure 3 for degrees of freedom in anaylsis of variance.

FIGURE 4



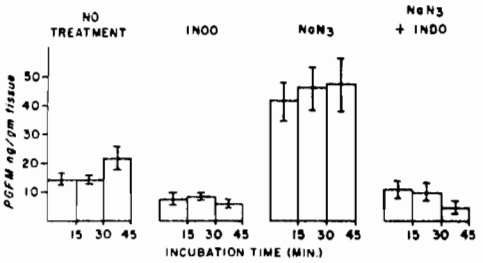

ANALYSIS OF VARIANCE

| Source | F ratio | |
|---|---|---|
| Day | 1.9 | n.s. |
| $NaN_3$ | 18.4 | S |
| Indo | 85.7 | P<.001 |
| Time | 27.0 | S |
| $NaN_3$ x Indo x Time | 23.0 | P<.001 |




ANALYSIS OF VARIANCE

| Source | F ratio | |
|---|---|---|
| Day | 2.2 | n.s. |
| $NaN_3$ | 34.7 | S |
| Indo | 87.6 | P<.001 |
| Time | 13.9 | P<.01 |
| $NaN_3$ x Indo | 31.2 | P<.001 |

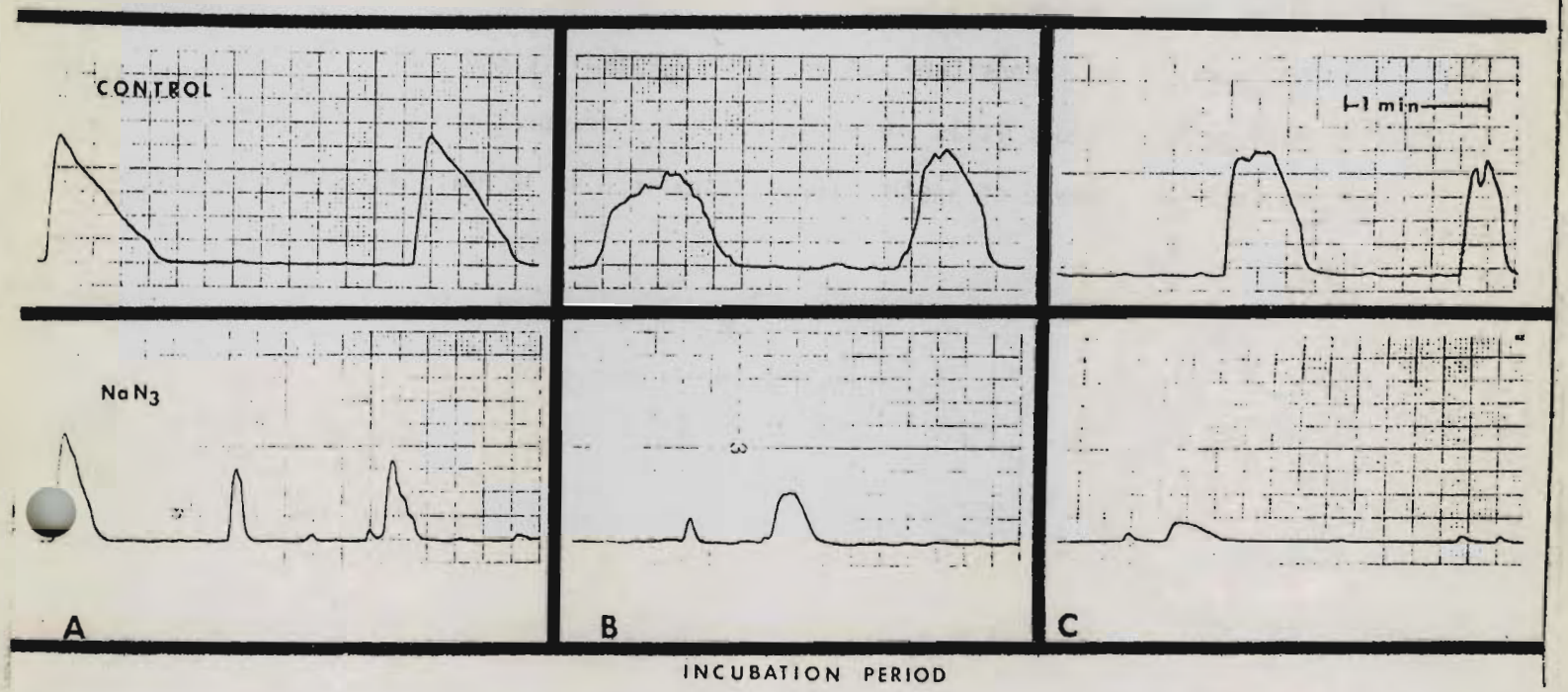



ANALYSIS OF VARIANCE

| Source | F ratio | |
|---|---|---|
| Day | 7.9 | S |
| $NaN_3$ | 67.7 | S |
| Indo | 159.0 | P<.001 |
| Time | 0.2 | n.s. |
| $NaN_3$ x Indo | 56.3 | P<.001 |

Figure 5. Representative portions of polygraph tracings of spontaneously contracting 21 day pregnant rat uteri (Control) and uteri with sodium azide ($NaN_3$, 20 mM) added to the bathing medium during the 1st (A), 2nd (B) and 3rd (C) 15 minute experimental period of incubation. Baseline is one major division from bottom of graph.

FIGURE 5



Study 3: Evaluation of Newer Prostaglandin Analogs

A study to test the efficacy of a 15 s 15 methyl $PGF_2a$ methyl ester suppository for abortion was completed. Enclosed for review is the report of that particular study.

In summary, the suppository was not overly effective and gastrointestinal side effects persisted. It is contemplated that a new protocol will be devised which will allow the use of another suppository in 8 to 12 hours if abortion has not occurred.

# EXPERIENCE WITH (15 s) - 15 METHYL PROSTAGLANDIN $F_2a$ METHYL ESTER SUPPOSITORIES

A number of studies have been carried out in an effort to investigate the efficacy and morbidity associated with the use of (15 s)-15 methyl prostaglandin $F_2a$ methyl ester (15 methyl $PGF_2a$). Bergstrom in Stockholm utilized the drug in a suppository form in doses of 0.5 and 1 mg for a total of 23 cases.[1] With the 1 mg suppository given at 6 hour intervals, 9 of 10 subjects aborted within 30 hours of treatment and five of these cases were complete abortions. Thirteen patients were treated with a regimen of 0.5 mg initially followed by subsequent doses of 2 mgs repeated at 3 hourly intervals. All 13 aborted within 24 hours with five patients aborting completely. Patients did experience, however, modest gastrointestinal side effects.

In another study, Bygdeman and Bergstrom reported on the use of the analog for 25 patients undergoing a midtrimester pregnancy termination.[2] These patients received 3 or 3.5 mg of the drug as a single vaginal suppository. Twenty-three of the 25 patients aborted without additional therapy with a mean treatment abortion interval of 13.$\pm$ 4.8 hours for nulliparous patients and 10.1 $\pm$ 3.6 hours for multiparous patients. The incidence of complete abortion was about 60%. Therefore because of the success in studies done at other centers, we elected to utilize the drugs in our study.

## MATERIALS AND METHODS

The medication consisted of 3 mg of 15 methyl $PGF_2a$ in a 2.2 gram Witapsol base to which was added a small amount of acetylsalicylic acid to insure stability. After routine pre-operative evaluation and laboratory determinations, 19 patients of gestational ages of 15 to 16 weeks from last menstrual period were selected for study.

The vaginal suppository was placed high in the posterior vaginal fornix. The patient was kept supine initially and at bed rest throughout the treatment interval. If she failed to abort within approximately 30 hours of initial treatment, other modalities were utilized to complete abortion such as intravenous oxytocin at 332 mu/min or curettage. Patients were monitored during the course of study with frequent vital signs. In addition, the incidence of complete, incomplete and failed abortion were analyzed as well as complications such as blood loss, gastro-intestinal side effects, fever and other untoward events.

RESULTS

The mean age of the patients was 21.3 with a range of 15 to 29 years. The racial distribution of the patients was 6 white patients and 13 non-white patients. Five patients were nulliparous whereas 14 had parities greater than 1 ranging from 1 to 4 previous term pregnancies. The mean duration of gestation as estimated from the last menstrual period and as documented by either sonography or fetal foot length was 15.6 weeks of gestation.

The results comparing the nulliparous and parous patients are indicated in Table I. As shown, for nulliparous patients, a failure rate of 80% was experienced; whereas for multiparous patients, the failure rate was reduced to approximately 36%. Overall, only slightly more than one-half of the patients experienced either an incomplete or complete abortion.

Table II lists the treatment intervals in hours according to parity. For the successful patients, as shown, the treatment abortion interval was approximately 18 hours for multiparous patients and the one nulliparous successful patient had a treatment abortion interval of 10.8 hours. When the failures were also examined, it was noted

that overall their treatment abortion interval averaged 37 hours. One of the failures was treated by oxytocin alone, 3 had oxytocin infusion followed by sponge-stick curettage to remove retained placentae, 3 patients required both oxytocin as well as vaginal evacuation to remove all the products of conception and one patient had a vaginal evacuation as the only means of managing her failed procedure.

## COMPLICATIONS

The mean blood loss associated with the procedure was 231.8 cc per patient with a range of 20 to 555 cc. Only 3 patients had blood loss exceeding 500 cc. Gastrointestinal side effects were also analyzed. Nine of 19 patients experienced no episodes of vomiting. Those patients that did experience vomiting had a mean number of episodes of 2.2 with the onset usually occurring approximately 6 hours after the placement of the suppository. Four of 19 patients did not experience diarrhea but for the 15 patients who did, the mean number of episodes was 3.5 with a mean onset 5.7 hours after initial treatment. Two patients experienced fever which subsided within 4 hours. Two additional patients experienced headaches, one patient was seen one week after abortion because of cramping, and another was seen for breast engorgement during the post-treatment interval.

The mean time from treatment to onset of uterine cramping was evaluated and was found to be 2.4 hours with no difference being noted between nulliparous and multiparous patients.

## DISCUSSION

The results from this small series utilizing 15 methyl $PGF_2a$ suppositories indicates that the drug as currently formulated and

utilized does not have a high degree of efficacy. The drug is reasonably well tolerated by most patients. However, gastro-intestinal side effects have not been eliminated. Review of our data suggests that perhaps the patients would benefit from a second suppository given somewhere between 8 and 12 hours after the initial suppository if active labor has not ensued.

## REFERENCES


1. Bergstrom, S., Bygdeman, M. (Karolinski Institute, Stockholm, Sweden - unpublished data).

2. Bygdeman, M., Bergstrom, S. et al. "Development of a Vaginal Suppository Suitable for Singel Administration for Interruption of Second Trimester Abortion," Contraception, in press.

TABLE I

| | OUTCOME* | | |
|---|---|---|---|
| | COMPLETE | INCOMPLETE | FAILURE |
| Para 0 | 1 (20.0) | 0 | 4 (80.0) |
| Para 1 or more | 2 (14.3) | 7 (50.0) | 5 (35.7) |
| TOTAL | 3 (15.8) | 7 (36.8) | 9 (47.4) |

TABLE II

TREATMENT INTERVALS (HOURS)

| | Complete or Incomplete Cases | Failure Cases |
|---|---|---|
| Para 0 | 10.8 | 38.6 |
| Para 1 or more | 17.5 | 34.9 |
| TOTAL | 15.7 | 36.5 |

Study 4: Infections

Study 4a:

This study is nearing completion and the data will then be analyzed. A total of 200 patients were in the study.

Substudy 4b and 4c: Culture Studies

These studies were completed and enclosed is a paper for review. The study demonstrated that cervical and vaginal cultures fairly closely reflected the endometrial culture results. Therefore, sophisticated culture collection devices probably are not warranted.

# BACTERIOLOGIC CULTURE RESULTS OBTAINED BEFORE AND AFTER ELECTIVE MIDTRIMESTER UREA ABORTION

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## ABSTRACT

Twenty patients undergoing elective midtrimester abortion by urea amnioinfusion had bacteriologic culture data obtained before and after the procedure. Despite the presence of pathogens in the vagina and cervix prior to the procedure, only 20% of patients exhibited endometrial growth following abortion. Factors that seem to favor such endometrial growth include increased injection-abortion intervals and the requirement for curettage to manage incomplete abortion.

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## INTRODUCTION

Several studies have documented that genital tract infections are a well-known complication of elective abortion. (1-6) In association with first trimester procedures, infection rates vary from 0.1 to 5.2%. Following midtrimester amnioinfusion, infection rates range from 1.5 to 18.5%, depending upon the criteria used to make the diagnosis.

A previous report from our institution indicated that the organisms most frequently isolated in instances of post-abortal endometritis included: Beta hemolytic Streptococci, E. coli, Neisseria gonorrhoeae, Staphylococcus aureus, and Bacteroides fragilis. (6) In addition, the report confirmed the previous findings that midtrimester induced abortion carries more risk for subsequent infection than do first trimester suction procedures. However, no data was obtained which indicated the frequency of potential pathogens in the genital tract of patients prior to an abortion procedure.

The current study reports bacteriologic culture data obtained from twenty patients before and after elective midtrimester abortion induced by urea amnioinfusion. The purpose was to determine the variation in culture results from different genital tract sites and to document the frequency of positive endometrial cultures after a midtrimester abortion. In addition, the study attempted to gain some estimate of the frequency of isolation of potential pathogens from the genital tract of patients prior to a midtrimester abortion and to determine the importance of such isolation.

## MATERIALS AND METHODS

The patients were selected from women presenting to the Fertility Control Center for midtrimester abortion. The standard practices previously reported for inducing midtrimester abortion with hyperosmolar urea and prostaglandin $F_2a$ (5 mg) were followed except for the obtainment of genital tract cultures before and after the procedure. (7)

Prior to the abortion procedure, specimens from the vagina and endocervical canal were obtained on each patient and following completion of the abortion, endometrial cavity specimens were also obtained. The vaginal specimens were collected by swabbing the posterior vaginal fornix with calcium alginate swabs. Endocervical specimens were collected by first wiping the ectocervix clean with a sterile gauze sponge. The calcium alginate swab was then inserted into the endocervical canal for approximately one to two centimeters and rotated gently. The endometrial specimens were obtained utilizing a double lumen tube system to reduce contamination from the cervix and vagina.

The collection device consisted of an inner small diameter cylinder of plastic which was placed inside a larger plastic cylinder with an outer flange. The outer cylinder also had a thin rubber diaphragm stretched over its endometrial end. In addition, a jel foam plug was placed into the endometrial end of the inner cylinder.

To obtain the endometrial specimen, the ectocervix was wiped clean with a sterile gauze sponge, and the outer cylinder was placed into the endometrial cavity. The inner cylinder then pierced the rubber diaphragm and a calcium alginate swab was placed through the inner cylinder pushing out the jel foam plug. The swab was then left for several seconds within the endometrial cavity before plating onto the selective media.

All swabs were placed onto selective media immediately after the specimens were obtained. For both aerobic and anaerobic isolations, a 5% sheep blood agar media, and supplemented thioglycolate broth media were used. Anaerobic isolation employed kanamycin-vancomycin-menadione laked blood agar media and phenyl-ethyl alcohol blood agar media. Aerobic culture media included modified Thayer-Martin media and McConkey's media. The aerobic media were placed in a candle extinction jar and incubated at 35° C. The anaerobic media were placed into a Gas-Pak* device at bedside prior to incubation at 35° C. Organisms identified and reported included: anaerobic Streptococci and Staphylococci, Clostridia perfringens, Bacteroides species, Neisseria gonorrhoeae, Beta hemolytic Streptococci, Staphylococcus aureus, and E. coli. Growth of other organisms were considered as non-pathogenic in this study and were subsequently labeled as "no pathogens."

Factors related to the abortion procedure were analyzed. These included: demographic characteristics of the population, injection-abortion intervals, utilization of curettage for incomplete abortion, and the occurrence of complications. Data were subjected to statistical analysis where applicable using Chi-square testing employing the Yates correction for small numbers. (8)

## RESULTS

Table I details the clinical characteristics. In addition to the data presented, 16 of the 20 patients were single, 13 of 20 patients were enrolled in a Federal Medical Assistance Program, and three patients were on antibiotics at the time of the procedure.

*BBL, Cockeysville, Maryland

TABLE I

CLINICAL CHARACTERISTICS*

| | |
|---|---|
| Age | 21.5 (16-36) |
| Para 0 | 12 |
| Para 1 or greater | 8 |
| Duration of gestation (weeks) | 19.3 (16-22) |
| Injection-abortion interval (hours) | 24.4 ( 5.6-50.5) |

* Ranges are given in parentheses

Tables II and III detail the bacteriological results. One patient who had no pathogens isolated at any site developed a fever to 102°F during the abortion procedure and underwent evacuation and curettage for removal of all products of conception. She was subsequently treated with parenteral penicillin and kanamycin and responded within 24 hours. However, despite the fever, none of her cultures were positive for any of the pathogens.

The patient who had Bacteroides fragilis isolated from only the cervical site experienced fever as high as 102° F for the first six hours following her abortion procedure. After obtainment of endometrial cultures, she was treated with intravenous ampicillin initially before being discharged on oral ampicillin after two days of hospitalization. Although Bacteroides fragilis was isolated from the cervix prior to the abortion, no pathogen was found in the endometrial cavity. However, a separate specimen sent to the routine hospital Microbiology Laboratory did reveal a light growth of an anaerobic, Gram-negative, pleomorphic rod which could not be further identified.

The last two patients in Table III with positive cultures for endometrial pathogens were receiving antibiotics prior to abortion. One patient was on parenteral penicillin and Streptomycin due to a prior history of rheumatic fever and presence of a heart murmur and one patient was treated with parenteral penicillin just prior to the abortion procedure because of a positive syphilis serology. However, despite the antibiotics, endometrial pathogens were still isolated from these patients.

Factors possibly contributing to isolation of endometrial organisms were analyzed. The injection-abortion intervals of patients with positive endometrial cultures were longer than patients with no growth. For example, the mean injection-abortion interval for patients with positive endometrial cultures was 35.8 hours as compared to 24.4 hours for all patients studied and 20.6 hours for those patients with negative endometrial cultures ($p < 0.05$).

Another factor analyzed was the requirement for curettage. Curettage is routinely employed for patients that have not spontaneously passed the placenta within two hours of fetal abortion. All patients with positive endometrial cultures had undergone curettage for retained placenta while in contrast, only 25% of patients without positive endometrial cultures for pathogens had undergone curettage.

TABLE II

BACTERIOLOGICAL RESULTS

| | No. | Percent |
|---|---|---|
| All three sites negative for pathogens | 6 | 30 |
| Cervix and vagina positive for pathogens only | 7 | 35 |
| Cervix or vagina only positive for pathogens | 3 | 15 |
| All three sites positive for pathogens | 3 | 15 |
| Endometrial cavity only positive for pathogens | 1 | 5 |
| | 20 | 100 |

TABLE III

PATHOGENIC BACTERIA ISOLATED BEFORE AND AFTER MIDTRIMESTER UREA ABORTION

| NUMBER | VAGINA | CERVIX | ENDOMETRIUM |
|---|---|---|---|
| 6 | No Pathogens* | No Pathogens | No Pathogens |
| 3 | E. coli | E. coli | No Pathogens |
| 2 | B. fragilis | B. fragilis | No Pathogens |
| 2 | E. coli<br>B. fragilis | E. coli<br>B. fragilis | No Pathogens |
| 1 | B. fragilis | — | No Pathogens |
| 1 | — | E. coli | No Pathogens |
| 1 | — | B. fragilis | No Pathogens |
| 1 | E. coli<br>B. fragilis | E. coli<br>B. fragilis | E. coli<br>B. fragilis |
| 1 | B. fragilis<br>E. coli | B. fragilis | B. fragilis<br>S. aureus |
| 1 | — | — | E. coli |
| 1 | B. fragilis<br>S. aureus | B. fragilis<br>S. aureus | B. fragilis |

* No pathogens indicates no growth of E. coli, N. gonorrhoeae, S. aureus and/or B. fragilis

Finally, the time between the occurrence of abortion and obtainment of cultures was also examined. Overall, 4.3 hours elapsed between time of abortion and obtainment of cultures. However, for those patients with positive endometrial cultures, the elapsed time was 3.7 hours as opposed to 4.4 hours for those with negative endometrial cultures. These differences were not significant.

## DISCUSSION

From this study, there appears to be a close correlation between culture results obtained from the vaginal and cervical sites prior to abortion. Sixteen of 20 patients had a 100% correlation of results obtained from the vaginal and cervical sites. Two patients had pathogens which grew from the cervix but not from the vagina and two patients had positive vaginal cultures but had negative growth of pathogens from the cervix.

In addition, of the total of six pathogenic isolates from the endometrium, four were present in both the vagina and cervix prior to abortion. This result suggests that most post-abortal endometrial pathogens arise from endogenous vaginal and cervical sites rather than from exogenous sources.

Despite the presence of pathogens in the vagina and cervix prior to an abortion procedure, only a small number of patients subsequently exhibit endometrial growth within the first few hours after completion of the abortion. Factors that would seem to favor the positive growth of endometrial pathogens include an increased injection-abortion interval and the need for curettage to manage incomplete abortion.

The significance of such bacteriological results is unclear. Two patients, despite being on antibiotics just prior to the initiation of the abortion procedure, grew pathogens from their endometrium and one patient, despite fever probably due to chorioamnionitis or endometritis, had no growth of pathogens from any site. This uncertainty regarding the clinical relevance of endometrial cultures has also been reported by Gibbs et al. in a study comparing bacteriologic results from patients with clinical endometritis versus normal postpartum women. (9) Thus, further study of the genital tract flora before and after gynecologic procedures will be required in order to clarify the significance of such results.

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Study 5:

Calcium Study

Two patients have been studied.

The first received 1 gm Ca as an augmentation agent along with urea. Amniotic fluid Ca levels increased from 6.1 mg/dl to 20 mg/dl following administration and remained high for an 8 hour period. Rhythmnic contractions began at 1.0 hours. $PGF_2a$ increased in amniotic fluid at 1.5 hours. Prostaglandins were not elevated in the blood at this time. The second patient received 2 gm Ca increasing the amniotic fluid levels from 7.1 to 42.0 mg/dl. Again, contraction began at 1 hour and PGF M levels rose beginning at 1.5 hours. Both of these patients failed to abort within 24 hours.

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# PROJECT IV

## STUDIES OF THE LATE SEQUELAE OF INDUCED ABORTION

Project IV

Evaluation of the risks following Cervical Laceration in Association with Mid-trimester Abortion.

Enclosed is a paper presenting our results of this particular study. At this point in time, no major conclusions can be made as to the future reproductive experience of patients who have suffered this particular complication. Although patients suffering cervical lacerations appear to experience more menometrorrhagia, the effect of their use of contraception may actually bias these results. Furthermore, this study shows that to evaluate risks of midtrimester abortion would require an expensive prospective study.

A previous report has presented in detail the protocol and questionnaire for this particular study. In addition, the interviewer's manual is enclosed.

# PRELIMINARY ANALYSIS OF DATA IN CERVICAL LACERATION FOLLOWING MIDTRIMESTER ABORTION VERSUS MIDTRIMESTER ABORTION CONTROLS

## I. Evaluation of Data Collection

Two main aspects of the data collection will be reviewed, a) locating the respondent and b) obtaining consent for the interview. The second section of this report will compare the cases and controls on a few characterisitcs and by response status.

### Locating the Subject

The length of time from the abortion until the interview varied from less than one year to four years. Many of the women had changed their addresses or phone numbers, and consequently, some tracing was necessary. In some cases, forwarding addresses obtained from the post office were valid, but often further tracing was necessary. Every source of information available to Survey Research Unit (SRU) staff was used. Indeed, this expertisc in this activity was reflected in the percentage (84%) who were eventually contacted or found to be out-of-state or deceased.

The amount of tracing effort has not been assessed in detail. There is a general impression from going through many of the interviews that some detective work, and not infrequently a substantial amount, was necessary. The tracing could be evaluated in more detail by checking the number of women who had moved and whether or not they were found through the post office, neighbors, contact persosn listed in the FCC records, street and telephone directories, or other institutional sources, (e.g. hospital records or Department of Social Services). There was a range of cooperation from people who were asked about the subjects's whereabouts. The extent to which contact persons in the FCC charts labeled as confidential might have been used should also be checked. This information was on the cards given to the SRU, in addition to the respondent's name, address, phone, birthdate, and confidentiality status.

The subjects who were not traced successfully (i.e. to the point of making direct contact with the woman) can be divided into four groups. There were 13 who had moved and for whom minimal tracing was possible. Four had moved out-of-state. Another three could not be found, even though there were several leads which seemed to have the potential of locating the subject. In three cases, contact was made with the women but it was impossible to make further contact after a couple attempts

to arrange a convenient appointment for the interview. There were six instances in which the subject was never contacted, and an intermediate person blocked efforts to communicate directly with the subject. These people were usually evasive about knowing the person in question or where she lived, suspicious about how the subject's name was chosen, or refused to give information that would enable direct contact to be made.

Obtaining Consent

The second aspect about the data collection is obtaining consent from the subject to conduct the interview. This topic includes concern on the part of the respondent for the way her name was obtained. A review of the interviews has not been completed, but the refusals have been examined. Interviewers were asked to describe the circumstances and the content of the conversations when the respondent declined to participate No particular reason for refusing was given by three women. Repeated broken appointments, which had been arranged between the interviewer and the respondent, accounted for four more. There were four women who said it was inconvenient to do the interview. The remaining eight refusals were due to various combinations of reluctance, suspicion, or concern about privacy issues.

Discussion

The reasons for refusing to participate and being unable to locate subjects make it clear that privacy is an issue for follow-up studies of women who have had induced abortions from the respondent's perspective. Information recorded by interviewers showed concern about privacy for six women with whom no contact was made and eight of those who did not agree to be interviewed, that is, 14 out of 28, or 50% of the ones refusing or traceable but not interviewed (Table 2). It is not possible to know how many of the 14 remaining women were thinking about these problems but did not verbalize them. The proportion who refused to be interviewed among all those approached directly is 15.6% (19 out of 122) excluding the cases who were contacted but not actually interviewed. This is 3.5 times greater than the comparable refusal rate in another sample drawn from the FOC population a year ago (Evans, unpublished data). This fact seems to suggest more reluctance to participate in a follow-up study than an interview study done at the time of the procedure. However, the proportion of refusals

among all women asked to join the study is comparable to refusal rates in a variety of different studies using personal interviews.

During the interview, some of the women who did agree to participate raised this issue of privacy. Apparently, they found the explanations and circumstances under which the interview was conducted to be satisfactory. A few of the respondents selectively gave consent to review medical records or denied access to their medical records. There were also some women who did not report the abortion in question as such in the interview.

Privacy is a delicate issue and one which should continue to be considered carefully in future efforts to contact former patients sometime after they have been seen at the clinic. We have already discussed obtaining permission for possible future contacts when patients come to the clinic. This consent is now being obtained routinely in the FCC. The data collection was carried out by telling the respondent or other persons that this was a health survey conducted by Johns Hopkins University. If questioned, the interviewer was allowed to say that some of the questions were about women's health, so a private interview was important. Interviewers did not say they were from the FCC, nor did they acknowledge any awareness of the woman's past medical history. This approach was taken for two reasons: to protect the confidentiality surrounding the woman's prior contact with the FCC and to avoid potential respondent bias from unduly attributing health problems to a medical history of induced abortion.

The major difficulty which interviewers reported in introducing the study was answering the question, "How did you get my name?" Depending on the interviewer's subjective assessment of the circumstances, responses included vital statistics, a random sample, or patient lists from clinics or hospitals in the area. Clearly, the acceptability of such reasons varied among the women and intermediaries. This aspect of the study was not particularly agreeable to some of the interviewers because they had to lie convincingly. It might be preferable to blind the interviewers about pregnancy history of the respondents for this reason. Unfortunately, blinding of the entire Survey Research Unit staff was not achieved for this round of data collection. Another methodology would be to inform only the respondent herself that the interviewer is from the clinic. Probably the reactions to this approach would vary

but at least the interviewers would be spared from lying. However the effect such a disclosure would have on the content of responses is unknown and would be difficult to assess.

This problem of "name source" might be more manageable with the larger study comparing pregnancy outcomes of young women with and without a history of induced abortion. Interviewers would not know beforehand whether or not a respondent had had an abortion. In fact interviewers should not be told that some women had abortions and others not. Nevertheless, the suspicion and reluctance of some respondents, presumably those having had abortions, might still be a problem, since such a reaction might tip off the interviewer that this could be a case with a history of a prior abortion. Another way to reduce the problematic nature of introducing the study would be to make it part of a health study with multiple objectives, not all necessarily related to reproductive health, and with a broader universe from which to select subjects.

## II. Comparisons of Cases and Controls and Relationship of Background Characteristics to Response Status

There were no differences between cases and controls in the proportions who were interviewed, who refused to participate, and who could not be located (Table I). The proportion interviewed increased with increasing age of the respondents (Table III). Refusals were associated with younger age more than with older age. It might have been more difficult for these respondents to obtain privacy, or to consent to the interview without parental agreement. The intermediate age group (20 through 24 years) was most often the hardest to trace. This seems plausible because the youngest women are most likely to live still with their parents. The intermediate age group is perhaps the most mobile due to marriage, jobs, or going away to school. The older women might be more likely to be establishing a family, living independently of parents, and somewhat more settled.

Race appeared to be related to interview status, also. White subjects refused to be interviewed more often than the Black women in the sample (Table IV).

There did not appear to be any strong trend in response status by previous pregnancies (Table V), although a somewhat larger proportion of women with two or more pregnancies before the abortion agreed to be interviewed. This finding is probably due more to the woman's age than her gravidity, since these two characteristics are usually highly correlated.

The longer the period of time from abortion until interview, the greater the likelihood that the subjects could not be traced successfully (Table VI). The slight increase in proportion of refusals with more recent abortions is probably due to the younger age of these respondents, compared to those who had their abortions over two years ago. Fluctuations in the proportion interviewed by year of the procedure did not show any distinct pattern.

The percentage distributions for cases and controls differed on age, race, and prior pregnancies (Tables VII, VIII, and IX). It was already known that significantly higher proportions of the cases than controls were younger and experiencing their first pregnancy. The reversal of the proportions in the racial categories is not easily explained. The

proportions for the comparison group reflect the distribution for the entire FCC population. Similar proportions of Blacks and whites might have been expected for both cases and controls. The relationship is almost statistically significant ($x^2 = 3.15$, d.f. = 1, $.05 < p < .1$), but still could be the result of random variation.

Generally speaking, the patterns of response status and background statistics observed for the entire sample also hold for cases and controls. Since there are only 46 cases, the percentages when broken down into categories of background factors and response status are not stable. These percentages would be expected to change somewhat with a larger number of cases. Hence, it does not seem particularly worthwhile to analyze these tables further.

Discussion

These results concerning response status for subjects in this pilot study are useful in considering modifications of the study design for a larger scale follow-up study of young women's pregnancy outcomes. The highest refusal rates were among white women and subjects less than 20 years old. The highest proportions of subjects who could not be located were in the 20-24 year old age group and had their abortions over three years ago.

In order to compensate for these factors, a couple choices for alterations in the research design are suggested. First, the sample size could be increased by a certain percentage to be certain that there were enough cases in the categories with anticipated higher drop-outs. Second, the sample could be stratified in such a way that certain categories would have a probability of being selected that is disproportionate to their actual share in the population from which cases are to be selected. Finally, the data obtained could be weighted after it is collected to account for the subjects who could not be studied. Such data would have been gathered with or without stratifications but the stratification would not have involved disproportionate sampling.

The first approach is simpler to carry out in terms of sampling procedures. However, it would increase substantially the duration of data collection and the expense of the study. Such practical considerations are not insignificant for a large study.

The second approach would be a more efficient way to insure sufficient numbers of cases from each subgroup for analysis. It would probably also be more efficient as far as time and money are concerned. The difficulty with this procedure arises during data analysis. As long as the subgroups are analyzed separately, there is no problem. If it is desired to combine the subgroups in the analysis, the disproportionate sampling must be accounted for by weighting the data. Weighted data requires special attention in the computation of statistics. The results obtained using weighted data are valid when done properly, but the interpretation of such results is not always as intuitively clear.

The third method of selecting the sample, weighting after the data collection, entails the same considerations about weighted data mentioned above. Since the proposed study would involve mostly teenage women, the refusal rate might be such that the percentage of non-respondents would raise concern about the representativeness of the sample obtained. Weighting after the data collection is one way to deal with this problem.

If it were decided to use an approach that would use weighted data, it would be advisable to seek the advice of statistician before designing the sample.

## III. Findings

### A. Health Effects

In the literature, there is mainly speculation about possible deleterious effects of having experienced a cervical laceration during an induced abortion in the second trimester. The possibilities include infection, unexpected bleeding, and obstetrical complications in subsequent pregnancies.

This complication does not occur often, and it has not been studied rigorously. Nevertheless, clinical concerns particularly about management of subsequent pregnancies, led to an attempt to examine the reproductive health of women who had this complication.

A retrospective, case-control design was used. In personal interviews which took place six months to almost four years after the abortion, women were asked for complete pregnancy histories and contraceptive use in the past two years. They were also asked questions about other aspects of their reproductive health. The control group consisted of women who had second trimester abortions without any laceration of the cervix.

Contingency tables were constructed and odds ratios (OR) were calculated to determine whether there was excess risk of having infections, menstrual changes, or poor pregnancy outcomes after a second trimester aboriton procedure that had a cervical tear as an immediate complication. The odds ratio estimates the relative risk of the negative outcomes which were studied following exposure to the risk factor, cervical laceration.

In order to assess what, if any, effects a repaired cervical laceration might have on women's health, several questions were posed:

1. Did the women experience any gynecological infections after the abortion?
2. Were there notable changes in patterns of the menstrual cycle after the abortion?
3. For those women who became pregnant again, were there any medical problems during the next pregnancy, and what was the outcome?

The null hypothesis for each of these questions is that there is no additional risk of these three health problems after having a tear in the cervix during a second trimester abortion. Only information reported by respondents is used as the basis for the data presented.

Gynecological Infections

Infections in the reproductive organs immediately after the abortion and later infections were counted. All types of vaginal, ovarian, tubal, or uterine infections were included regardless of whether or not a health practitioner was seen and/or whether or not the infection was treated. Obviously, there is a range of severity in this broad definition. Information is generally available about severity or could be obtained from medical records to assess the occurrence of infection.

The table below seems to suggest that the null hypothesis cannot be disproved.

Relationship Between History of Cervical Laceration and Occurrence of Gynecological Infection After the Abortion

| | Case | Control | Total |
|---|---|---|---|
| Infection | 8 | 17 | 25 |
| No Infection | 24 | 54 | 78 |
| TOTAL | 32 | 71 | 103 |

O.R. = 1.059

Changes in Menstrual Cycle

Reported changes in menstrual patterns which were counted included noticeable changes in the duration of menstrual flow and/or of the time between periods, in the severity of cramps and in unexpected bleeding. These changes were tabulated according to all contraceptive use for the entire time period after the abortion. This method of defining contraception in relation to alterations in menstrual patterns could be improved. There is information available in the interviews concerning contraception at the same time the reported changes happened. Since intermittent use and substitution of methods are widespread in this sample of women, it might be helpful to tabulate the changes in relation to contraception at the time changes were reported to have occurred.

This table shows the distributions of all contraceptive methods used since the abortions for both cases and controls. It should be noted that these two distributions are similar in terms of the proportions of the two groups using a particular method.

Distribution of Cases and Controls by Contraceptive Use Since the Abortion

| | Case N | Control N | |
|---|---|---|---|
| Pills only | 18 (56.3) | 33 (46.5) | |
| IUD only | 1 ( 3.1) | 7 ( 9.9) | |
| Other methods only | 0 ( 0.0) | 5 ( 7.0) | |
| Pills/IUD and other methods | 8 (25.0) | 17 (23.9) | |
| None | 2 ( 6.2) | 6 ( 8.5) | |
| Sterilization | 3 ( 9.4) | 3 ( 4.2) | |
| TOTAL | 32 (100 %) | 71 (100 %) | 103 |

The next two tables show the difficulty in trying to reach any conclusions about the relationship between cervical tears and subsequent changes in menstrual cycle, primarily because of the confounding factor of contraceptive use and the small sample size currently available. The odds ratio among pill/IUD users currently suggests a 2.5 to 3 fold increased risk of menstrual changes among women who had a cervical laceration than among women who did not. To what extent the average younger age of cases might account for this result has not yet been tested.

Menstrual Changes Among Case and Controls Who Ever Used Pills and/or an IUD for Contraception after the Abortion

| | Case | Control | Total |
|---|---|---|---|
| Menstrual changes noted | 23 | 38 | 61 |
| No menstrual changes noted | 4 | 18 | 22 |
| TOTAL | 27 | 56 | 83 |
| | | O.R. = 2.72 | |

Menstrual Changes Among Cases and Controls Who Never Used Pills and/or IUD for Contraception after the Abortion

| | Case | Control | Total |
|---|---|---|---|
| Menstrual changes noted | 2 | 9 | 11 |
| No menstrual changes noted | 0 | 2 | 2 |
| TOTAL | 2 | 11 | 13 |

(O.R. cannot be calculated)

Next Pregnancy

Since only three women who had a cervical laceration during the abortion became pregnant again, it is not possible to estimate the relative risk of poor outcome and difficulties during pregnancy. The indicators of unusual problems while carrying the next pregnancy were defined as advice to take bed rest, hospitalization related to the pregnancy, medication besides iron and vitamins, or infection.

This table shows the distribution of cases and controls by the status of the pregnancy.

Problems During the Next Pregnancy Among Cases and Controls

| | Case | Control | Total |
|---|---|---|---|
| Problems present | 0 | 8 | 8 |
| No Problems | 3 | 23 | 26 |
| TOTAL | 3 | 31 | 34 |

The outcomes of the pregnancy immediately after the index abortion are presented in the next table.

Pregnancy Outcomes Among Cases and Controls

| | Case | Control | Total N | % |
|---|---|---|---|---|
| Live birth vaginal del. | 1 | 9 | 10 | (29.4%) |
| Live birth-C-section | 0 | 3 | 3 | ( 8.8 ) |
| Induced abortion | 2 | 8 | 10 | (29.4 ) |
| Spontaneous abortion | 0 | 4 | 4 | (11.8 ) |
| Still pregnant | 0 | 7 | 7 | (20.6 ) |
| TOTAL | 3 | 31 | 34 | (100 %) |

These data reveal that almost 30% of the sample which became pregnant again terminated that pregnancy.

B. Reporting of the Index Abortion

One of the reasons for conducting this pilot study was to estimate what proportion of respondents from this type of population might not report to an interviewer a prior induced abortion. It was possible to determine if a woman reported the index abortion on the basis of the date and location given for abortions which she listed. In addition, it was clear when no induced abortion was reported. A fairly stringent rule was applied for those abortions listed which could have been the index abortion. The woman had to identify Johns Hopkins as the facility and the date she gave had to be within one year, erring either way, from the actual date. If she gave a less accurate date but could identify Johns Hopkins and the type of abortion as an instillation procedure, then that was counted as reporting the index abortion. In addition, if the procedure could not be identified but the date was fairly accurate and the location was correct, this respondent was also counted as reporting the abortion of interest.

This table shows that both cases and controls reported the abortion which was the basis for their selection into the sample almost equally often. Overall, about 13% of the respondents did not report this abortion.

Reporting of Index Abortion by Cases and Controls

| | Case | | Control | | Total |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Reported | 30 | ( 93.8) | 60 | ( 84.5) | 90 |
| Not reported | 2 | ( 6.2) | 11 | ( 15.5) | 13 |
| TOTAL | 32 | (100 %) | 71 | (100 %) | 103 |

In reviewing the interviews it becomes clear that most of the women who failed to report the abortion did not disguise it as a miscarriage, a different kind of abortion, or an abortion obtained at another facility, although these ways were seen a few times. That pregnancy was simply not mentioned at all. Naturally, this finding suggests that there would also be some under-reporting of

abortions besides the one which was the starting point for this analysis.

C. Discussion

In this section several suggestions for further analysis of the available data will be made. In addition, implications of some of the results for the research design of a larger study of this problem will be explored.

Further Analysis

Although it is unlikely, given the results as they stand now, that any striking trends will be uncovered from this body of information, several steps might be taken to refine our understanding of the results. The severity of gynecological infections reported could be classified roughly. Further information might be desirable. It could be obtained from the health practitioners for whom the respondents gave consent for us to request medical records. Closer scrutiny of the information concerning menstrual cycles might be helpful. Again, a rough categorization of severity might be useful. Contacting health care providers whom the woman saw could also clarify the nature of the problem. A careful look at the method of contraception being used at the time the changes occurred might result in a somewhat different tabulation of the data. The pregnancy outcomes were not reported here in detail. Information on birthweight and length of gestation could be obtained from hospitals where the women delivered.

A few issues concerning the conduct of the research could be reviewed with an eye toward streamlining data collection and understanding the quality of information given by respondents. Many of the interviewers found that they did not make extensive use of the cards with all the questions about pregnancies and contraception. It would be worthwhile to count how many interviews used them. Nevertheless, the cards should be available for those few instances when they would be necessary to complete the interview successfully.

The quality of the data could be assessed further in two ways. The amount of discrepancy between the actual date of the abortion and the date given in the interview might indicate the relative accuracy of other dates provided. The levels of suspicion and

non-cooperation expressed verbally or sensed by the interviewer, when recorded, could be compared to the confidential status in the FCC chart for both respondents and refusals. The information in the chart might be a good or poor predicator of the quality of interview to expect.

Implications for Research Design

If it were decided that a larger scale study of this health problem should be conducted, this pilot study could be used in several ways to guide the design of the second effort. Some of these ways have been discussed in earlier parts of this report.

It might be advisable to stratify the sample of controls on the basis of age and parity. This procedure would improve their comparability to the cases which tend to be more often younger and of zero parity, relative to the FCC client population as a whole.

A substantial proportion of the sample decided to abort the next pregnancy. The effect of this outcome on sample size as well as the dependent variables should be considered.

These results provide a foundation to determine statistically what sample size would be required in order to obtain significant results. Estimates of the prevalence of the gynecological infections and changes in menstrual patterns are now available. Besides these estimates, the probabilities of pregnancy outcomes can be approximated for this patient population.

The number of cases is limited by the relatively infrequent occurrence of cervical lacerations. Simply proposing a larger sample size, however, will have little effect on the number of cases which could be included.

Another question discussed when the current study was being planned was the optimal length of time from the abortion to the end of follow-up in which subsequent deliveries might occur. Clearly, in the analysis presented here, the cases were the limiting factor, in the sense that so few of them had become pregnant again. The time element is certainly one reason for the small number of pregnancies.

Related to the issue of the total length of time post-abortion is the matter of using a second, or third, follow-up interview. Selected questions from the present interview could be used again at some defined time interval from the date of the abortion. This longitudinal approach might reduce some of the under-reporting of pregnancies and the inaccuracy in reporting of dates and contraceptive use. On the other hand, the more frequent questioning might produce relatively more positive responses about menstrual changes and infections. It might be hard evaluate how much of these changes are normal fluctuations in such a group of women.

Serious consideration should be given to eliminating some questions in order to shorten the interview. At times, the choice will not be so easy to make. However, the responses of the present interview can be studied from the perspective of which questions yielded most completely the basic information sought. Also, the questions which succeeded in bringing out significant information occasionally could be identified. These would also have value. Consideration should be given to eliminating questions when their use in data analysis is unclear. These are not hard-and-fast rules, but merely a few observations on how to approach trimming of the interview itself.

TABLE I

RESPONSE STATUS OF SUBJECTS FOR THE TOTAL SAMPLES AND FOR CASES AND CONTROLS
(Excluding 2 subjects known to live out-of-state)

| | Cases | Controls | Total |
|---|---|---|---|
| Refusals | 5 ( 10.9) | 14 ( 13.2) | 19 ( 12.5) |
| Inability to locate | 9 ( 19.5) | 21 ( 19.8) | 30 ( 19.7) |
| Completed Interviews | 32 ( 69.6) | 71 ( 67.0) | 103 ( 67.8) |
| TOTAL | 46 ( 100% ) | 106 ( 100% ) | 152 ( 100% ) |

TABLE II

REASONS FOR NON-RESPONSES DUE TO REFUSALS
AND INABILITY TO LOCATE

| | REFUSALS | NUMBER |
|---|---|---|
| * | Repeated broken appointments | 4 |
| * | Reluctance, suspicion, concern about privacy | 8 |
| * | Inconvenience, no time | 4 |
| * | No reason given | 3 |
| | TOTAL: | 19 |

| | INABILITY TO LOCATE | |
|---|---|---|
| | Information obtained helped but didn't lead to contact with subject | 3 |
| * | Timing: Unsuccessful attempts to rearrange appointment after direct contact with respondent (without refusal to participate) | 3 |
| * | Persons contacted in tracing were suspicious and blocked direct contact with subject | 6 |
| | Moved (including 4 who moved out-of-state) | 17 |
| | TOTAL | 29 |

* 28 subjects who were actually contacted directly or indirectly

TABLE III

RESPONSE STATUS BY CURRENT AGE OF SUBJECT

(Cases and Controls Combined)

| Current Age Years | RESPONSE STATUS | | | |
|---|---|---|---|---|
| | Interviewed | Refused | Couldn't Locate | Total |
| 14-19 | 28 (63.6) | 9 (20.4) | 7 (15.9) | 44 (100%) |
| 20-24 | 44 (65.7) | 7 (10.5) | 16 (23.9) | 67 (100%) |
| 25-29 | 31 (75.6) | 3 ( 7.3) | 7 (17.1) | 41 (100%) |
| TOTAL | 103 | 19 | 30 | 152 |

TABLE IV

RESPONSE STATUS BY RACE OF SUBJECT
(Cases and Controls Combined)

| | RESPONSE STATUS | | | |
|---|---|---|---|---|
| | Interviewed | Refused | Couldn't Locate | Total |
| Race | | | | |
| White | 41 (59.4) | 15 (21.7) | 13 (18.9) | 69 (100%) |
| Black | 61 (74.4) | 4 ( 4.9) | 17 (20.7) | 82 (100%) |
| TOTAL | 102 | 19 | 30 | 151* |

* 1 unknown

TABLE V

RESPONSE STATUS BY NUMBER OF PREVIOUS PREGNANCIES
(Cases and Controls Combined)

| | RESPONSE STATUS | | | |
|---|---|---|---|---|
| | Interviewed | Refused | Couldn't Locate | Total |
| No. of Previous Preg. | | | | |
| 0 | 59 (66.3) | 12 (13.5) | 18 (20.2) | 89 (100%) |
| 1 | 21 (61.8) | 6 (17.7) | 7 (20.6) | 34 (100%) |
| 2+ | 23 (79.3) | 1 ( 3.5) | 5 (17.2) | 29 (100%) |
| TOTAL | 103 | 19 | 30 | 152 |

TABLE VI

RESPONSE STATUS BY YEAR OF ABORTION

(Cases and Controls Combined)

| | RESPONSE STATUS | | | |
|---|---|---|---|---|
| | Interviewed | Refused | Couldn't Locate | Total |
| Year | | | | |
| 1973, 1974 | 31 (63.3) | 5 (10.2) | 13 (26.5) | 49 (100%) |
| 1975 | 32 (74.4) | 4 ( 9.3) | 7 (16.3) | 43 (100%) |
| 1976 | 28 (65.1) | 7 (16.3) | 8 (18.6) | 43 (100%) |
| 1977 | 12 (70.6) | 3 (17.6) | 2 (11.8) | 17 (100%) |
| TOTAL | 103 | 19 | 30 | 152 |

TABLE VII

PERCENTAGE DISTRIBUTIONS OF CASES AND CONTROLS BY CURRENT AGE

| Current Age (years) | Cases | Controls |
|---|---|---|
| 14-19 | 41.3 | 23.6 |
| 20-24 | 45.6 | 43.4 |
| 25+ | 13.1 | 33.0 |
| | 100% | 100% |
| | (46) | (106) |

TABLE VIII

PERCENTAGE DISTRIBUTIONS OF CASES AND CONTROLS BY RACE

| Race | Cases | Controls |
|---|---|---|
| White | 56.5 | 41.0 |
| Black | 43.5 | 59.0 |
| | 100% | 100% |
| | (46) | (105*) |

* 1 unknown

TABLE IX

PERCENTAGE DISTRIBUTION OF CASES AND CONTROLS
BY NUMBER OF PREVIOUS PREGNANCIES

| No. of Previous Pregnancies | Cases | Controls |
|---|---|---|
| 0 | 84.8 | 47.2 |
| 1 | 10.9 | 27.4 |
| 2+ | 4.3 | 25.4 |
| | 100% | 100% |
| | (46) | (106) |

# INTERVIEWER'S MANUAL

**Front Sheet and Introduction**

1. All contacts and attempted contacts should be noted.

2. Most of the respondents were chosen at random, but some were chosen because of their health history. None of the Survey Research Unit's staff knows which way was used to select any of the subjects.

3. The topics of this interview could be very sensitive to the respondent. Therefore, if at all possible, arrange to conduct the interview alone.

4. The questionnaires, consent forms, and front sheets are all kept separately from each other in locked file cabinets to which only the research staff has access. No respondent names are written on the questionnaires themselves.

5. The purpose of the consent form is to inform the respondent of the conditions under which an interview is conducted, how the information obtained in the interview is handled subsequently, and that she does not give up any personal rights if she agrees to the interview. The Clinical Investigations Committee of the Johns Hopkins Hospital expects a written statement to be signed by the respondent, and research staff are enjoined to uphold the statement's provisions. This committee monitors all research done by the Hospital's staff to ensure the protection of the personal rights of subjects.

**Page 1: Q.1**

1. Number of years old on the day of interview. If her birthday were the next day, she would still be the age she has been during the past year.

**Q.4,6**

1. "Technical or business school" means secretarial schools, computer data processing schools, beautician schools, automotive mechanics training, nurse's aid training, etc., but not college degree (AA, BA, BS etc.) programs (these would be included in Q.3).

**Q.6a**

1. Note whether the number given is for college or technical school.

Q.7 1. If a second source of health care is mentioned, this question can be shortened the 2nd. time to, "where do you usually go?"

2. Ask the set of questions (7-10k) in sequence for one provider, and then go through them again if the woman names a second provider. Note at top of second answer column if you came from Q. 11a or Q. 27f.

Page 2: Q.7a 1. "Inapp" means inapplicable, and is equivalent to NA throughout the questionnaire.

Q.7b 1. If there is any doubt as to which category, make a note in space provided for other, listing types of services, payment mechanism (e.g. prepaid, fee-for-service, etc.), and any other helpful descriptive information.

2. Military hospitals-Ex: Kimborough, Naval Hospital (Annapolis), Walter Reed, Fort Meade, V.A. Hospital.

3. Employer's health service - Ex: JH University Health Service, industrial plant infirmary.

4. COFLAC - family planning clinic at Chester and McElderry Streets in Baltimore. (Often mispronounced as Coflax)

5. Neighborhood FP Center - Neighborhood Family Planning Center on East North Avenue, across from Sears.

6. "Free Clinics" - Ex: People's Free Medical Clinic on Greenmount in the Waverly section of Baltimore; Rockville Free Clinic; but not city and county health department clinics (even though they do not charge fees).

7. Other Ex: All other, including Public Health Service (note it as such).

Q.8 1. The distinction here is between a specialist ("only certain problems") and general practitioner ("most health problems...family"). If respondent asks or needs explanation, "most health problems...family" means a variety of health needs; general practitioner is synonymous with family doctor, doctor you go to "for everything".

2. Questions refer only to the respondent; if family has a GP but the respondent does not go to this doctor, then this MD would not be included as a regular source of health care for her.

Page 3: Q.9 1. Any time in the past.

Q.10 1. If the visit was for a check-up, say for FP, or post-partum or prenatal care, we don't need much detail unless the woman was having a particular problem. However, since it might not be clear from her response if a problem existed, Qs. 10a and 10b should be asked.

| | | |
|---|---|---|
| Q.10a,b | 1. | If she answers positively to either of these questions and gave no indication of a health problem in Q.10, probe to find out what the problem was which was treated. |
| Q.10a | 1. | For Ex.: blood tests, pap smear, urine tests, cultures - probe to find out purpose of blood or urine tests - if the woman knows(i.e., what the doctor was trying to diagnose). Also, X-rays, special radiological tests such as barium enema, intravenous pyelogram, EKG, biopsy, etc. |
| Q.10b | 1. | The doctor could have given the woman a drug sample or other medication in the office. If pills, creams, or shot, try to find out what type - hormones, antibiotics, vitamins and minerals, tranquilizers. Respondent might not know what type of medication but she might be able to describe well what it looks like. |
| Page 3: Q.10c | 1. | Since some diagnostic and therapeutic procedures are done on an outpatient basis without an overnight stay, this question is phrased as it is. Most of the time, responses will probably apply to a stay in the hospital. |
| Page 4: Q.10h | 1. | For example: ultrasound examination of the pelvic region, laparascopy, biopsy, special cultures, EKG etc. |
| Q.10i | 1. | You could explain: that is, an IV, intravenous medication, a needle that stayed in your vein and was connected by plastic tubing to a bottle of medication. |
| Qs. 10f-10k | 1. | These questions are meant to give us an idea of the severity of the condition and to let us know what treatment was given during the hospitalization. |
| Q.11 | 1. | This is a filter question to see if the woman uses a second health care provider. If she does, then you go back to Q.7 and ask this set of questions again, recording her responses in the second answer column. |
| Q.11a | 1. | It might be obvious by the time you reach this filter question that the woman has an ob-gyn provider. Since a number of women have 2 sources of women's health services, this question could be modified when appropriate - "do you have <u>another</u> doctor or clinic...go?" |
| | 2. | Proceed to Q.12 if the response is "no". |
| Page 5: Q.12b,c | 1. | There are 20 cigarettes in one pack. |
| Q.14 | 1. | Read instructions carefully. |

Page 6:

2. Cancer: Ask respondent in what part of the body she had cancer diagnosed if she says she has a medical history of cancer.

3. Breast disease: all types (e.g. mastitis, cystic breast disease) except breast cancer.

4. Parathyroid disease: a disease syndrome which includes bone problems (calcification), other physical symptoms and some psychological changes. A person will know if they have this disease, can be hyper-, or hypo-parathyroid. With hyper - there is often renal involvement with calcium deposits. These glands are next to thyroid gland.

5. Hepatitis: serum or infectious.

6. Sickle cell disease: sickle cell disease is a genetically determined ailment. If a child receives the sickle cell trait from both its father and mother, it will manifest the disease. If the child obtains the trait from one parent, it is said to have the trait, but the child doesn't develop the complete clinical picture of the disease.

7. The "4" code has been deleted for diseases that do not have surgery as a mode of treatment.

Q.14a

1. Record whatever other diseases the woman mentions that she feels are "major." Examples might be, tuberculosis (TB), or gonorrhea.

Page 7: Q.15

1. D&C stands for dilatation and curettage. This is a common operation in which the lining inside of the womb (uterus) is scraped off by an instrument called a curette. Dilatation refers to widening the opening to the womb, that is, the cervix.

2. This question is aimed at surgical D&C's performed when a woman is not pregnant. However, since this technique is also used for abortions, keep it in mind if the woman tells you about an abortion here. In a later section of the questionnaire, information is requested about induced abortions. In such a case, appropriate answer to Q.15 is "no", but make notation that she had a D&C for the purpose of ending a pregnancy.

3. The woman might tell you about a menstrual extraction. This is removal of contents of womb by suction through a small plastic tube when pregnancy is suspected, but too early to confirm by common pregnancy tests. If so, record information same as for abortion in this and following related questions (but it will not be included in pregnancy section unless woman knows that pathology exam of tissue showed presence of a pregnancy). Appropriate answer is "no".

Q.15b 1. Sometimes this procedure is done for severe irregular bleeding, removal of placental tissue, termination of pregnancy, or after miscarriage. (TOP = abortion)

2. It is appropriate to record D&C's done for all reasons except termination of pregnancy.

Q.15c,d 1. If the D&C was for the purpose of abortion or due to miscarriage, skip these two questions, but be sure in the section on pregnancies that this pregnancy is accounted for.

Q.16 1. Sometimes women can not remember exactly. Try to associate it with her year in school and find out how old she was then, as one technique.

Q.17 1. Since the response choices are rather long here, read each one aloud as the respondent looks at the card.

2. If none of the descriptions seems to fit, write down how she would describe her usual menstrual cycle.

3. Cycle starts with menstrual flow and subsequent period of a few weeks until she has her next menstruation (means "period"). You can use Card 20 to show what you mean by period.

Q.18 1. A woman might say that her usual period could be described by a combination of these terms. This is fine, simply write down what she says on the lines next to "other". Probe sufficiently to obtain a clear meaning.

Q.19 1. This means the number of days she has menstrual flow, from beginning to end of the flow.

Q.20 1. This question refers to the # of days between successive menstrual bleeding times. Card 20 illustrates the distinction between these 2 questions.

Page 8: Q.21 1. Time period covered here would be the last 5 years.

2. We are interested in changes that she felt were unusual for herself.

3. Such changes might have happened more than once. If so, we would like to know this - and ask 21a,b,c for each additional time.

4. If she says "yes", probe to find out if it had ever happened to her <u>before</u> the time she mentioned.

Q.22 1. This question refers to the length of time the woman experiences menstruation.

2. Any changes in last 5 years; if she answers yes, probe to see if it has ever happened before the time she mentioned. Note this fact, if it is the case.

3. For multiple occurrences, ask 22a,b,c about each time and record in box below instructions in capital letters, for events in last 5 years.

4. Be sure to record any spontaneous remarks that are relevant to the question - such as factors that might have contributed to the change, in her view.

Page 9: Q.23 — 1. Same comments are relevant for this question as for Q.21 and Q.22.

Qs. 24,25 — 1. Space is provided to write down what qualifying remarks a respondent might make. For instance, in Q.25 she might say that she has cramps only during certain parts of her period.

Q.26 — 1. Time period of primary interest here is the last 5 years. If she says "yes" to the question, probe to find out if it had ever happened to her before the time she mentioned. Note this information, please.

2. An unusual change is meant to be in terms of her own perception of the change (to her it seemed unusual).

3. Such changes might have happened more than once (within last 5 years, or further back in the past).

Qs. 26b,c,d — 1. If changes in last 5 years occurred more than once, then ask these questions about each separate occurrence.

Page 10: Q.27 — 1. This question is about irregular bleeding, or break-through bleeding, between periods. This often happens when the Pill is being taken for contraception, but it can happen at other times as well.

2. Again, we are most interested in the last 5 years, but if it has happened before that time, please note that fact, since that will be useful information for us to evaluate her situation.

Qs. 27c,d,e, f,g,h — 1. Ask, as appropriate, for each occurrence in the last 5 years.

Q.27~~f~~h — 1. If a name and address are given, when you go back to 7a, check to see if the series of questions have been asked yet. If questions 7a-9 have been answered about the provider, they need not be repeated. In this case, start with Q.10a. Be sure to mark conspicuously that you have asked these because of the responses to Q.27. Go through all questions 10b-10k, as appropriate.

2. If there is no more room, write responses on extra sheets of paper.

Q.28

1. This question begins a series of potentially sensitive questions, particularly if the interview can not be conducted in complete privacy.

2. Do not read the question aloud. Record her response and proceed to the next question, which should also be done using a card.

3. Theoretically, all of these women should answer "yes" to this question, but if a respondent says "no", simply ~~proceed to Q.29.~~ skip to 56.

Q.29

1. Hand respondent card 29. Do not read the question aloud. Record her response.

2. If she says she has had no pregnancies, skip to Q. ~~57~~ 56.

3. Response should include number of live births, miscarriages, induced abortions and stillbirths.

Q.30

1. The purpose of using cards is to enable the woman to complete the interview in situations which may arise when there are other people around the place where the interview is conducted. Other people in the house might not be aware of any pregnancies experienced by the respondent, or of a particular pregnancy, that ended in an abortion. The respondent's right to keep this fact secret must be protected.

2. Use of cards can be discontinued at any time the respondent wishes to. You can mention this to her - that even though she wants to use the cards now, she is not required to continue using them if she wants to switch back to your asking the questions.

Page 11: Q.31

1. This question attempts to find out when the woman became pregnant.

2. Generally, pregnancy occurs in the month after the last normal period. Sometimes a woman will continue to have a period after she becomes pregnant, or she might have spotting, or scant bleeding. If these conditions are true, then note this and try to get dates for last normal period and for spotting.

3. If the woman can't answer this question - because she can't remember the month defined this way, or for some other reason, then ~~use the~~ probe ~~which is specified. It is not necessary to ask the probe if a satisfactory answer was given before.~~

4. If the respondent cannot give you a response to the probe either, then ask her whether it was fall, spring, winter, or summer that she became pregnant.

Q.32

1. Induced abortion means an abortion which the woman decided to have for medical or personal reasons, as compared to spontaneous abortion, that is, a miscarriage.

2. Stillborn refers to delivery of a dead fetus after 28 weeks of pregnancy.

3. Tubal (ectopic) refers to a pregnancy that grows in one of the fallopian tubes instead of in the uterus. Usually, these pregnancies must be removed by an operation through the abdominal wall.

4. Live birth - now dead means that the baby was born alive but died anytime after it was outside of the mother.

5. Live birth - now alive is, by this point, obvious.

6. If the child was born alive but the mother gave it up for adoption and doesn't know if child is still alive, note this situation.

Q.33

1. For example, for a live birth, it would be the child's birthday.

2. It's not unlikely that women who have had pregnancies end in other ways might not be able to recall this date as well. Simply obtain the best information possible, which might be the month and year. Probe to ask "how far along" she was when the pregnancy ended - and note her response in <u>weeks</u> so we can reconstruct an answer to this question.

Q.34 1. Information should be obtained about all kinds of infections.

2. Find out:
   a. what body part was involved;
   b. how severe it was - if it required medication or extra doctor visits;
   c. if it was acute (sudden onset) or chronic (woman has had infection for sometime - has seen doctor before about it).
   d. if it was gynecological in nature - was there bleeding when she had it, and did she become anemic from it.

Q.35 1. Did the woman go to a clinic or doctor during the pregnancy at regular intervals to be checked for the progress of the pregnancy?

Q.35a 1. This question refers to the first, second, third, fourth, etc. month when the woman had her first prenatal visit.

Page 12: Q.37 1. This question is concerned with hospitalizations before the actual labor which resulted in a birth. Sometimes women have false labor before they have true labor resulting in a birth. False labor is not what is meant by "labor" in this question. If she was hospitalized for false labor, that should be taken as a "yes" answer.

2. It would be helpful to probe briefly for what problem she was hospitalized, and to write in this reason.

3. We are interested in hospitalizations during the pregnancy, other than to deliver the baby, and which were pregnancy - related or not pregnancy - related.

4. Be sure to obtain name and address of hospital where she went for this reason.

Q.38 1. Any level of consumption should be recorded as "yes."

Q.38a 1. Earlier in the interview, the woman is asked about typical consumption of alcohol. This question asks her to compare how much she drank during the pregnancy to the usual consumption already described. Then she should decide if she drank more than that, less than the usual amount, or about the same amount during the pregnancy.

Q.39 1. Any level of smoking should be taken as a "yes" answer.

Q.39a 1. The same instructions given about Q.38a apply here to smoking - comparing usual habit to smoking during this pregnancy.

Q.40 1. Any drugs taken should be counted as a "yes" response.

2. Probe to determine as best she can tell you what they were, from aspirin to sominex, from barbiturates to heroin, from antibiotics to blood pressure pills. Sometimes they can tell you for what condition they were taking medicine, and what the pills (liquid, etc) looked like, even though they don't know the name of the medicine. Probe for this type of information.

3. If the pregnancy ended in a miscarriage, skip from here to Q.45.

Q.41

1. This question should be asked if the delivery was a live-birth or stillbirth. If delivery was at home or other place (not in a hospital), note this.

Page 13: Q.42

1. This question is to find out how the baby was delivered.

2. Use the probe if she is initially unsure how to answer. If the delivery was normal, that is, through the vagina, then probe is not necessary, and you should also skip Q.42a.

Q.42a

1. Some typical reasons for a C-section are: long labor (greater than 24 hours), baby too big to get through birth canal or pelvic opening, excessive or prolonged bleeding, serious infection in the mother. These reasons can be used as specific probes.

Q.43

1. If she doesn't know exactly, obtain the best estimate, and note that the respondent was not sure of the birth-weight.

Q.44

1. If no breastfeeding or less than 2 weeks, record as zero.

2. If she says 2 1/2 months, for example, write in the fraction.

3. Since the next two questions are only about miscarriages and induced abortions, skip to Q.47.

Q.45

1. Sometimes a woman who has a miscarriage does not go to the hospital because of it. Then the answer would be "no."

2. If she had a miscarriage and just went to her regular doctor, for instance, to be examined afterwards, response would be "no".

3. If she went to the hospital after a miscarriage for observation and/or a D&C, then her response would be "yes."

4. The phrase "when this pregnancy ended" has been used because this question could apply to two possible outcomes (miscarriage or abortion), and we wanted to use a tactful phrase.

5. Some induced abortions are performed in clinics that are not part of any hospital.

6. If the woman is unwilling to provide the name and address of the clinic or hospital, please remind her that all of the things she tells you are kept confidential and are not used for any other purpose besides this research project. If she still refuses, tell her that it's OK, she does not need to answer the question, and proceed to the next question.

Q.46

1. The only time a "no" answer is appropriate is if she went because of a miscarriage but no operation (e.g., D&C) was done.

2. For induced abortions one of the types of abortion procedures should be the appropriate response:

   a. D&C (surgical) - done in first 12 weeks of pregnancy usually. The womb is scraped with a sharp instrument to remove the pregnancy and some of the lining of the uterus that has built up during the pregnancy.

   b. Suction D&C - also usually done in first 12 weeks of pregnancy. The pregnancy is removed with a tube connected to a machine that produces a vacuum (the same principle as a vacuum cleaner). Then the doctor will scrape the womb a little to be sure all the tissue is out. Also called vacuum aspiration, vacuum abortion, suction abortion or suction curettage. Some hospitals will do suction abortions up to 16 weeks.

   c. Saline abortion - this type of abortion is done from 16 to about 24 weeks of pregnancy (i.e., second trimester). Also called injection abortion, abortion with needles, salting out, Saline. It involves exchanging some aminotic fluid with saline solution through a needle in the abdomen. After some time the woman goes into labor and delivers the dead fetus. Usually the woman is in the hospital 1-3 days.

   d. Urea/PG abortion - also done during the same period of time in the pregnancy. Also called injection abortion, abortion with needles, or Urea. Some women might mistakenly call a Urea abortion as a saline abortion. Frequently, they might not remember which medication was used. The way the abortion is carried out and what happens is the same as for saline abortion. Instead of saline solution, drugs called Urea and Prostaglandin (PG) are used together.

   e. Second trimester abortion (method unknown) - use this choice if she can't tell you which type of injection procedure, as described above, she had. Only abortions done between 16 and 24 weeks of pregnancy would be included here.

   f. Other - rarely, some other methods are used. If the abortion was illegal and was not done by one of the

methods described already, then note that here. If a surgical incision in the abdomen was made and the fetus removed this way, this type of induced abortion would be noted here. If the woman had an injection - type abortion and she knows that it didn't work and that the fetus was removed either through an incision, by suction, or D&C, note that here.

3. Sometimes a woman who has a miscarriage will have a D&C afterwards to remove any tissue that might stay inside the womb. If this is true, then you would circle the code number for either D&C (surgical) or suction D&C, or note in other if she can't remember exactly what was done.

Page 14: Q.46a

1. The purpose of this question is to find out if she had any immediate complications after the operation. Examples would be an infection in the pelvic area of the body or excessive bleeding. We are interested in problems like these which were severe enough to require an additional visit to a doctor or hospital, and which the woman was told about by the practitioner who saw her. Sometimes a second suction procedure is done or a woman is given antibiotics, depending on the problem and its severity.

Qs. 47-53

1. For your convenience in determining the time period to ask about for these questions, a space has been provided to transfer the date in Q.33. Then figure out in your head 3 months after that date. Use this month and year to ask the questions. Do not say to the woman "3 months after that" or "3 months after the pregnancy ended." Simply say only the month and year which occurred 3 months after the pregnancy ended.

2. These questions are meant to cover the time period between pregnancies, or since the last pregnancy (if there were no subsequent pregnancies).

Q.48

1. By "period" is meant what the woman perceived to be her normal menstrual bleeding.

Q.48b-52

1. These questions repeat an earlier series of questions about changes in menstrual patterns.

Page 15: Q.54

1. This question needs to be asked only once, to be sure all pregnancies have been accounted for.

2. Check Q.30 and be sure that Qs. 31-53 have been asked for the same number of pregnancies. Then ask Q.54 in case any have been missed.

3. Be sure to use the card if they have been used for the Questions on pregnancies.

Page 15: Q.55

1. This question refers to infertility, when a couple is trying to have a child and for one reason or another, pregnancy does not occur.

Page 16: Q.56

1. Since you might not be able to interview in complete privacy, cards are provided for this section which is about contraception. You should be aware of the possibility that members of the household do not know that she uses birth control. The choice is up to the respondent; she can later change to cards or to your asking the questions aloud later in the interview.

2. Q.56a - which appears on a card, is an explanation of the time frame and types of contraception in which we are interested. No response is recorded.

Q.57

1. First, go down the list of various methods. Only the methods will be listed on card 57. Circle N or Y for "no" or "yes" after each one.

2. Then, ask in which months the methods were used, starting with the current month. Even if the interview is conducted right at beginning of a month, consider that to be the current month. E.g., if the Pill was used: "Did you use the Pill this month (in December)? How about last month, that is November? etc.

3. By rhythm is meant use of calendar, or temperature charts, or guess work about "safe period."

4. Withdrawal means that the man pulls out of the woman before he has an orgasm.

5. Abstention means not to have sexual relations by conscious choice.

6. Note that the part of the table which deals with months used will be filled out from right to left. Write in names of the months in squares at the top. The months which correspond with the numbers will not be the same as the order of the month in a calendar year. (December will be month 0, November month 1, etc.) Circle the number for that month in the line for each contraceptive used during that month. If that method, is not used, no number should be circled.

7. If woman was not using any method during a month, circle the number of the month. Likewise, circle numbers for the months she was pregnant.

8. If it is not possible to complete the table with a respondent put a check in the open parentheses at the bottom of the table.

9. More than one method could be used in any month.

Page 17: Qs. 58-65b

1. Qs. 58-60 concern use of birth control pills in the last year. If the respondent has not used them, skip to Qs. 61-64b.

2. Qs. 61-64b are about use of an IUD in the last year. If no IUD was used in the last year, skip to Q.65.

3. Example: a woman is currently taking pills. Ask Q.58, then go to Q.61. If she did not use an IUD in the past year, skip to Q.65.

4. You will know from Q.57 - the completed table, which questions are appropriate to ask.

5. Skip patterns are indicated on the questionnaire as interviewer instructions.

6. Cards might continue to be used for these questions. Therefore, you should be prepared to have respondent write answers on separate paper for open-ended questions, e.g., Qs. 58, 59, 62, 64a, as well as "other" responses.

Q.59

1. This question is asked if the woman took pills for part of the year but stopped using them.

2. After obtaining an answer, skip to Q.61.

3. Probe to find out if health practitioner advised her to stop taking birth control pills, and if so, what the practitioner's reason was to advise this.

4. If she says something vague about side effects, probe to find out how severe they were, and if a doctor, etc. was consulted about them.

5. "Continuing to use pills" would be circled if this question is not asked because the woman is continuing pill use.

Q.60

1. This question means use of birth control pills at any time in her life but not during the past year.

2. This question would be asked only if the woman did not mention pills in Q.57 (the table on methods of birth control).

Q.61

1. If woman mentioned IUD use at some point in the last year (Q.57, again), ask Q.61 and Q.62.

Page 18: Qs. 63-64b

1. These questions are asked only if an IUD was used for part of the year covered in Q.57.

| | | |
|---|---|---|
| | Q.63 | 1. This question refers to expulsion of the IUD. |
| | Q.64 | 1. This question refers to removal of the IUD. |
| | Q.64b | 1. Multiple reasons for removal of an IUD are possible; therefore, circle all answers that apply to respondent's situation. Be sure to probe for any additional reasons. |
| | | 2. The choice "IUD perforated uterus" refers to the rare occurrence when the IUD can move into the abdominal cavity through a hole in the wall of the womb. This hole usually is made inadvertently during insertion. Sometimes surgical removal of the IUD is necessary. |
| | | 3. After this question is answered, skip to Q.66. |
| Page 19: | Q.65 | 1. This question is asked if the respondent did not mention an IUD in Q.57 (the table on contraceptive methods). |
| | | 2. If she answers "yes," then Qs. 65a and 65b should be asked. |
| | Q.65a | 1. Number of years of actual use of an IUD is requested here (<u>not</u> number of years since it was used). |
| | Q.66 | 1. All respondents should be asked Q.66. |
| | | 2. If using cards, hand her card 66 instead of asking it out loud. Then give her card 57, which lists the methods, and ask her for the letter next to all of the methods she used in the year before last, (e.g., from November 1975 to November 1976). |
| | Q.67 | 1. This question deals with any kind of sterilization operation Sometimes it is done only for the purpose of ending child-bearing. Sometimes an operation is performed for other medical reasons (e.g., cancer) but it also makes the woman unable to become pregnant anymore. |
| | Qs. 67a, 67b | 1. Ask only if woman says "yes" to Q.67. (Other-wise, skip to Q.68.) |
| | | 2. Some synonyms for different types of operations are listed in parenthesis. Usually, the operation solely for the purpose of sterilization is tubal ligation - cutting and tying off tubes, applying clips, or burning out a section of each tube. |
| Page 20: | Q.68b | 1. This question asks about "cohabitation," or common law marriage. |
| | Q.69 | 1. Use whichever choice is appropriate - either husband <u>or</u> sexual partner. |
| | | 2. The name of the operation for sterilization of a man is called "Vasectomy." |

3. If no particular man is mentioned in Q.68, ask Q.69 anyhow. If woman says the question does not apply to her situation, circle the "2" code.

Q.70

1. Again, choose the appropriate term to call the man.

2. If there is no particular man mentioned in Q.68, ask Q.70 anyhow; if woman indicates question doesn't apply to her situation, circle "2" for "no."

Q.71

1. This question asks about somewhat common gynecological and obstetrical problems which a woman might have had recently, and for which she sought medical care.

2. If she had a spontaneous miscarriage or a premature baby, then be sure questions in the pregnancy section have been answered about this pregnancy.

Q.71a

1. Instructions here are to remind you to check earlier parts of the interview to be sure information has been obtained.

2. If infection or ectopic pregnancy, check Q.7 to see if that provider is listed. Then check Q.9 to see if the date corresponds to one that might be there already. If both of these are true, then check Q.10 for the condition. If this contact was reported, then go on to Q.72.

3. If the provider is not listed, ask Qs.7a or 7b, 8, 9, not 10, 10a-10k, according to their appropriateness (i.e., if woman did not go to hospital, then skip ahead to Q.72).

4. Indicate at top of column that responses were obtained after Q.71 was asked.

5. Fill in information for Qs. 7 and 10, but you don't need to ask because they duplicate Qs. 71 and 71a.

Put in a separate column

6. If the provider is listed, but for another time or condition, then ask only Qs. 9, 10a - 10k, again as appropriate. Also, follow comments #4 and #5 above.

7. If miscarriage or premature delivery, the pregnancy series should be checked. Look first at Qs.32 and 33. If the pregnancy has been reported, proceed to Q.72. If not, ask Qs. 31-46a, as appropriate. Don't ask respondent questions which duplicate information you already have - e.g., Q.32, 41, 45 (no need to get name and address again).

Page 21: Q.72

1. If a woman presently has a job (part-time or full-time) that she works regularly, her answer would be "yes."

Q.72a-c

1. Sometimes the conditions surrounding employment are not clear. These questions clarify her employment status.

2. If it is still not satisfactorily explained, provide notes about her situation. (likewise for questions which follow about her mother, father, and husband/male housemate).

Q.73

1. This question is about the type of work the respondent does - her job title, or occupation.

2. A space is provided to write in the type of business, or industry in which she works. Be sure always to probe for this information.

Q.73a

1. This question tries to get a description of the activities she performs on the job. A good probe might be, "On a typical day, what might you do at work?"

2. You could also probe for full-or part-time status, i.e., the number of hours worked per week - but this is not as important as a good description of what she does.

Q.74

1. If her mother is not working, there are several possibilities for her employment status. These are listed as additional responses to Q.74. Circle all that apply. Probe enough to find out about the mother's situation. If none of the categories fits, then write in a clear explanation.

Qs.75-75b

1. The same comments as for Q.73-73b apply here.

2. Sometimes people will tell you how much they make an hour, but usually such information is not known for parents; therefore, it is not being asked in this questionnaire.

Page 22: Qs. 76-77b

1. The comments concerning the meaning of these questions and content of appropriate answers are the same for Qs. 73-75b.

Pages 22-23: Qs. 78-79b

1. These questions should be asked about a man to whom the woman is married, or who lives with her and contributes to their joint household expenses. If neither of these conditions holds, then skip to "closing of interview."

2. Use appropriate terminology (choice within the parenthesis) according to the woman's situation, which should have been clarified by now.

3. Remarks concerning the substance of questions and acceptable answers are the same as previously mentioned for Qs. 73-75b.

Closing of Interview

1. Read the statement to the respondent.

2. Follow the instructions. A checklist of question numbers to review for names and addresses appears on Page 24.23.

Page 23-24

1. As you go through the questionnaire, check that all questions appropriate to her situation have been answered.

2. Circle Y or N for each question number in the list.

3. If a provider was mentioned, ask her if she would give us permission to obtain her medical records from there, and then circle Y or N according to her response.

4. Fill in the name of the provider on the consent form and then have her sign and date it. If she doesn't object, wait to fill in the address and dates of interest on the form until later, unless it would become confusing to you. It will just take time here.

5. Then ask her about any possible phone contact for office use. If she refuses, we must accept that - but be sure to read the next paragraph which explains our desire to keep the interview confidential.

6. Specifically, find out who we may and may not speak with even in a general way, the best time of day or evening to reach her, if we could leave a message for her to call us back.

7. Specify relationship of any contact persons to the respondent.

8. Then thank her, using the closing remarks.

9. After leaving the home, note the ending time, her ethnic group and any observations that would help us understand her situation or what happened during the interview.

THE JOHNS HOPKINS UNIVERSITY

December 15, 1977

MEMORANDUM #1

[redacted]

FROM: Ann, Isobel, and Shirley

1. Remember to write subject # on all extra sheets.

2. Do not write subject # on Consent Form. If name is illegible, print under it.

3. Go back to Q7 after Q.11, 27, 71a. If a new Health Provider is mentioned, note number of question on top of column.

## Explanations of Questions

Qs. 7-10

1. for multiple providers, especially dermatologists, allergists, etc., ask 7, (&a &b), and 8 only. You won't need to obtain consent for medical records for these places.

2. Each clinic is recorded separately.

3. If provider has died, or respondent has moved away from the last provider, ask 7, (7a & b), and 8. Add a note that the doctor has died or is not seen now because the respondent has moved. In the latter case, get the doctor's or clinic's full address anyhow. If the doctor has died recently, i.e. last 5 years, we would like consent for him/her, (especially if gyn, GP, internist, or family planning).

Q. 7b

4. If at 7b you learn respondent has gone to Hospital Outpatient Clinic, be sure to go back to 7a.

Q.10a-10k

1. Only asked if for Family Planning or OB-GYN.

Q.10b

1. Ask name or description of medication.

Q.10c

1. If the provider is a hospital outpatient clinic, then this question is redundant to ask. You can confirm that she was at a hospital clinic, and the answer to be circled will be yes.

| | | |
|---|---|---|
| Q.10e | 1. | Days are equivalent to nights, outpatient = 0. |
| Q.11 | 1. | Always ask Q.11a even if no provider mention in Q7. |
| Q.14 | 1. | Current medications will mean within 6 months of the interview. |
| | 2. | List whatever disease respondent tells you about in Q.14a. It can be crossed out later by us if we don't need that information. |
| Q.15 | 1. | Have you <u>ever</u> (no time limit) except for pregnancy. If answer is no, skip to Q.16. |
| Qs. 16-20 | 1. | All refer to when not taking the Pill or using IUD. |
| | 2. | If having an IUD substantially changed her bleeding pattern, then note this and direct respondent to tell you about her typical pattern without the IUD. |
| | 3. | When you read the choices in Q.17, replace "I" with "you" as you read. |
| | 4. | Card 20 can be used before Q.20 as an aid to explaining "period" and "cycle". Be sure that the respondent understands how we are using these words. Sometimes women think that "period" means the whole menstrual cycle. |
| Q.21 | 1. | Refers to last 5 years. Write it in. |
| | 2. | Probe - "Did this happen more than once," or "was there another time it happened?" |
| Q.28 | 1. | If answer is no, skip to Q.57. Forget Q.29. |
| Q.30 | 1. | Tell respondent that we will be asking a series of questions about each of "these events," so she can decide whether she wants to use cards for each one or only selected ones (i.e. only some of the pregnancies). Also, if she wants to go back to using cards after you have started to go through the questions aloud, that option is available. |
| Q.32 | 1. | Cross out skip instruction after response choice of "induced abortion." Write in the following under Q.33: IF ABORTION, SKIP TO 45. Be sure to ask Q.32 and Q.33 of all. |
| Q.35 | 1. | Regular Prenatal Care means <u>ANY.</u> |
| Q.47-52 | 1. | <u>DO NOT SAY 3 MONTHS LATER</u>, Use proper month and year. If 2 pregnancies, write 2nd. pregnancy 3 month date under the first so you can refer easily to that date. |
| Q.48 | 1. | Even if using the Pills. |

Q.55 1. Before asking Q.55, insert this filter question:

Have you been trying to get pregnant in the last year? Yes . . . 1 No . . . 2

IF NO, SKIP TO 56.

Q.57 1. Questions refer to contraception. Even if respondent is alone, interviewer must still show her card 56 because it is not written in.

2. SLASH LINE through 12th month of the Grid.

3. Interviewer must indicate if none or pregnant by circling months.

4. Re: douching. Write No for response if only used for personal hygiene reasons. You might need to probe about how, ie., under what circumstances, she usually douches. Douching would be Yes only if she douched immediately after having sex, although she may or may not expressly say that she does it to keep from getting pregnant.

5. The same comments hold for douching in Q.66.

Qs. 68a-b 1. If woman is separated, divorced, or widowed, ask Q.68b before proceeding to Q.69.

Qs. 69-70 1. Before asking Q.69, insert the following as a filter question:

In the past year, have you had at least one sexual partner? Yes . . . 1 No . . . 2

IF NO, SKIP TO 71.

2. If more than one current sexual partner, rephrase the questions, for example:

Q.69. Has your current sexual partner, that is, the one you see most often, ...?

Probably the woman would cue you in to rephrase the questions.

Q.73 1. If answer to Q.72 is No, add a filter question:

How long has it been since you last held a job?

If greater than one year, skip to Q.74.

2. Same for Qs. ~~75~~ 74, ~~77~~ 76, ~~79~~ 78.

Questions referring to health provider and consent form, change

15b to 15c
27f to 27h
55b to 55c

Manual

p. 4, Q. 15 #3.--change to read: but it will <u>not</u> be included.....

p. 5. Q.15b 1&2 - Termination of pregnancy means Abortion.

Consents

1. Obtain consent forms for deceased M.D.'s

2. Be sure to get providers in the last 5 years, who are for family planning, gyn, general health care, internal medicine, although providers in these categories from further back in time should also be on consent forms. In particular, be sure to get consent for major abdominal surgery, even if it was some time ago.

THE JOHNS HOPKINS UNIVERSITY

January 5, 1978

MEMORANDUM #2

TO: Interviewers

FROM: Ann Merrill

1. Circle all 9's where necessary, ex. in SKIP patterns.

2. Be careful that slash marks don't look like 1's.

3. Please don't ask questions that should be skipped. Ex.: Don't ask 10a - 10k if it's not an ob-gyn problem; skip to 11.

4. Double check age of Respondent as well as other dates such as of pregnancies (make sure full term pregnancies are approximately 9 mos.).

5. Q.23 - Fill in answers at bottom (consent forms).

6. Q.34-44 - Don't ask in abortion cases.

7. Q.56 - Please circle the appropriate answer.

8. Follow MEMO #1. Attention to, Q.21, Q.55, 1; Q.69-70, 1.; and Q.73, 1. Use these filter questions consistently.

# PROJECT V

# IMPROVED CLINICAL APPROACHES TO SURGICAL TERMINATION OF PREGNANCY

Project V

Study 1: Management of first trimester abortions.

These studies have been completed and were recorded in the previous report. An additional study by Cohen et al is also included.

Study 2: Continued studies of pharmacological means of cervical dilation.

A manuscript regarding the work-force instrument is included in this report.

# GESTATIONAL TROPHOBLASTIC DISEASE WITHIN AN ELECTIVE ABORTION POPULATION

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ABSTRACT

Gestational trophoblastic disease, most commonly hydatidiform mole, is an unusual condition within the United States. The in cidence of hydatidiform mole has been reported to vary from 1 in 1200 to 1 in 2000 pregnancies. The report describes 8 cases of hydatidiform mole and 1 case of choriocarcinoma among 4829 patients presenting for elective first trimester abortion. Factors which might account for the frequency of 1 in 600 for hydatidiform mole are discussed. Although the clinical course for patients with hydatidiform mole appears benign, gross examination of tissue obtained at suction curettage and the liberal use of histologic evaluation in questionable cases is required to make the diagnosis.

## Introduction

With the liberalization of elective abortion in the United States since the early 1970's, the number of patients presenting for pregnancy termination has shown a gradual increase. For example, about 900,000 cases were completed in 1974 in the United States while currently the annual case load is estimated to be in excess of one million procedures.[1] In addition, the percentage of patients undergoing first trimester suction procedures has shown a gradual increase to approximately 90% of all patients. At least 50% of first trimester cases are performed in outpatient free standing clinics.[1] Such clinics, in order to offer services at a reasonable cost to patients, frequently do not submit specimens for pathological examination unless faced with a diagnostic problem. In contrast, hospital based programs, in order to meet national hospital accreditation criteria, routinely submit all operative specimens for pathologic evaluation.

One of the potential problems faced with clinicians performing elective abortions is the occurrence of gestational trophoblastic disease. Although the classic gross features such as grape-like clusters in the absence of a fetus are usually readily recognized, unfortunately, not all instances of trophoblastic disease have these findings. Furthermore, in pregnancies of 8 weeks or less duration from last menstrual period, one may have difficulty in grossly identifying fetal parts. Therefore, unless all specimens are submitted for histological evaluation, one may fail to recognize some cases of gestational trophoblastic disease.

The purpose of this present report is to review the experience in the Fertility Control Center with gestational trophoblastic disease from July 1, 1973 through June 30, 1976. Since all specimens routinely undergo pathologic evaluation, the study enabled us to determine the frequency of this problem within our abortion population as well as to determine the clinical characteristics of these patients.

## Materials and Methods

From July 1, 1973 through June 30, 1976, 4829 patients underwent suction curettage within the Fertility Control Center. About 75% were 12 weeks or less duration of gestation while the remaining 25% were beyond 12 weeks as determined by fetal foot length measurements. However, the vast majority of these latter pregnancies did not exceed 14 weeks. Midtrimester amnioinfusion patients were not part of this current study. Such patients routinely had determination of the presence of fetal heart tones. In the absence of fetal heart tones, sonography was carried out and if an abnormality such as gestational trophoblastic disease or an ovarian cyst was found, the patients were referred to the gynecology division.

All patients presenting for abortion were screened with a history, physical examination, and laboratory tests including a urine pregnancy test. The suction curettage procedures were carried out utilizing paracervical block anesthesia and intravenous analgesia. All obtained tissue was submitted for pathologic review.

The criteria utilized to diagnose gestational trophoblastic disease included presence of placental villi with edema, loss of vasculature, and varying degrees of trophoblastic proliferation.[2] Once the diagnosis of gestational trophoblastic disease was made, patients were followed by the Division of Gynecologic Oncology.

Patients with hydatidiform mole were followed weekly with the Beta-subunit of serum human chorianic gonadotropin until titers were negative, then monthly for a minimum of 1 year. In addition, chest roentgenograms were obtained initially and at 6 months. The course of one patient with choriocarcinoma is described in detail.

## Results

Eight patients with hydatidiform mole and one patient with choriocarcinoma were diagnosed during the 3 year period of study. During this same time period, 4829 cases of suction curettage were completed. Table I lists the demographic characteristics of this latter group of patients.

Table II details the characteristics of the 8 patients with hydatidiform mole. The frequency of hydatidiform mole within the group of patients presenting for suction curettage was about 1 in 600. As shown, 75% were age 16 or less, 75% were Black, 87.5% were single, and 62.5% were nulliparous. In addition, 75% were receiving Medical Assistance, a characteristic utilized in this study to grossly estimate socio-economic status. The majority of patients were asymptomatic although 2 patients complained of mild vaginal spotting and 1 patient presented with galactorrhea. Three patients had durations of pregnancy by menstrual data that were two or more weeks less than the examiner estimated.

The operator was able to grossly identify tissues suspicious of molar pregnancy in 75% of the cases, but in one case a gross diagnosis of decidua and no fetal parts was made, while in another the gross diagnosis of tissue normal for a pregnancy termiantion was made. In these two latter instances, the histology showed hydatidiform mole.

All patients with the diagnosis of hydatidiform mole developed negative titers within 6 weeks and no patient required chemotherapy. One patient underwent abdominal hysterectomy at a later date for symptomatic myomas and her pathology did not reveal evidence of trophoblastic disease. Another patient has presented to the Fertility Control Center on three subsequent occasions for pregnancy termination without problems. The patient with galactorrhea had disappearance of the symptoms several weeks after evacuation of her molar pregnancy.

In addition to the 8 cases of hydatidiform mole, an unusual case of choriocarcinoma presented during the time period of study:

She was a 17 year old single, Black patient, para 0010, not on Medical Assistance who presented to the Fertility Control Center at 15 weeks duration of gestation from her last menstrual period with a history of several days of spotting and request for pregnancy termination.

Her past history was positive only in the finding that she had undergone dilatation and curettage for elective abortion in an outpatient clinic 15 months prior. Physical examination by two examiners indicated a somewhat irregular anterior positioned uterus which was 10 to 13 weeks in size. Routine laboratory tests were normal and the urine pregnancy test was positive.

Clinical Course:

A sonogram reported a uterus 9 weeks in size with a pattern suggestive of a missed abortion. Two subsequent suction curettages obtained only scant tissue which was histologically identified as decidua. In the presence of a persistently positive urine pregnancy test, an examination under anesthesia and laparoscopy were then performed. The pelvic examination revealed ill-defined thickening

in the base of the right broad ligament. Laparoscopy revealed normal pelvic structures except for possible thickening of the right broad ligament.

A repeat sonogram reported the presence of a 6 x 5 centimeter cystic mass in the right pelvis separate from the uterus. At laparotomy, this cystic cavity was identified adjacent to the cervix, in the base of the broad ligament, just beneath the right uterine artery. The cavity was entered with the release of gestational-like tissue which was later histologically identified as choriocarcinoma.

Her post-operative course was complicated by fever of unknown origin. In addition, a post-operative intravenous pyelogram showed narrowing of the right ureter at the uretero-vesical junction with hydronephrosis. A metastatic work-up was negative and after 5 courses of methyltrexate, negative titers of the Beta subunit were obtained. At 27 months after surgery, she remains asymptomatic with negative titers.

Discussion

Gestational trophoblastic disease, most commonly hydatidiform mole is an unusual condition within the United States. The estimate usually noted is 1 in 2000 pregnancies.[2] However, a study from North Carolina[3] and one from Rhode Island[4] indicate incidences of 1 in 1200 and 1 in 1450 pregnancies respectively. The current report shows that among elective abortion patients presenting for first trimester suction curettage, a frequency of 1 in 600 may be realized.

One explanation for this increased incidence in our population may be that patients destined to abort molar pregnancies are

undergoing curettage with subsequent submission and identification of material as gestational trophoblastic disease. Such patients might otherwise spontaneously abort at home and not have tissue pathologically evaluated.

It has been suggested that a number of factors such as age, parity, nutritional status and race may influence such rates. When one compares the patient with gestational trophoblastic disease with other patients requesting first trimester pregnancy termination within our population, very little difference can be seen. Like the general group of patients, the patients with gestational trophoblastic disease are more frequently single, Black teenagers of low parity. These characteristics are different than those characteristics sometimes associated with gestational trophoblastic disease in that increased incidence of the disorder has been reported to be associated with increasing maternal age and parity.[4] However, one report does indicate an apparent increased frequency of the condition among Blacks.[4]

One distinction, although not statistically significant, is that patients with gestational trophoblastic disease more frequently receive Medical Assistance and therefore are probably from a lower socio-economic class than the general group of patients. Low socio-economic status and poor nutrition may be related to the occurrence of hydatidiform mole.[5] However, at least one report suggests that this relationship may not be important for cases occurring within developed countries.[6]

Although durations of gestation as noted by menstrual data and pelvic examination show variation in some instances, this finding is not too dissimilar from our experience with patients who do not have trophoblastic disease.

With the exception of the patient with choriocarcinoma, the disease and clinical course of most patients appears to be relatively benign. However, careful evaluation and follow-up for at least one year is of obvious importance. Finally, it should be noted that hydatidiform mole or an abnormality was suspected by gross examination at suction curettage in all but one case. However, the recognition of this entity still requires that the operator carefully inspect all tissue obtained at suction curettage. Fetal parts should be identified and in the absence of fetal parts or in the presence of abnormal appearing tissue, histological evaluation is mandatory.

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TABLE I

DEMOGRAPHIC CHARACTERISTICS OF PATIENTS UNDERGOING SUCTION CURETTAGE IN THE FERTILITY CONTROL CENTER FROM JULY 1, 1973 THROUGH JUNE 30, 1976

| CHARACTERISTIC | PERCENT |
|---|---|
| Age | |
| 15 or less | 10.1 |
| 16-20 | 43.8 |
| 21-30 | 34.4 |
| 31 or greater | 11.7 |
| Race | |
| White | 42.2 |
| Black | 57.8 |
| Marital Status | |
| Single | 62.3 |
| Other | 37.7 |
| Parity | |
| 0 | 51.3 |
| 1-3 | 38.3 |
| 4 or greater | 10.4 |
| Pay Status | |
| Medical Assistance | 39.0 |
| Other | 61.0 |

TABLE II

CLINICAL CHARACTERISTICS OF PATIENTS WITH HYDATIDIFORM MOLE IN THE FERTILITY CONTROL CENTER FROM JULY 1, 1973 THROUGH JUNE 30, 1976

| AGE | RACE | MARITAL STATUS | PARITY | PAY STATUS | DURATION OF GESTATION BY LMP | DURATION OF GESTATION BY PELVIC-EXAM | SYMP-TOMS | IMMED. POST-OP DX. OF OPERATOR | DURATION OF FOLLOW-UP |
|---|---|---|---|---|---|---|---|---|---|
| 15 | White | Single | 0 | M.A.* | 10.5 | 12 | None | Hydatidiform mole | 14 months |
| 32 | Black | Other | 2 | M.A. | 16 | 10 | Spotting | Hydatidiform mole | 12 months |
| 15 | Black | Single | 1 | M.A. | 12 | 11 | None | Hydatidiform mole | 12 months |
| 16 | Black | Single | 1 | M.A. | 11.5 | 8.5 | Galac-torrhea | Hydatidiform mole | 12 months |
| 15 | Black | Single | 0 | Other | 7 | 7 | None | Hydatidiform mole | 12 months |
| 23 | Black | Single | 0 | M.A. | 8.5 | 8.5 | None | Normal | 12 months |
| 16 | White | Single | 0 | Other | 8.5 | 9 | None | Hydatidiform mole | 12 months |
| 16 | Black | Single | 0 | M.A. | 10 | 6.5 | Spotting | Decidua; no fetal parts | 12 months |

* M.A. Stands for Medical Assistance

# Forces Associated With Cervical Dilatation At Suction Abortion: Qualitative and Quantitative Data In Studies Completed With a Force-Sensing Instrument

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Abstract

Cervical dilatation is one of the most common surgical procedures. It is completed in a blind manner and requires clinical skill for it to be completed safely. This study attempts to evaluate factors associated with cervical damage during cervical dilatation. The results of cervical dilatation utilizing a force-sensing cervical instrument is reported.

Twenty-two cervical lacerations occurred in a population of 1602 suction abortions, an incidence of 0.12%. The incidence in nulliparous and multiparous patients was 2.7 and 0.23 percent respectively. A small number of uterine perforations were doucmented to be related to cervical trauma.

Thirty-five nulliparous and multiparous patients underwent cervical dilation with a force-sensing instrument. Required work expressed in kgs per sec. was greatest in nulliparous patients dilated with Pratt dilators. Less work was required to effect dilatation of multiparous patients. Cervical dilatation initiated by laminaria in nulliparous women markedly reduced the required work to effect dilatation to 10 mm's with Pratt dilators.

These results would suggest that it would be prudent to utilize laminaria in most nulliparous patients seeking pregnancy termination by suction curettage.

In 1976, an estimated 1 million elective abortions were carried out in the United States with 93% of the procedures being completed by cervical dilatation in combination with sharp or suction curettage (1). Thus, safe and effective dilatation of the cervix is an essential step in the uterine evacuation process.

In general, cervical dilatation is carried out over a three to four minute interval utilizing a set of graduated, tapered metal dilators, such as the Pratt dilator. In addition, laminaria tents are utilized on occasion either alone or in conjunction with dilators to effect cervical dilatation (2).

The dilatation process is performed "blindly" with the operator being dependent upon "feel" combined with experience in order to determine how much mechanical force to exert. Excessive force may result in cervical lacerations through avulsion of the cervical stabilizing clamp or by rapid over distention of the internal cervical os. In addition, dilatation without careful determination of the direction of the cervical canal may also result in formation of a false passage with the potential for cervical laceration and uterine perforation.

Patients who seem at particular risk for cervical damage during dilation include nulliparous women with hypoplastic

cervices and parous women with scarred cervices.

The present study attempts to evaluate factors potentially associated with cervical damage following cervical dilatation and suction curettage for elective abortion. In addition, the results of cervical dilatation utilizing an instrument designed to measure both force and work are presented in an effort to correlate quantitative measurements with some of the subjective impressions experienced by operators.

## Materials and Methods

Patients undergoing suction curettage abortion in the Fertility Control Center (FCC) of the Johns Hopkins Hospital during 1976 were studied. The study group consisted of 1602 patients of which 740 (46.2%) were nulliparous and 862 (53.8%) were multiparous.

All procedures in the FCC are carried out in a standardized fashion by board eligible or certified faculty members. Meperidine, 50 mgs, and Diazepam, 10 mgs, is slowly given intravenously. After pelvic examination and surgical prepping, a circumferential, submucosal paracervical block is performed utilizing 10 cc's of 1% Lidocaine. The cervical canal is then sounded for direction and cervical dilatation is carried out over a three minute interval utilizing Pratt dilators. Upon completion of cervical dilatation, suction curettage followed by brief, sharp curettage is performed.

Laminaria tents are primarily employed for patients with hypoplastic appearing cervices or nulliparous patients with pregnancies felt to exceed 12 menstrual weeks. In most instances, a 5 mm laminaria tent* is inserted three to four hours prior to the above described dilation and curettage procedures. On occasion, a smaller laminaria tent may be utilized.

In order to quantitate factors associated with cervical dilation, 20 nulliparous and 15 multiparous patients underwent cervical dilation with a force-sensing cervical dilation instrument. This instrument developed by the Applied Physics Laboratory of Johns Hopkins University is designed to measure force and work associated with cervical dilation utilizing modified Pratt dilators.** As shown in Figure 1, a force sensing transducer is housed in a receptacle which accepts Pratt dilators modified into a single ended format. The force sensing transducer is in turn connected to the unit which houses the computation and display circuitry. The final component of the system is an OEM recorder module containing two channels.

Although the transducer measures only force of insertion, the tensile strength of the cervical wall is related to insertional force due to the taper of the dilators. Since Pratt dilators' diametral taper varies linearly with length, tensile strength of the cervix is approximated by the instrument in the following fashion:

*Berkeley Corporation

**The Force-Sensing Instrument was developed by J. G. Chubbuck and H. R. Bittner of the Staff of the Applied Physics Laboratory of The Johns Hopkins University.

$$\text{Cervical Tension} = \frac{\text{Insertion Force}}{2\pi\,(\text{Dilator Taper})}$$

The transducer provides linear dc voltage output proportional to the force being applied to the cervix at a sensitivity of one volt per kilogram of applied force.

As a means of accumulating and storing the time integral of force over the extended dilatation time period for each dilator, the time integral is computed in increments and converted to digital form. Discreet pulses, each representing 0.1 kg-seconds of force impulse, are generated in the design of a pulse resetting integrator. The sensitivity of the integrator is 10 pulse-per-volt-second as viewed at the output.

In order to discount the accumulation of forces not associated with dilation such as occur during changing of dilators, a foot switch is provided to the operator which interrupts the accumulator during such intervals.

Although the instrument contains outputs which displays dilator insertion force and measured force over the time of the total dilation process, accumulated force with time with the use of each dilator is the measurement of greatest interest. Accordingly, the work in kilograms per second at each dilator interval was plotted for each study patient. In addition, for all patients in this series, data relating to age, parity, duration of gestation, use of laminaria tent, and complications relating to cervical injury or uterine perforation were collected.

Results

Table I details the number of cervical lacerations occurring in the FCC population undergoing suction curettage in 1976. As shown, the frequency of laceration in nulliparous patients was about 10 times the rate seen in multiparous patients. This difference was significant ($P>0.001$). During 1976, 25% of pregnancies terminated by this method were gestations of greater than 12 weeks duration as determined by fetal foot length measurement, and 36.4% of the cervical lacerations occurred in this population as shown in Table 2 (3). The reported lacerations include not only those due to over distention, but also those secondary to cervical clamp avulsion. Two lacerations, both of which occurred in nulliparous patients, required sutures. It should be noted that laminaria tents were utilized for 225 (30.4%) of the nulliparous patients. The most frequent indication was pregnancy of greater than 12 weeks duration although a small percentage of patients had laminaria employed due to a hypoplastic appearing cervix. No patient in this laminaria group experienced a cervical laceration.

During this time interval, six (0.15%) uterine perforations occurred in the study group. Three patients were only observed, three required laparoscopy to ascertain damage and complete the abortion, and one of these patients required laparotomy to control bleeding. Of note was the observation that two of the lacerations, as documented by

laparoscopy, were at the level of the internal cervical os and were associated with the creation of a false passage during cervical dilatation. Both patients were nulliparous and a laminaria tent had not been utilized.

Table III summarizes some of the characteristics of the 35 patients undergoing cervical dilation with the force-sensing instrument. The age distribution and abortion history of this study group is comparable to that of the general FCC population.

The nulliparous patients who underwent dilation in combination with laminaria tent placement, all had laminaria inserted due to pregnancies of greater than 12 weeks. The The average time from insertion to Pratt dilation was 3.5 hours with a range of 2.9 to 4.6 hours.

Table IV summarizes additional characteristics of this study group. The size of the dilator when resistance was first noted was utilized as an estimate of inherent cervical resistance or tension for patients without laminaria and an estimate of laminaria effect for those receiving the device. For three out of the ten nulliparous patients receiving laminaria, resistance was first perceived at Pratt size 27, while five had resistance first noted at size 33. For nulliparous patients without laminaria, in only two instances was first resistance noted at Pratt size 33. In addition, it should be noted that the time required to effect

dilation was longer in this group. For multiparous patients, resistance to the Pratt dilator was observed as early as size 15 and in all cases, by size 27.

In summary, nulliparous patients treated with laminaria for an average of 3.5 hours, experienced effective cervical dilation. The multiparous and nulliparous patients subjected to dilation alone, demonstrated increased cervical force when first resistance was used as an estimate of inherent cervical resistance. Time required for complete cervical dilation varied considerably within the three groups.

The work recorded in kilograms per second with each size of Pratt dilators may be noted in Figures 2, 3 and 4. As shown in Figure 2, the work required to effect cervical dilation in nulliparous patients where laminaria were not utilized, showed considerable variation from patient to patient with high values of 25.0 and 27 kg. per sec. being recorded in one instance each. In nulliparous patients receiving laminaria (Figure 3), the greatest work required to effect cervical dilation was less than 9 kg.per sec. and the variation in work required was markedly less. Finally, in multiparous patients (Figure 4), the majority of patients demonstrated small quantities of force required to effect cervical dilation. However, three patients did show strikingly increased quantities of work as compared to the remainder of patients in that group. It should be noted that the patients requiring the greatest work had normal appearing cervices.

A number of the nulliparous patients demonstrated a marked increase in force to effect dilation with a number 27 Pratt dilator, with a subsequent decrease with larger dilators and a second increase associated with utilization of a 33 Pratt dilator. These changes also occurred in the multiparous patients. In the nulliparous patients treated with laminaria, the first marked increase in work required occurred with a 33 Pratt dilator.

Figure 5 displays the composite of the individual determinations for the three groups. Due to the extent of variability, no standard error of the means are indicated on this figure. Thus, it can be seen that the work to effect cervical dilation in nulliparous patients not receiving a laminaria tent is higher than the other two groups. When laminaria tents are utilized in nulliparous patients, it appears that the work curve is not dissimilar from multiparous patients. However, it should be noted that the duration of gestations did show considerable variation and that patient selection was not randomized, therefore, these differences could in part reflect differences in cervical behavior secondary to duration of gestation.

Discussion

Cervical injury continues to occur in association with cervical dilation and suction curettage primarily because of the subjective and blind nature of a procedure which is effected by force. Because of the increased morbidity and mortality rates of intra-amniotic injection procedures, increasing numbers of mid-trimester pregnancy terminations are being effected by dilatation and evacuation (1). This procedure requires even more extensive cervical dilatation than first trimester pregnancy terminations. The reported incidence of cervical injury with dilatation completed in association with suction abortion varies between 0.1 and 5% (4,5,6). The data of this current report demonstrates an overall incidence of cervical injury of 1.4% with a 10 fold increased incidence in nulliparous patients. However, most literature dealing with cervical laceration do not delineate the severity of the laceration nor is the incidence of cervical injury related to parity.

Clinical experience with laminaria would indicate that effective cervical dilatation may be accomplished by the utilization of these devices for relatively short periods of time prior to the initiation of suction procedures. There have been reports that the use of laminarias is associated with a reduced incidence of cervical trauma as compared to dilatation

effected by metal dilators in a rapid manner (7,8). These findings have been substantiated for nulliparous patients in this report. As a result of this experience, it may be appropriate to effect cervical dilation in most nulliparous patients with laminaria in order to minimize cervical injury. However, it should be noted that no data exists at present which indicates what the long term potential for injury or cervical incompetence may be when one utilizes these devices.

There have been several efforts directed towards quantitation of force and work required to produce cervical dilatation (9,10,11). Hulka et al documented in a population undergoing first trimester suction curettage that considerably more force was required to effect cervical dilatation with Hegar dilatators as compared to tapered Pratt dilators (9). In his study, the cervix offered the greatest resistance at 9 mm dilation (equivalent to Pratt size 27) then at larger dilations. These findings suggested that significant damage to the substance of the cervix may have occurred by dilating the cervix to 9 or more mm's during the first trimester of pregnancy. Furthermore, age, race and gravidity did not appear to effect the force required to accomplish cervical dilatation. Of interest was the lack of apparent difference in work required to dilate the cervices of primigravida versus multiparous patients. It was suggested that the failure to detect a difference between these two groups was possibly related to

the fact that no distinction was made by history between women who have previously delivered and were parous and women who had previously aborted but were nulliparous. Finally, Hulka presented evidence that as pregnancies advanced in the first trimester, less force was required to effect dilation.

The data presented in the present report is at variance with Hulka's findings (9). The first resistance of cervical dilation was noted at a smaller diameter dilator on the average when multiparous patients were compared with nulliparous patients. Furthermore, the work required to effect cervical dilatation was considerably greater in nulliparous patients as compared to multiparous patients except when laminaria tents were utilized. In the multiparous population, seven of the 10 patients showed a total of less than four kg's per sec. of work to effect cervical dilation to a Pratt size 33 dialator, while in the remaining three patients, the work required to effect cervical dilatation was as high as 9.5 to 15.3 kg's per sec. Thus, the work required to effect cervical dilatation of the multiparous cervix is variable and may be related to the presence of scarring from previous obstetrical incidents. The difference in work required in the multiparous patients could not be explained by differences in pregnancy duration. Also, operator difference was not a factor since all of the cases with the device were completed by a single operator. In the nulliparous population not receiving

laminaria, an even greater quantity of work was required in order to effect dilatation.

In a number of instances, both in multiparous and nulliparous patients, there was increased resistance with a number 27 Pratt dilator, but, there was always a second peak of resistance encountered with a number 31 Pratt dilator (equivalent of 10 mm's of dilatation). This second and usually larger peak of increased resistance was not shown in Hulka's study. It is assumed that the decrease in resistance is related to the occurrence of cervical damage that may well be of such a nature that it is not perceived clinically. This possibility finds support in the work of Liu et al in his in vitro cervical dilation study (10). This study indicated a "yielding" of the parous non-pregnant cervix at 9 to 11 mm's dilation in a substantial number of cases.

A third study determined cervical status six weeks after suction curettage by measuring the internal os diameter with dilators (11). In this study, if cervical dilatation at the prior abortion procedure was limited to 10 mm's or less, the cervical measurements at the six week follow up were normal. However, when the cervix was dilated to greater than 12 to 14 mm's, many of the patients had large cervical measurements at the follow-up period. Thus, there is evidence that cervical damage may occur at approximately 9 to 10 mm's of dilation in the first trimester of pregnancy. However, further study noting pre-abortion dilation measurements would be of importance.

It is of interest in the current study that nulliparous patients treated with laminaria did not show a substantial increase in resistance until a number 33 Pratt dilator was utilized (equivalent to more than 10 mm's of dilatation). It is unknown whether dilation effected by laminaria and Pratt dialators allows recovery of the cervix after the procedure. In addition, to facilitate safe termination of more advanced pregnancies, further study with longer treatment intervals or multiple laminaria are needed to determine if resistance can be further decreased and that the cervix regains it's pre-abortion status.

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## Legends

Figures:

1. A Force-Sensing Cervical System.
2. Work in Kg./sec. to effect Cervical Dilatation with Pratt dilators in nulliparous patients.
3. Work in Kg./sec. to effect Cervical Dilatation with Pratt dilators in laminaria pretreated nulliparous patients.
4. Work in Kg./sec. to effect Cervical Dilatation with Pratt dilators in multiparous patients.
5. A Summary of the work required to effect Cervical Dilatation in the three groups of Patients.

Table 1

Cervical Laceration Following Suction Curettage*

| | Para 0 | Para 1 or greater |
|---|---|---|
| Total patients | 740 | 862 |
| Number of Lacerations | 20 (2.7) | 2 (0.2) |

*Percentage in parenthesis

Table II

Distribution of Cervical Laceration

By Parity and Pregnancy Duration

| Pregnancy Duration | Para 0 | Para 1 or > |
|---|---|---|
| < 6 - 7 | 6 | |
| 8 - 9 | 3 | 1 |
| 10 - 11 | 3 | |
| 12 | 1 | |
| 13 | 2 | 1 |
| 14 | 1 | |
| 15 or > | 4 | |
| | 20 | 2 |

Table III

Age, Parity, and Past Abortion History of Patients Undergoing Cervical Dilation With the Force-Sensing Instrument

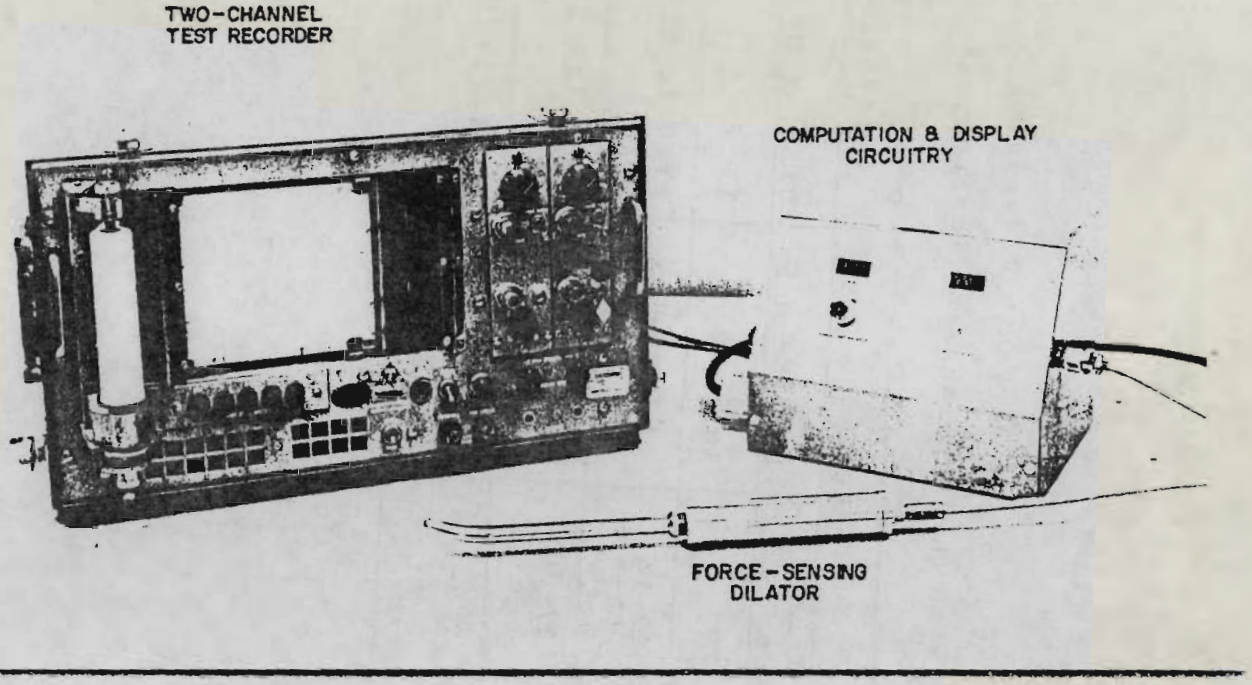
| Age | Para 0 | | | | Para 1 or Greater | |
|---|---|---|---|---|---|---|
| | Laminaria + Dilation | | Dilation Only | | Dilation Only | |
| | Never Preg. | Past ABN | Never Preg. | Past ABN | Never Preg. | Past ABN |
| Less than 15 | 1 | - | 1 | - | - | - |
| 15-19 | 5 | 1 | 3 | 2 | 4 | 1 |
| 20-24 | 2 | 0 | 2 | 1 | 5 | 1 |
| 25-29 | 1 | 0 | - | - | 1 | 1 |
| 30-35 | - | - | - | - | - | - |
| Greater Than 35 | - | - | 0 | 1 | 1 | 1 |
| TOTAL | 9 | 1 | 6 | 4 | 11 | 4 |

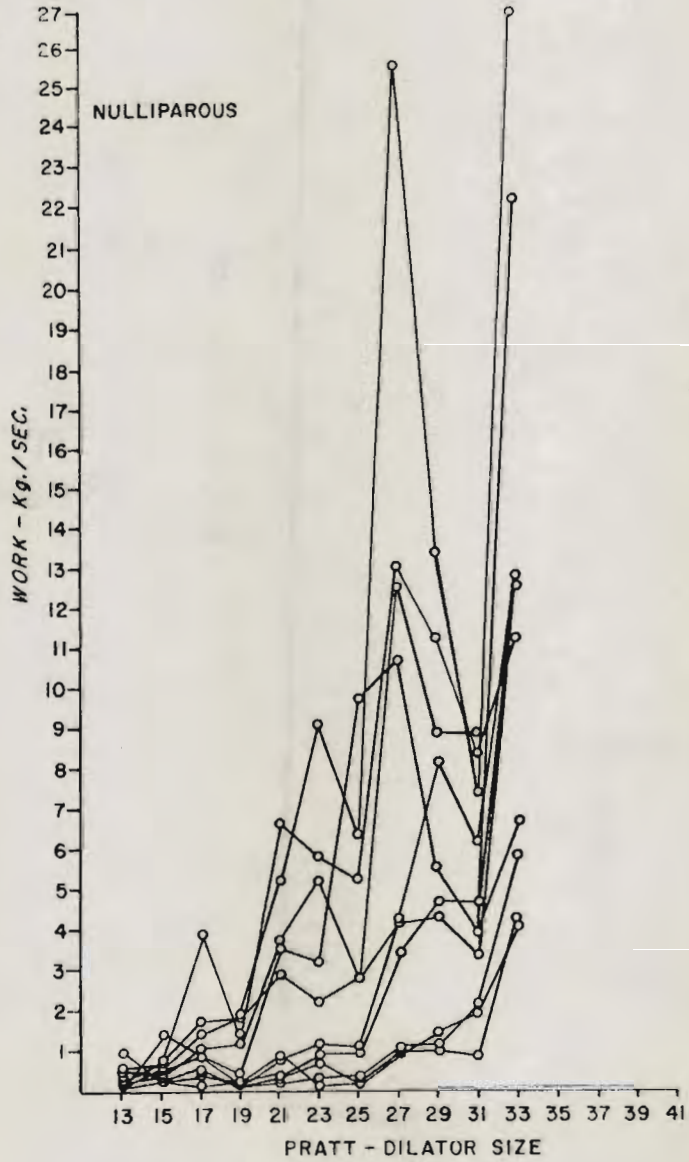
Table IV

Clinical Characteristics of Patients Undergoing Cervical Dilation with the Force-Sensing Instrument*

| | Nulliparous | | Multiparous |
|---|---|---|---|
| | Laminaria + Dilator | Dilator Only | Dilator Only |
| Patients | 10 | 10 | 15 |
| Pregnancy Duration (Weeks) | 12.7 (12-14) | 9.0 (6-12) | 11.0 (8-12) |
| First Resistance (Dilator Size) | 31.3 (27-39) | 25.6 (21-33) | 23.7 (15-27) |
| Cervical Dilation Time (Minutes) | 3.5 (2.9-4.6) | 3.9 (3-7) | 3.4 (2-6) |

*Range in parenthesis


TWO-CHANNEL
TEST RECORDER
COMPUTATION & DISPLAY
CIRCUITRY
FORCE-SENSING
DILATOR


NULLIPAROUS
WORK - Kg./SEC.
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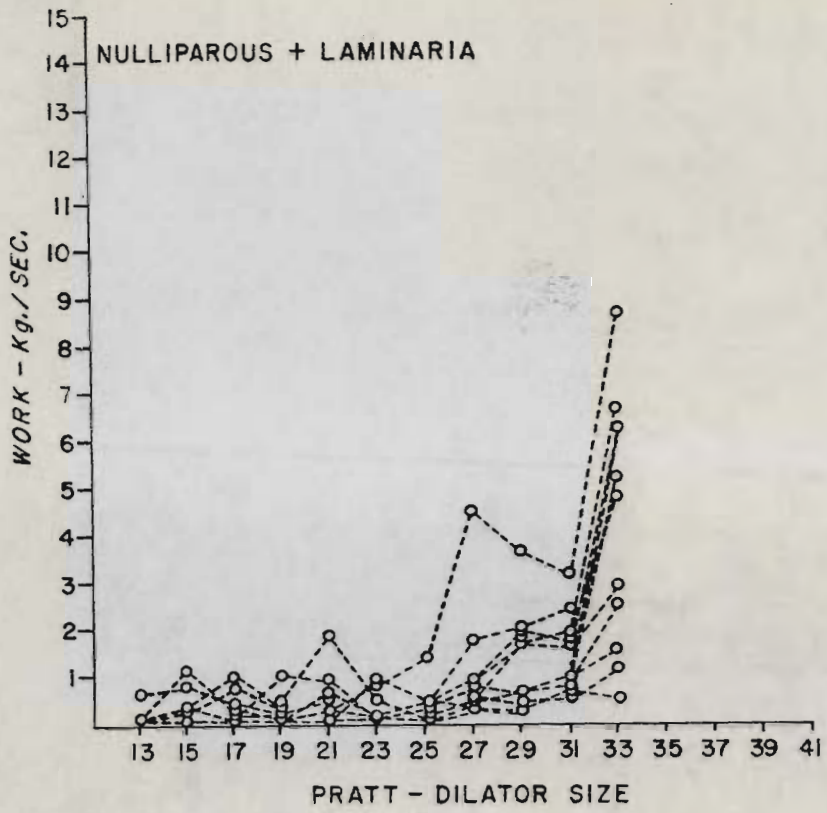
PRATT - DILATOR SIZE


NULLIPAROUS + LAMINARIA
WORK - Kg./SEC.
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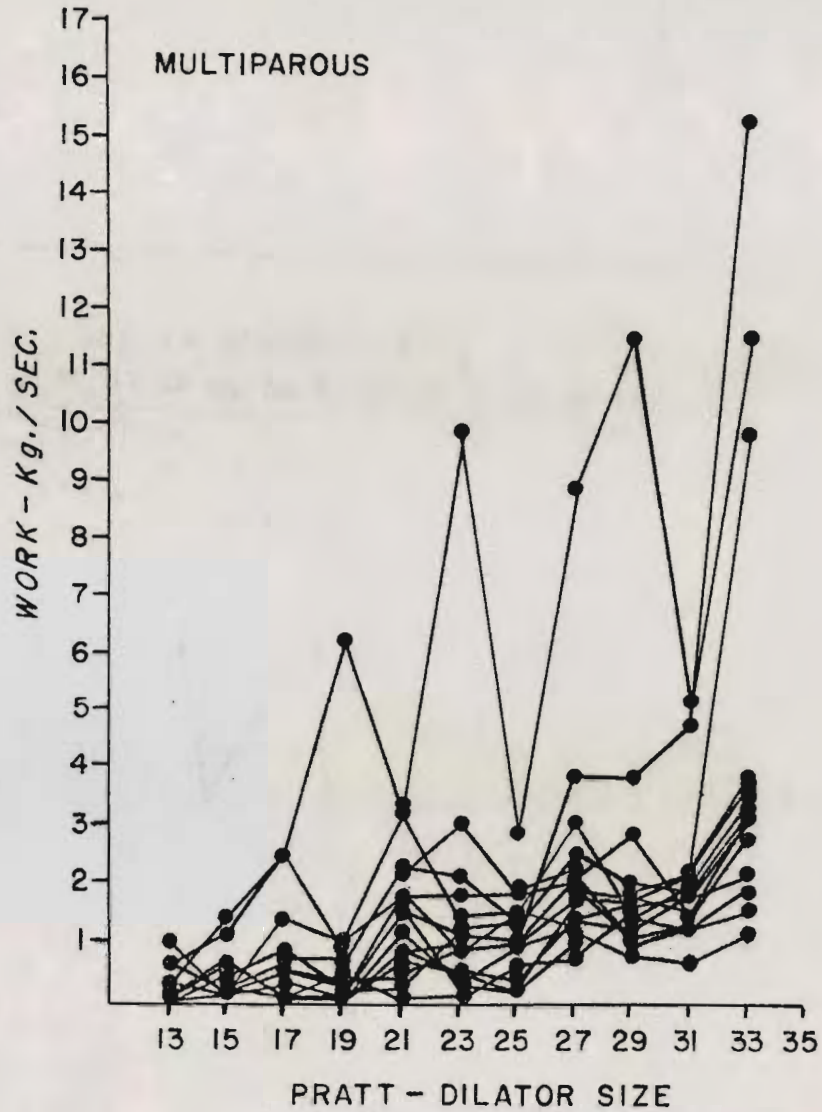
13 15 17 19 21 23 25 27 29 31 33 35 37 39 41
PRATT - DILATOR SIZE


MULTIPAROUS
WORK - Kg./SEC.
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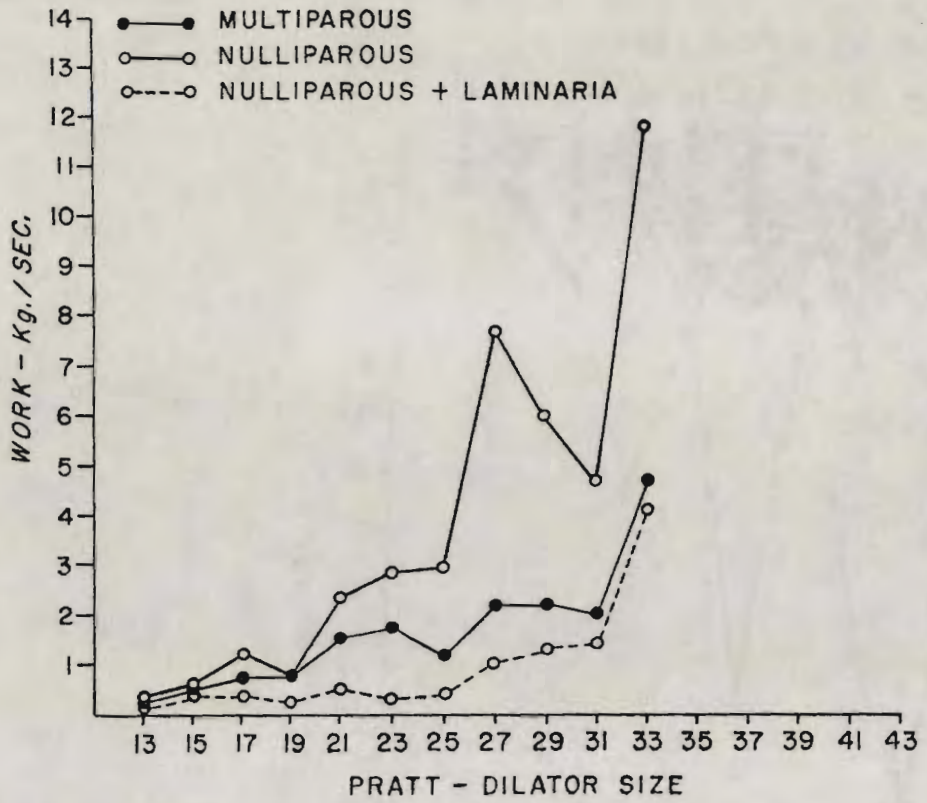
PRATT - DILATOR SIZE


MULTIPAROUS
NULLIPAROUS
NULLIPAROUS + LAMINARIA
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PRATT - DILATOR SIZE

Study 3: New Equipment, Battelle Corporation Dilator

No further studies have been run utilizing the various types of two-bladed dilators. After review of our data to date, it was determined that further study would not be overly profitable.

Attempts are being made now to obtain and utilize the "in situ" Battelle dilator. This would be advantageous since we have large numbers of potential patients for study and, in addition, because we have the Applied Physics Laboratory Device to test the effectiveness of the dilator.

AID SUPPORTED PUBLICATIONS

(1972-1978)


1. Intramyometrial prostaglandin $F_2a$. A. C. Wentz and T. M. King. Amer J Obstet Gynecol 114: 112, 1972.

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