BIBLIOGRAPHIC DATA SHEET

1. CONTROL NUMBER	2. SUBJECT CLASSIFICATION (695)
PN-AAJ-446	PC00-0000-0000

3. TITLE AND SUBTITLE (240)

Simplified techniques of fertility management; semi-annual report, Oct. 1979 - March 1980

4. PERSONAL AUTHORS (100)

5. CORPORATE AUTHORS (101)

Johns Hopkins Univ. School of Medicine

6. DOCUMENT DATE (110)	7. NUMBER OF PAGES (120)	8. ARC NUMBER (170)
1980	66p.	613.943.J65c-1979/80

9. REFERENCE ORGANIZATION (130)

Hopkins

10. SUPPLEMENTARY NOTES (500)

11. ABSTRACT (950)

12. DESCRIPTORS (920)

Fertility
Sterility
Abortion
Sterilization

13. PROJECT NUMBER (150)

932054800

14. CONTRACT NO.(140)

AID/DSPE-C-0054

15. CONTRACT TYPE (150)

16. TYPE OF DOCUMENT (160)

AID 590-7 (10-79)

613.943
J65c
Oct. 1979-
March 1980

RESEARCH ON SIMPLIFIED TECHNIQUES

OF FERTILITY MANAGEMENT

SIX MONTH REPORT

4/1/80

PROJECT I: Comparison of Optic Visor Versus the Surgical Microscope for Reconstructive Surgery of the Fallopian Tube

The objective is to determine which method will prove the most effective and less expensive for tubal sterilization reversal.

Principal Investigator: Dr. John A. Rock

During the past six months, 24 women met the criteria to be included in the randomization of optic visor - loop versus microscope. These pairs have been forwarded to the statistician, Dr. Allyn Kimball and follow-up is accumulating.

Pathology specimens have been supplied to Dr. Tim Parmley so as to meet the criteria for his study of fallopian tubes after sterilization. The current protocol is on schedule and there should be no difficulty in accumulating enough patients to answer this question.

Attached is a listing of the ordered pairs, their identifying letters, age, sterilization method, segment of tube anastomosed, method of magnification, interval to pregnancy and pregnancy outcome.

ORDERED PAIRS	IDENTIFYING LETTERS	AGE	STERILIZATION PROCEDURE	ANASTOMOSIS (SEGMENTS)	MAGNIFICATION	PREGNANCY INTERVAL	PREGNANCY OUTCOME
PAIR #1							
Old	C.C.	22	Pomeroy	Ampullary-Isthmic	Hood and Loop	4 mo.	Lost at 6 mo.
New	E.M.	28	Falope Ring	Ampullary-Isthmic	OMPI-6	6 mo.	Term delivery
PAIR #2							
New	C.K.		Pomeroy	Ampullary-Isthmic	Hood-Loop	3 mo.	Spontaneous abortion at 6 weeks.
Old	M.A.	27	Single Burn Electrocautery (Monopolar)	Ampullary-Isthmic	OMPI-6	2 mo.	Term delivery
PAIR #3							
Old	J.W.	30	Modified Irving	Ampullary-Isthmic	Hood-Loop	5 mo.	In Progress
New	A.N.		Double Burn Electrocautery	Ampullary-Isthmic	OMPI-6	9 mo.	Term delivery
PAIR #4							
Old	J.T.	29	Pomeroy	Ampullary-Ampullary	Hood-Loop		
New	F.W.		Falope Ring	Ampullary-Ampullary	OMPI-6	5 mo.	In Progress
PAIR #5							
New	B.G.	29	Pomeroy	Ampullary-Ampullary	OMPI-6		
Old	M.U.	26	Pomeroy	Ampullary-Ampullary	Hood-Loop	10 mo.	In Progress

ORDERED PAIRS	IDENTIFYING LETTERS	AGE	STERILIZATION PROCEDURE	ANASTOMOSIS (SEGMENTS)	MAGNIFICATION	PREGNANCY INTERVAL	PREGNANCY OUTCOME
PAIR #6							
New	J.S.		Single Burn Electrocautery	Ampullary-Isthmic	OMPI-6	3 mo.	In Progress
Old	J.C.		Single Burn Cautery	Ampullary-Isthmic	Hood and Loop	6 wks.	Right ectopic pregnancy
PAIR #7							
New	A.E.	24	Falope Ring	Ampullary-Isthmic	OMPI-6	3 mo.	In Progress
Old	D.S.	29	Electrocautery	Ampullary-Isthmic	Hood	2 mo.	In Progress
PAIR #8							
New	A.A.	32	Electrocautery	Ampullary-Isthmic	OMPI-6		
Old	S.S.	27	Electrocautery	Ampullary-Isthmic	Hood		
PAIR #9							
Old	L.J.	32	Electrocautery	Ampullary-Isthmic	Hood		
New	D.P.	25	Pomeroy	Ampullary-Isthmic	OMPI-6		
PAIR #10							
Old	S.G.	35	Pomeroy	Ampullary-Isthmic	Hood		
New	L.J.	27	Electrocautery	Ampullary-Isthmic	OMPI-6		IN Pregnancy
PAIR #11							
Old	B.M.	35	Pomeroy	Bilateral Ampullary-Isthmic	Hood		
New	D.K.	32	Cautery	Bilateral Ampullary-Isthmic	OMPI-6		

ORDERED PAIRS	IDENTIFYING LETTERS	AGE	STERILIZATION PROCEDURE	ANASTOMOSIS (SEGMENTS)	MAGNIFICATION	PREGNANCY INTERVAL	PREGNANCY OUTCOME
PAIR #12							
New	P.T.	31	Pomeroy	Ampullary-Ampullary	OMPI-6		
Old	A.S.	27	Pomeroy	Ampullary-Ampullary	Hood		

PROJECT II: Histopathological Description of Fallopian Tubes Following Various Methods of Tubal Occlusion in Which There Have Been Both Subsequent Success and Failure

The Objective of this study is to develop a hypothesis for the improvement of safety and efficacy of tubal ligation.

Principal Investigator: Dr. Timothy H. Parmley

Since the onset of the contract, we have accessioned 30 cases. Five of these are cases in which previously ligated tubes have been removed incidental to subsequent pelvic disease. Twenty-five of the cases are tubal reanastomoses. By a variety of injection techniques, we have studied these cases for the presence of tubo-peritoneal fistulae. We are in the process of preparing for publication this initial experience.

Amongst the five cases of tubal removal, we have successfully injected India ink through two fistulae. In both cases, microscopic sections subsequently revealed the ink to be in endometriosis-lined channels.

Amongst the twenty-five cases evaluated for reanastomosis, there were fourteen tubes with a fistula and twenty-four with endometriosis. Most of these have occurred in those cases previously sterilized by laparoscopic methods. Tables I, II, and III summarize this data.

Our tentative conclusions, which will require many more cases to confirm, are:

1) Tubal fistulae develop in one to four years in almost 50% of cases.
2) They develop primarily in those cases in which the proximal segment of tube is less than 4 cm.
3) They develop on the basis of endometriosis which is, in fact, even more prevalent than the fistulae.

TABLE I - Incidence of fistula and endometriosis vs. type of ligation

Type of Ligation	# of fistulae/ # tubes studied	# tubes with endometriosis/ # tubes studied
Cautery	12/26*	15/24*
Pomeroy	2/18	5/18
Falope Ring	0/6	4/6

* One case not submitted to pathology

TABLE II - Incidence of fistula and endometriosis vs. length of remaining proximal tubal segment

Length of remaining proximal tubal segment	# of fistulae/ # of tubes studied	# tubes with endometriosis/ # tubes studied
Less than 4cm.	13/29*	20/27*
Four or more cm.	1/21	4/21

* One case not submitted to pathology

TABLE III - Incidence of fistula and endometriosis vs. time since original ligation

Time since original ligation (yrs.)	# of fistulae/ # of tubes studied	# tubes with endometriosis/ # tubes studied
< 1	0/4	0/4
1	0/0	0/0
2	1/2	1/2
3	6/14	10/14
4	3/8	7/8
5	1/6	1/6
6	2/8	3/8
7	1/4*	0/2*
8	0/4	2/4

* One case not submitted to pathology

PROJECT III: The Comparison of Dilatation and Evacuation with Intra-Amniotic Injection for Midtrimester Pregnancy Termination

The objective of this study is to identify and evaluate safer and more practical abortifacients.

Principal Investigator: Dr. Theodore M. King

Attached is the designed experimental protocol for dilatation and evacuation vs. intraamniotic urea and prostaglandin F_2a.

We have started perfecting our technique for evacuation of pregnancies of 16 weeks duration. We view that we will start the randomization of cases in the coming eight weeks.

FORM A

RPN. No. _____
J.H.U.M.S.

Investigator(s) _____

DO NOT WRITE IN THIS BLOCK

JOINT COMMITTEE ON CLINICAL INVESTIGATION

RESEARCH PROJECT NOTIFICATION

(New Project or Project Approved More Than Three Years Ago)

Principal Investigator: Theodore M. King (must be a faculty member)

Co-Investigators: Milagros F. Atienza

Ronald T. Burkman

TITLE OF PROJECT: Comparative Study of Dilatation and Evacuation vs. Intraamniotic Injection in Midtrimester Abortion

Yes	No	
	x	Will drugs be used? If YES, and an investigational new drug is involved, append: a) summary of the preclinical data and any available clinical safety information, or an explanation of why this is unavailable; b) the IND No.; & c) the Sponsor (if applicable).
	x	Will subjects be exposed to any source of ionizing radiation (x-rays, radioisotopes)? IF, YES, complete and attach the RCU Form 5 and return with this application.
	x	Will research on pregnant women or fetal tissue be involved? If YES, complete and attach the Fetal Tissue Committee form with this application.
	x	Will any infectious or biohazardous agents be used: If YES, append details of containment procedures.
	x	Will the project use any device which must be reviewed by the Medical Devices Committee (either at JHH or APL)? If, YES, append the necessary information for review.
x		Is this project being submitted for funding by the federal government (eg. DHEW, NIH, FDA, etc.)? If YES, attach a copy ONLY of the "Human Subjects" portion of the "Methods of Procedure" section from the grant application. This is the required section dealing with the clinical studies and details responsibility for patient selection and care.

Identify subjects to be used: Women in the second trimester of pregnancy, whether nulliparous
(types of Pts, volunteers*)

or multiparous, requesting termination of pregnancy.

Age Range of subjects: 11-50 years old

Source of Patient Population: Fertility Control Center
(JHH, OPD, Volunteers*)

*If Volunteers are to be used, in the Research Plan, include a deposit of the recruitment procedure and any remuneration for the subjects.

No. of subjects (experimental & controls) expected to be enrolled: First year 100
Total Study 200

RESEARCH PLAN

I. Specific Research Question to be Addressed by this Protocol:

Whether Dilatation and Evacuation Procedure in the Midtrimester Pregnancy is as safe and effective when compared to the standard method of termination by use of Intraamniotic Hypertonic Urea-Prostaglandin $F_{2}a$.

Sections II, III, and IV, of the RPN form should be detailed in an attachment of no more than three pages and provided in consecutive order. Submission of a more elaborate description is not an acceptable fulfillment of this requirement, although supporting data, relevant publications etc. may be included as an appendix.

II. Rationale: State the problem, the state of present knowledge relevant to it, and the aims of the proposed study as relating to the research question.

III. Protocol: Briefly describe the experimental design. Identify all procedures involving research subjects, and distinguish between routine procedures for medical care and those instituted primarily for the purpose of the research.

IV. Risks: Describe major and minor risks and their expected frequency, along with any measures that will be taken to minimize and deal with them. In studies without therapeutic benefits to the subjects, both minor risks and possible late effects from the research should be included.

V. Informed Written Consent: Consent must be obtained from all participating subjects (or guardians). An original and one copy of the completed consent form (Form C) must be attached to this application.

If you believe that written consent need not be obtained for this particular study, please attach a justification.

Source of Research Support & Identifying Number:
(IF NONE, PLEASE SO INDICATE)

Title of grant or contract: The Comparative Study of Dilatation and Evacuation vs. Intraamniotic Injection in Midtrimester Abortions

Awarded 9/29/79 Pending _____ (Date of Submission to Sponsor 7/12/79)

IT IS UNDERSTOOD THAT IT IS THE INVESTIGATOR'S RESPONSIBILITY TO NOTIFY THE COMMITTEE IMMEDIATELY OF ANY SIGNIFICANT UNTOWARD RESULTS OF THIS STUDY SO THAT THE MATTER MAY BE REVIEWED.

Signature of Responsible Investigator — Date 1/17/80

Signature of Departmental Chairman — Date 1/17/80

The completed, typed form with original signatures should be sent to:
Secretary, The Joint Committee on Clinical Investigation
The Johns Hopkins University School of Medicine
Room 125 Administration Bldg./720 Rutland Avenue
Baltimore, Md. 21205 Phone: 955-3008

Revision: 6/79

II. Rationale:

The standard methods of terminating second trimester pregnancies utilize the intraamniotic instillation of hypertonic saline, hyperosmolar urea and prostaglandin F_2a. Depending upon the method used, serious complications persist such as cervical laceration, fluid and electrolyte imbalance, abortion of live fetuses, coagulopathy, and in addition, less serious problems like gastrointestinal side effects which have not been totally eliminated. Our experience in the Fertility Control Unit has shown that intraamniotic instillation of a combination of urea and prostaglandin F_2a 5 mg has resulted in a short injection-abortion time and a decreased incidence of systemic side effects attributed to higher doses of PGF_2a. The intracervical application of laminaria tents prior to the instillation of the drugs have greatly decreased the incidence of cervical laceration.

Since 1973, a modified method of Suction Curettage now termed Dilatation and Evacuation has been performed by skilled operators in the termination of pregnancies in the early second trimester (up to 16 weeks gestation). By inserting Laminaria tents in the cervical canal several hours prior to the abortion procedure, the incidence of cervical laceration has been reduced. The incidence of uterine perforation while not totally eliminated has also been reduced.

While published data from CDC seem to show that D&E may be safer than intraamniotic injection techniques, no actual study designed to evaluate the question of comparative safety between different techniques, has been undertaken.

Thus, by doing a randomized clinical trial using either Dilatation and Evacuation of Intraamniotic instillation or urea and prostaglandin F_2a in the termination of early midtrimester pregnancies we expect to be able to determine which is the safer and more effective method.

III. Protocol:

Women 11-50 years of age, whether nulliparous or multiparous and without the stated conditions are eligible for inclusion in the study.*

Following admission and signing of the appropriate informed consent, each patient will be randomly assigned to either the Dilatation and Evacuation group or the intraamniotic urea-prostaglandin group. All patients will undergo a complete medical and surgical history, physical examination and collection of laboratory work that will include hematocrit, Rh determination, STS, cervical culture for gonorrhea, testing for pregnancy and sonography for evaluation of gestational age. Laminaria tents will be inserted 24 hours prior to the abortion procedure. Fetal heart tones must be documented prior to the procedure.

*Conditions resulting in exclusion from the study are listed on pg 2.

Dilatation and Evacuation Cases. Laminaria tents will be removed immediately prior to the abortion procedure. Intravenous fluids containing oxytocin will be given prior to the abortion procedure. Systemic analgesics and a local anesthetic will be given followed by further dilatation of the cervix, if necessary, with K-Pratt dilators. Removal of the products of conception will be made with the use of the largest suction cannula.

Blood loss during the procedure will be measured. Any complication will be documented.

Intraamniotic Urea and Prostaglandin F2a. Twenty-four hours after insertion of Laminaria tents, the patient will undergo amniocentesis in the routine manner followed by intraamniotic injection of 80 gm of urea dissolved in 134 cc of 5% dextrose and water. This will be followed by 5 mg of Prostaglandin F2a given in 2 doses 5 minutes apart. Intravenous fluids will be given to the patient only if she is unable to maintain oral fluid intake.

All patients in this group who are unaborted by 24 hours will receive PGE_2 vaginal suppositories given at 3 hour intervals.

Those still unaborted by 48 hours will undergo dilatation and evacuatio

Amount of blood loss, any systemic side effects including management of such problems will be recorded.

IV. Risks:

All induced abortions are associated with some risk which include hemorrhage, infection, retained products of conception (which sometimes requires uterine curettage), cervical laceration, uterine rupture or perforation which can require abdominal surgery or hysterectomy. These complications may be serious enough for death to occur.

Conditions Resulting in Exclusion from the Study:

1) Patients with serious medical illnesses such as cardiovascular or pulmonary disease including bronchial asthma
2) Those who have undergone prior uterine surgery such as myomectomy, cesarean section or hysterotomy
3) Those with known prostaglandin allergy
4) Those currently on prophylactic antibiotics
5) Those with active gonorrhea
6) Those with allergies to local anesthesia
7) Diabetes Mellitus
8) Threatened Abortion
9) Those with hematocrits less than 30%
10) Those with fetal death in-utero

Patient I.D. Plate

FORM C
J.H.U.M.S.

CLINICAL INVESTIGATION CONSENT FORM

Title of Research Project: Comparative Study of Dilatation and Evacuation vs. Intraamniotic Injection in Midtrimester Abortion

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Explanation of Project to Subject:

It is my understanding that I have been asked to participate in an abortion research project designed to determine the effectiveness and side-effects of two different methods for terminating my pregnancy. I am aware that I have been considered for this project because I requested and have been approved for abortion. I understand that if I participate in this project, the method used to perform my abortion will involve either Dilatation and Evacuation or the Intraamniotic Instillation of Hypertonic Urea and Prostaglandin F_2a. The choice of either of these two methods will be determined by chance.

I understand that the possible beneficial effect of my participation includes the termination of my pregnancy. As I understand it, undergoing abortions carry risks including: possibility of hemorrhage, infection, drug reaction and retained after-birth; some of these complications may be serious and life threatening.

The possible risks related to dilatation and evacuation are cervical laceration and uterine perforation (puncture of the womb). The treatment of any complication can include abdominal surgery or even a hysterectomy. Complications may even result in death.

The possible side effects related to urea include diuresis (increased urination), nausea and vomiting. The side effects of using Prostaglandin F_2a may include vomiting, fever and diarrhea.

There is, of course, in any abortion technique, a chance of failure which would require the utilization of other techniques of abortion. I have been advised that it is impossible to predict with certainty the time required to achieve an abortion and that if the method should result in failure, some alternative method of abortion will be necessary.

I am participating in this project of my own free will and understand that I may withdraw at any time.

D & E Versus 1A Urea PG 5 MG

Purpose: To compare the efficacy and safety of Dilatation and Evacuation and Urea - PGF_2a Amnioinfusion in Elective Midtrimester Abortion

Basic Study Design:

The basic design is that of a randomized clinical trial with standard definitions and management protocols with the quantification of the immediate, early, and delayed complications as well as side effects associated with the procedures.

Protocol:

All patients whose pregnancies are over 14 weeks to no more than 16 weeks in duration (at the time of abortion procedure) as determined by last menstrual period, physical examination and sonography will be selected for study. The duration of gestation shall be increased to include patients with pregnancies of 18 weeks duration after safety of D&E performed at 16 weeks has been ascertained.

These patients will give informed consent for participation. Patients less than 18 years of age will require parental consent to participate. The informed consent is attached.

Exclusions from the Study:

1) Patients with serious medical illnesses such as cardiovascular or pulmonary disease including bronchial asthma
2) Those who have undergone prior uterine surgery such as myomectomy, cesarean section or hysterotomy
3) Those with known prostaglandin allergy
4) Those currently on prophylactic antibiotics

5) Those with active gonorrhea

6) Those with allergies to local anesthesia

7) Diabetes Mellitus

8) Threatened Abortion

9) Those with hematocrits less than 30%

10) Those with fetal death in-utero

All patients will undergo routine history and physical examination and laboratory data collection to include: hematocrit, Rh determination, urinalysis, syphilis serology, gonorrheal cervical culture and pap smear. Presence of fetal heart tones must be documented prior to initiation of either abortion procedure.

D&E Procedure

After immediate preoperative examination to confirm prior screening findings, the vagina will be prepped with an antiseptic solution, the endocervical canal will then be sounded and as many laminaria as can be introduced without causing extreme discomfort to the patient, will be inserted. Patients will be placed on bed rest in the Unit for at least 24 hours. After which time, the laminaria tents will be removed and intravenous fluids will be started containing oxytocin to be administered at 50 cc per hour delivering oxytocin at a rate of 332 mu/minute. The patient will then receive intravenous analgesia using between 5 to 10 mg of diazepam and 25 to 100 mg of meperidene.

A paracervical block will be carried out in a circumferential fashion utilizing 1% lidocaine. The D&E procedure will then be completed using the largest cannula possible. In addition, extraction forceps, such as Ring forceps or Sopher forceps will be utilized.

All tissues obtained will be examined for completeness. Fetal foot length will be routinely measured and recorded.

Upon completion of the procedure, the patients will be observed in the recovery room for 2-4 hours and then will be discharged.

Follow-up will occur at two weeks initially followed by four weeks. Contraception will be started at the two week follow-up visit.

Urea Administration

Patients in this group will undergo insertion of laminaria tents in a fashion similar to that described for dilatation and evacuation. This is to be followed 24 hours later by urea amnioinfusion.

The abdominal wall is prepped routinely followed by local anesthesia using 1-2 cc of 1% lidocaine; a 16-gauge tuohy needle is then introduced through the anesthetized skin into the amniotic cavity, removing between 100 to 200 cc of amniotic fluid. This is followed by the instillation of 80 grams of a 59.7% solution of urea into the amniotic cavity; when this is done, a total of 5 mg of PGF_2a will be instilled in two divided doses to rule out possible hypersensitivity.

Patients receiving urea and PGF_2 will be managed in the routine fashion:

a) A maximum of three attempts at amnioinfusion will be undertaken prior to obtaining repeat sonography for sac location.

b) Incomplete abortion - defined as excessive bleeding, fever or failure to pass the placenta within two hours after fetal expulsion. In such instances, ring forceps-curettage will be carried out using the previously described intravenous analgesia.

c) Patients unaborted by 24 hours will have prostaglandin E2 suppositories (10-20 mg) inserted into the vagina every three hours.

d) Patients unaborted by 48 hours will undergo dilatation and evacuation

e) Cervical inspection will be carried out in all patients after completion of abortion to determine the presence of cervical laceration or avulsion and the need for surgical repair.

Patients in this group are discharged 4 to 6 hours after completion of abortion.

Side Effects and Complications:

The following will be documented:

1) Nausea, vomiting, diarrhea

2) Blood loss:

Excessive blood loss will be defined as 500 cc; the need for blood transfusion and/or curettage

3) Infection:

Endometritis - defined as utilization of antibiotics except for conditions such as urinary tract infections or upper respiratory infections.

4) Cervical Laceration - its extent and its management

5) Uterine rupture or perforation

6) Reaction to anesthesia and other untoward effects

SURVEILLANCE FORM

History #_____ FCC #:_____

Name:_____

Date:_____

Age:_____ Parity:_____ Race:_____ Status:_____ Payment_____

Gestage: Dates:_____wks Exam:_____wks Sono:_____ F.L.____mm_____wk

Laminaria: Number inserted _____ Duration of Insertion:_____ hpours

Technique Used:

A. Dilatation and Evacuation:

Analgesia: Valium_____mg Demerol_____mg Lidocaine 1% (local)_____cc

Intravenous Fluids: _____cc Oxytocin_____units

Cervical Dilatation: Dilator size at 1st resistance_____

Maximum dilator used (size)_____ Cannula size_____

Duration of procedure:_____

B. Amnioinfusion:

Technique used:_____removal of fluid followed by Urea and PGF a IA

_____exchange of fluid with Urea and PGF a IA

Needle: Type_____ Length_____ Gauge_____

Amnicentesis:_____1st attempt,clear fluid bloody tap No. of taps

Amount of fluid removed_____cc Amount of Urea_____cc Amount of PGF a

Rupture of Membrane:_____SRM_____ARM _____No.of hours after injection

Abortion of Fetus: Date_____ Time_____ Hour_____

Placenta: Date_____ Time_____ Hour_____

Total Abortion Time:_____ Hours

Placental Delivery:_____Spontaneous _____ Manual from Vagina

_____Spongestick or Manual from the Cervix

_____Spongestick from Fundus _____Currettage

C Complications:

Estimated Blood Loss:_____cc. Transfusion _____Yes _____ No

Temperature 100°:____Yes ____No Diagnosis_____

Cervical Laceration:_____Yes _____No Treatment _____

C. Complications: (continuation)

Uterine Damage: _____ Yes _____ No Diagnosis: _____

Treatment: _____

Coagulation Defect: _____ Yes _____ No Treatment: _____

Intravascular Injection: _____ Yes _____ No SUN (Serial) ____ stat ____ 4hrs

Symptoms: _____ Treatment: _____

Currettage after Complete Abortion: _____ Yes _____ No

Indication: _____

Other: _____

FOLLOW-UP

Two Weeks Post-Procedure: Date:

Interval History:

Physical Exam: Vital Signs: BP _____ Temp _____ PR _____ RR _____

Pelvic Exam:

Contraception:

Weeks Post-Procedure: Date:

Interval History:

Physical Exam: BP _____ Temp _____ PR _____ RR _____ LMP _____

Pelvic Exam:

Contraception :

PROJECT IV: The Animal Testing of New Abortifacients

The objective of this study is to identify and evaluate safer and more practical abortifacients.

Principal Investigator: Dr. Norman H. Dubin

EACA

The testing of epsilon amino caproic acid (EACA) has been completed in the rat. Intrauterine administration appeared to be effective, but day specific. Systemic administration was ineffective. An early draft of a manuscript relating to this study has been included in this report. Because of the day specificity, it has been decided to leave monkey testing of this drug in abeyance. It has been noted that some IUD's contain EACA to control excessive bleeding. While no evidence is available to suggest greater efficacy of this type of IUD over those not containing EACA, the presence of the drug may also act as additional contraceptive protection.

Silver Nitrate

The randomized trial of intrauterine administration of 1% silver nitrate versus 0.9% saline has been completed. The protocol and results are included. In addition, 3 monkeys were injected with silver nitrate and had the reproductive tracts removed at various times within a week of injection. The pathology on these tissues is currently being done and will be included in the next report.

In Progress

Dr. Cecil Robinson is currently synthesizing some suicide substrates (seco-steroids) which act as a 3 β-ol enzyme inhibitor and prevents progesterone production. We will test the drug initially in the rat. In the interim we have also received corticosterone antiserum from Dr. Walter Morishige, University of Hawaii, and are in process of validating the radioimmunoassay in our laboratory. This will allow us to monitor the effect of the drug on adrenal function since corticosterone is the major corticoid in the rat. Our laboratory has already validated the progesterone radioimmunoassay.

We have also received literature from AID on LH-RF analogues as abortificients. We will use this as the next drug in our monkey studies. In the interim, any pregnant monkeys obtained will be placed on protocol for the cervical studies (Part VII).

EFFECT OF EPSILON AMINO CAPROIC ACID, A PLASMINOGEN INHIBITOR, ON IMPLANTATION AND FETAL VIABILITY IN PREGNANT RATS

N.H. Dubin

D.C. Cummings

T.M. King

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The Johns Hopkins University
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Baltimore, Maryland 21205

INTRODUCTION

The implantation process involves a complex series of events involving both the blastocyst and uterus (Weitloff, Animal Models in Reproduction, 1979). Trophoblastic involvement in the process may itself involve distinct biochemical processes from the time it attaches to the uterine epithelium to the time it establishes its relationship with the maternal circulation.

Many migratory cells produce plasminogin activator which is believed to play a role in their tissue invasiveness. Recent evidence indicates that the blastocyst contains plasminogen activator activity and the invasiveness of the trophoblast may depend upon fibrinolytic activity. Most evidence for this depends upon *in vitro* studies (see Discussion). The present experiments provide *in vivo* evidence that plasminogen activator activity may be important in early stages of pregnancy in the rat.

MATERIALS AND METHODS

Rats were purchased timed pregnant from Sprague Dawley (C.D.®), Madison, WI. The day sperm were observed in the vagina was defined as pregnancy Day 1. On various days of pregnancy, groups of rats were lightly anesthetized with ether and laparotomized abdominally under aseptic conditions. The uterine horn to be injected was grasped lightly with forceps near the cervical end and a sterile 1 ml tuberculin syringe equipped with a 25 gauge needle was inserted through the uterine wall into the lumen. Various doses of EACA or vehicle (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 backflow. The abdominal wall was sutured and the skin closed with wound clips. The rats were returned to their cages until autopsy on day 15. At autopsy animals were decapitated. Corpora lutea, implantation sites and viable fetuses (based on size and gross appearance) were counted. Appearances of normal and

resorbed sites are photographically illustrated in another publication (Dubin et al., Biol. Reprod. 21:47, 1979).

Study 1. Effect on Day of Injection

Rats were injected on one occasion between day 3 and 8 of gestation. Groups of rats received either EACA or the saline vehicle. Other groups were sham treated, that is, the needle was inserted into the uterine lumen but nothing was injected. The EACA dose used in this study was determined by the maximal solubility (30 mg/0.1 ml) and the volume that would yield an acceptable viability rate when the vehicle alone was injected (see Discussion). EACA was prepared fresh on the day before injection in 0.9% sterile sodium chloride.

Study 2. Effect of EACA concentration

All rats received EACA in one uterine horn on day 6 of pregnancy. Groups received either 0.3, 0.6, or 3.0 mg EACA in a 0.01 ml volume. The contralateral horn received 0.01 ml saline.

Study 3. Effect of Plasmin

On pregnancy day 6, rats received intralumenal injections of either EACA (3 ml), EACA plus plasmin (KABI), plasmin alone, or saline in a 0.01 ml volume. In this experiment, both horns of the uterus were injected with the same substance. Lyophylized plasmin was reconstituted in sterile 0.9% sodium chloride immediately before injection. Doses are expressed in casein units (CU; Sqouris et al., Vox Sang. 5:357, 1960).

Study 4. Effect of Systemic EACA

Groups of rats received subcutaneous injections of either 0, 15, 30 or 60 mg of EACA daily from day 3 to 10. The concentration of the injection fluid was always 30 mg/0.1 ml.

RESULTS

Study 1. Effect on Day of Injection

In untreated rats the implantation rate was 82.2 ± 6.7% while the viability rate of those implantations was 93.8 ± 4.9% (Table 1). Sham intrauterine injections on any one day between pregnancy day 3 and 8 did not affect the implantation rate, however, viability was affected when a sham injection was performed on day 4 (46.1 ± 10.7%) or day 5 (8.9 ± 6.1%). Injection with normal sterile saline (0.1 ml) reduced percent implantation when injection was made on day 4. Saline injections affected viability on days 4 and 5 although this effect could be accounted for by merely inserting the needle since the percent viability in these groups were not significantly different from the sham injections on comparable days of pregnancy. Injection of 0.01 ml saline on day 6 decreased percent viability below the level of the sham control (47.3 ± 11.5 versus 82.7 ± 5.5%). Injection of 3 mg EACA in the same volume of saline further reduced the percent viability to 13.3 ± 6.0%. EACA did not affect viability on any other day tested nor did it affect implantation on any day tested. EACA was not tested on day 4 or 5 because of the low viability rates encountered with sham injections.

Study 2. Effect of EACA concentration

When intrauterine injections were made on day 6, the percent viability was dependent upon the dose of EACA administered (Figure 2). In this study all injections were made in 0,01 ml saline.

Study 3. Effect of Plasmin

In another study, intrauterine injections of 3 mg EACA again resulted in reduced viability (13.9 ± 4.3%) versus 54.1 ± 5.4% for saline control, $P < .001$). Injection of EACA along with plasmin resulted in 36.5 ± 9.6% which was significantly higher than EACA, yet less than the saline injected rats ($P < .05$).

Plasmin itself appeared to reduce viability although statistically this was not significant probably due to the greater standard error.

Study 4. Effect of Systemic EACA

No difference in either implantation or fetal viability were observed following systemic injection of up to 60 mg EACA/day from day 3 to 10.

DISCUSSION

Implantation involved a complex set of interactions between trophoblast and uterine tissue (Weitloff, 1979). The trophoblastic function itself may actually involve numerous events which depend on different biochemical mechanisms. For example, the attachment of the blastocyst to the uterus may involve sialic acid residues or immunologic factors since this may be prevented by Concavalin A or neutrophil mobilization. The penetration of the epithelium involves proteolytic enzymes other than fibrinolysis since trypsin will prevent this process (Kubo et al., 12th Meeting SSR, Abstr. 78, 1979) but EACA does not. Invasion through the intracellular matrix represents yet another trophoblastic process which appears to be, in part, dependent upon fibrinolytic activity.

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 activator. 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.

We have tested *in vivo* the effect of EACA, a plasminogen inhibitor on both implantation and fetal viability in pregnant rats. One approach was to inject the drug directly into the uterine lumen around the time of ovulation.

Technical difficulties were encountered because of non-specific effects. We have previously showed that intraluminal injections of 0.1 ml of normal sterile saline will result in a significant reduction in implantation rate when administered on day 4 (Dubin et al., 1979). Furthermore, fetal viability was affected when saline was injected on days 4, 5, 6 or 7. On day 6, almost all implantation sites were resorbed from horns receiving 0.1 ml saline. This effect was volume dependent. Since day 6 is of particular interest because trophoblast invasion is occurring about this time (Enders and Schlafke, 1976), the highest concentration of EACA in the samllest volume (3 ml/10 ml) was injected at this time.

Interuterine EACA administration did not affect implantation rates when injected on day 6, but it did increase the number of resorptions, possibly by preventing normal trophoblastic invasion. This effect was dose-dependent and could be partially reversed by simultaneously injecting plasmin along with the EACA, providing evidence for the specificity of the response.

Although EACA is orally effective in women in reducing menstrual bleeding (Callendar et al., Br. Med. J. 4:214, 1970; Kullander, Fertil. Steril. 21:48L, 1970), in our study systemic administration of EACA had no effect on fetal viability. This might be because of inaccessability of the drug to the blastocyst residing within the uterus.

It has been noted that IUD insertion increases plasminogen activator activity in the endometrium (Kasonde and Bonner, Br. J. OBGYN 83:315, 1976). EACA reduces IUD induced memorrhagia (Kasonde and Bonner, Br. Med. J. 4:17, 1975). There is no documentation that IUD's containing EACA are more effective in reducing fertility, however, it is hypothesized that such devices provide additional protection because of EACA's effect on suppressing trophoblast invasiveness.

The present experiments support the hypothesis that plasminogen activator activity is involved in the implantation process and provide the first *in vivo* evidence for its physiologic importance.

Figure 1: The Fibrinolytic System

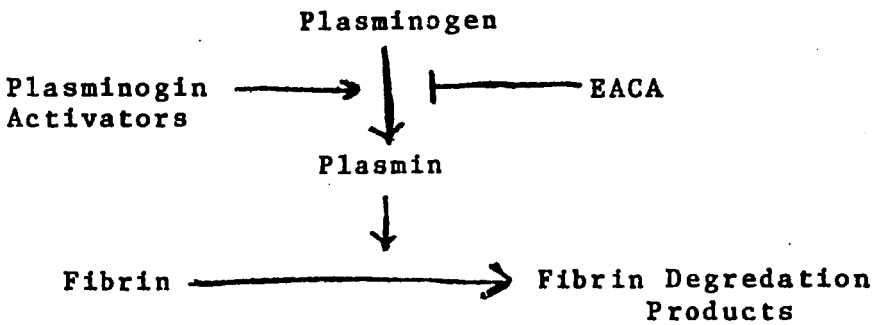


Figure 2

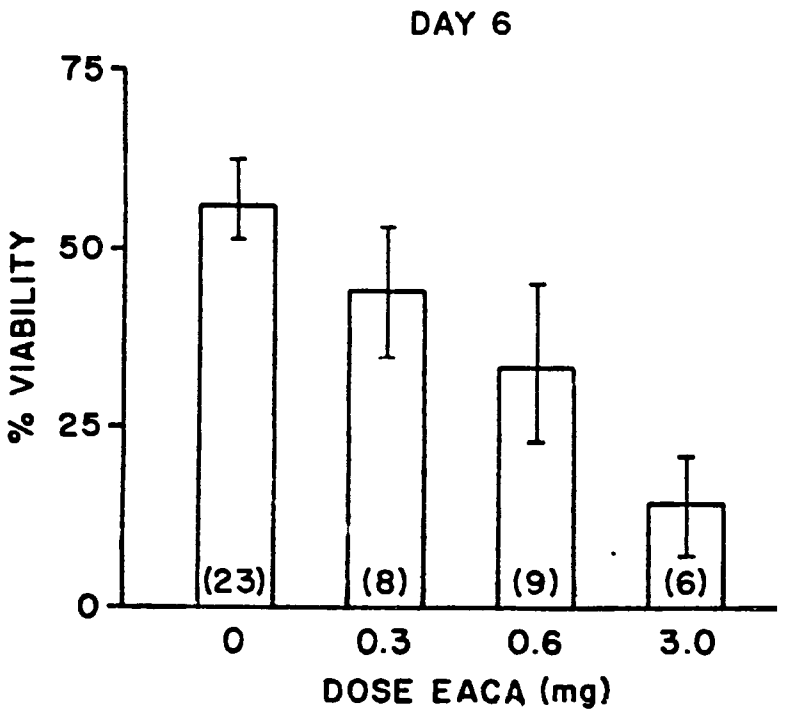
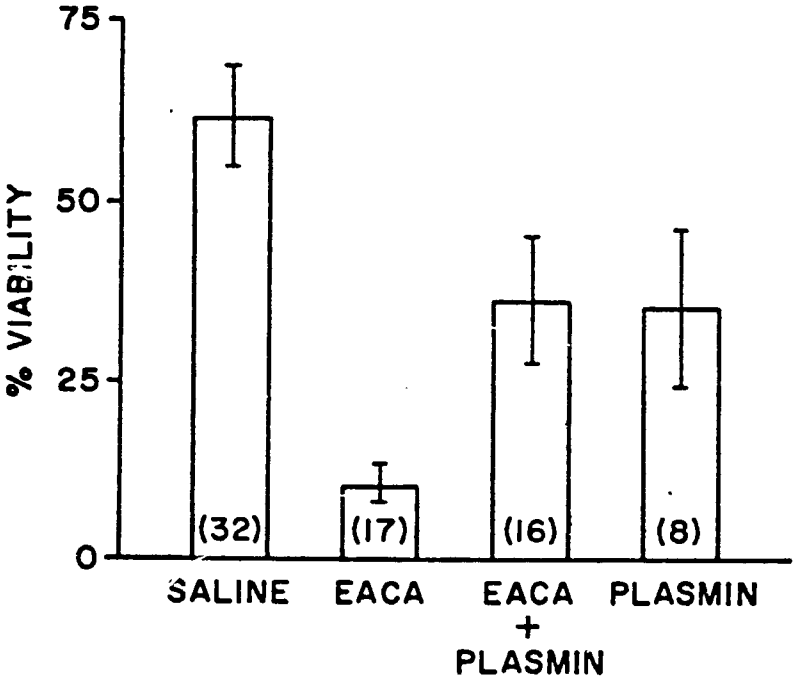


Figure 3



EACA vs SALINE $p < .01$

EACA vs EACA + PLASMIN $p < .01$

EACA + PLASMIN vs SALINE $p < .05$

Table 1

Treatment Day	Treatment	Number	Percent Implantation	Percent Viability
-	Untreated	6	82.2 ± 6.7	93.8 ± 4.9
3	Sham	5	92.0 ± 8.0	89-4 ± 6.8
	Saline	12	85.1 ± 4.4	88.0 ± 3.8
	EACA (30 mg)	9	89.1 ± 7.3	86.1 ± 11.3
4	Sham	10	80.9 ± 9.8	46.1 ± 10.7*
	Saline	10	62.6 ± 5.7x	25.0 ± 10.4*
	EACA	not determined		
5	Sham	9	85.8 ± 6.1	8.9 ± 6.1*
	Saline	6	65.9 ± 7.6	24.17 ± 12.9*
	EACA	not determined		
6	Sham	20	78.0 ± 4.9	82.7 ± 5.5
	Saline	7	65.2 ± 9.2	47.3 ± 11.5*†
	EACA (3 mg)	6	70.4 ± 10.9	13.3 ± 6.0**
7	Sham	17	78.8 ± 4.6	86.7 ± 6.7
	Saline	8	74.9 ± 8.6	75.9 ± 11.4
	EACA (3 mg)	8	70.9 ± 9.0	82.4 ± 5.9
8	Sham	8	68.2 ± 9.2	70.4 ± 15.4
	Saline	11	77.6 ± 8.6	77.8 ± 9.1
	EACA (30 mg)	9	81.9 ± 8.7	91.0 ± 5.6

x = $P < .05$ vs. untreated control

* = $P < .01$ vs. untreated control

† = $P < .01$ vs. sham control for the same day

** = $P < .05$ vs. saline control for the same day

Table 2

EFFECT OF SYSTEMIC EACA ON IMPLANTATION AND FETAL VIABILITY

Dose of EACA (mg)	Number	% Implantation	% Viability
0	12	77.2 ± 5.9	90.3 ± 3.7
15	11	80.3 ± 3.2	87.4 ± 5.1
30	12	76.7 ± 4.6	95.0 ± 1.9
60	10	81.3 ± 3.2	89.6 ± 2.3

SILVER NITRATE STUDY

MATERIALS AND METHODS

Cynomolgus monkeys (Macaca fascicularis) were obtained from Primate Imports (Port Washington, NY). They were fed Monkey Chow (Charles River Breeding Labs, Inc., Wilmington, MA), supplimented with fresh fruit. All monkeys were individually caged except during timed mating periods. Animals were checked daily (including weekends) for vaginal bleeding with cotton swabs. They were cohabitated for 8 days beginning 12 days after the onset of vaginal bleeding. 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 caretaker of primates. At the same time, urine was collected from the bladder by urethral catherization and tested for monkey chorionic gonadotropin (mCG) by a hemagglutination inhibition test (Ortho Diagnostics Inc., Raritan, NJ).

If monkeys were documented pregnant by palpation or mCG testing, they were placed on the study protocol. Day of pregnancy was counted from the midpoint of the cohabitation period. Thus, cohabitation day 5 was counted as pregnancy day 1. At approximately 1 month (range 27-43 days) of pregnancy, the animal was immobilized with an intramuscular injection of 4.4 mg per killogram of body weight of Ketamine hydrochloride (Ketaset). A 12 cm long, 18 gauge, 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 1% silver nitrate or 0.9% saline was injected. The treatment was randomized and the investigator performing the injection and the monkey caretaker was unaware of which solution was being administered.

The monkeys were observed daily for vaginal bleeding. Blood samples were taken from a peripheral vein prior to treatment, on day 1, 2 and 3 following treatment and at month after treatment for blood chemistry and hematologic

evaluations. Blood samples were analyzed by the Division of Clinical Laboratories of the Johns Hopkins Hospital. Also, 1 month after treatment, monkeys were repalpated to determine the progression of pregnancy.

RESULTS

All monkeys receiving intrauterine injections of 1 ml 1% silver nitrate between 27 and 43 days of pregnancy showed vaginal bleeding beginning one or two days after treatment. The bleeding laster for an average of 5.3 days (range 4-7 days). These monkeys were found to have non-enlarged or subnormally enlarged uteri at the time of repalpation indicating that abortion had ensued. Continued observation of these monkevs confirmed that they were no longer pregnant.

Monkeys receiving 0.9% saline had variable outcomes. Two animals (#2 and #3) showed some spotting after injection but pregnancy progressed. These animals delivered normal healthy fetuses at 165 and 181 days. #6 had an uneventful pregnancy and a healthy infant was born at 148 days. #4 also had uneventful pregnancy until delivered by C-section at 108 days of a living fetus as part of another study. Monkey #2 demonstrated vaginal bleeding on days 6 and 7 following injection and aborted. Monkey #5 passed fetal tissue 29 days after injection.

Hematology and blood chemistry determinations revealed no significant difference between silver nitrate and saline treated monkeys (Tables 1 and 2).

Figure 1: Effect of intrauterine administration of 1 ml 0.9% saline on pregnancy in cynomolgus monkeys. The following legends apply to this figure and figure 2. Solid bars represent normal progress of pregnancy. Arrow indicates time of treatment. Stippled bar represents vaginal bleeding. A = abortion; B = birth of normal healthy infant; T = pregnancy artifically terminated but fetus viable.

Figure 2: Effect of intrauterine administration of 1 ml 1% silver nitrate on pregnancy in cynomolgus monkeys.

Figure 1

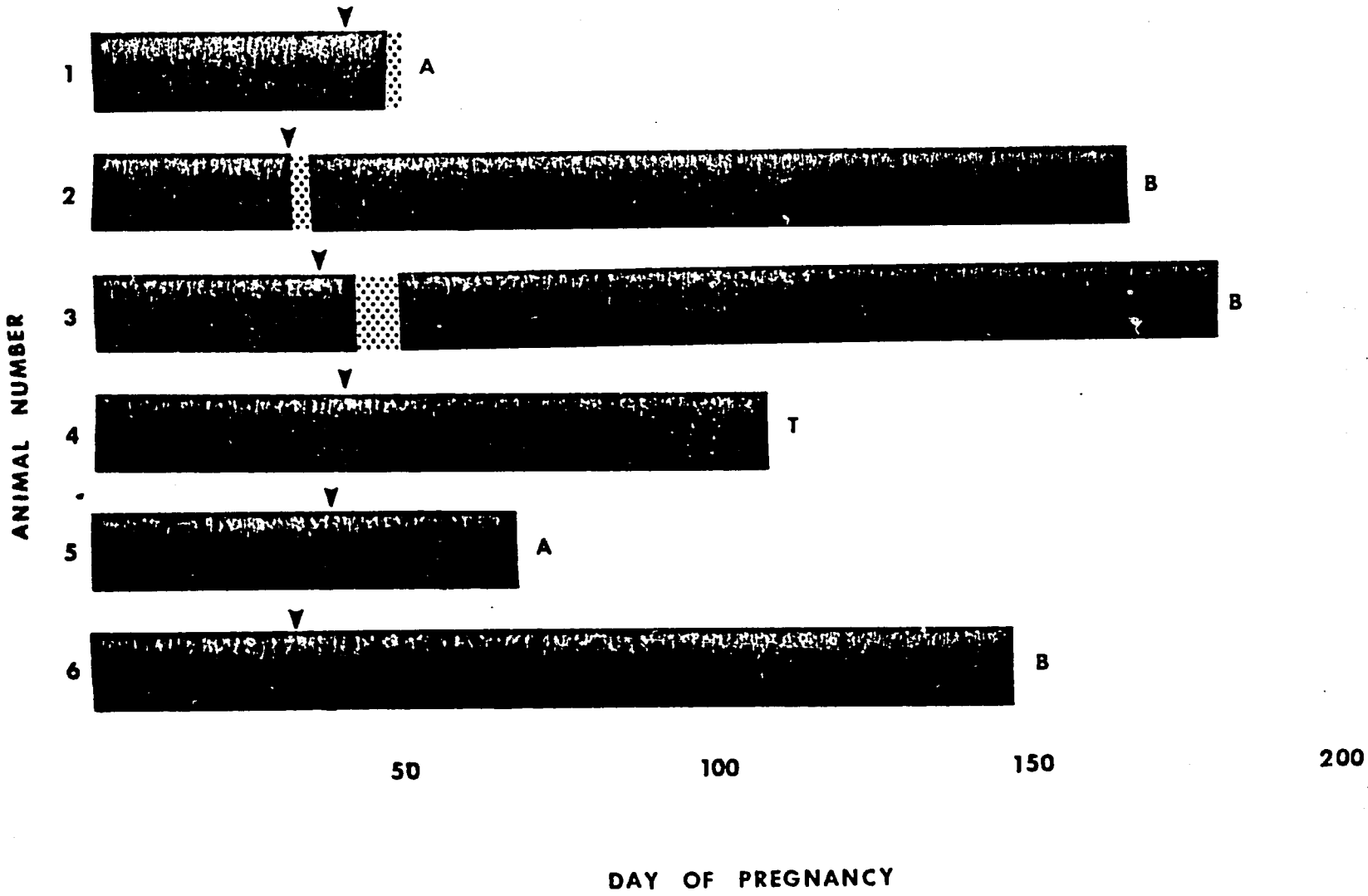


Figure 2

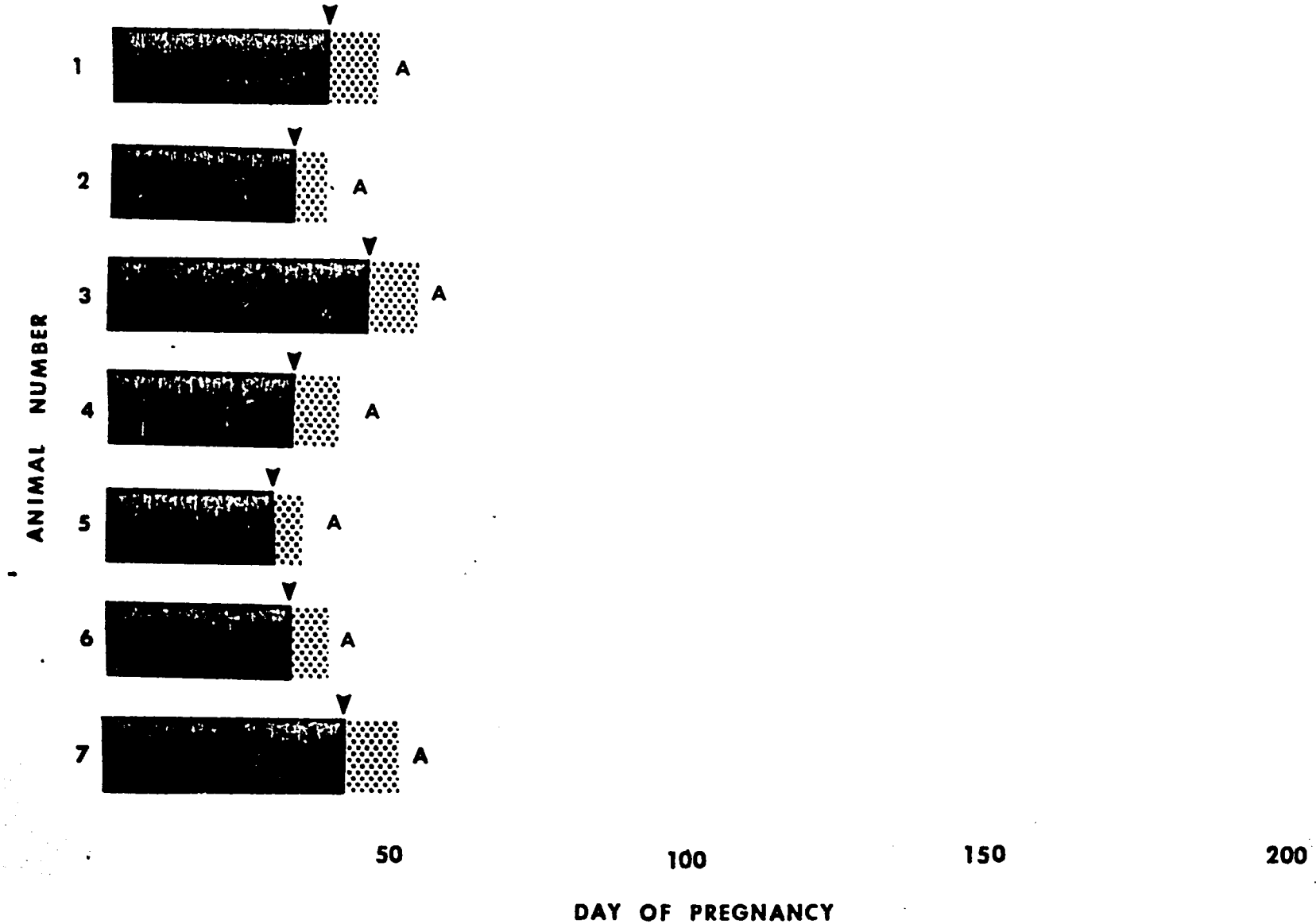

ANIMAL NUMBER
1
2
3
4
5
6
7
A
A
A
A
A
A
A
50
100
150
200
DAY OF PREGNANCY

Table 1

Hematology Following Administration of 1 ml 1% Silver Nitrate or 0.9% Saline

			Time after Drug Administration			
	Mating	Drug Admin	24 hr	48 hr	72 hr	1 month
White blood cells Count X 10^3						
Silver Nitrate	9.3±0.6	8.0±1.1	9.2±0.7	8.0±0.9	8.8±0.7	9.0±.5
Saline	8.7±1.0	8.4±1.3	8.1±1.6	8.6±1.0	8.8±1.0	
Red Blood Cells Count X 10^6						
Silver Nitrate	5.8±0.1	5.2±0.3	5.5±0.1	5.6±0.1	5.4±0.2	5.9±.3
Saline	5.8±0.3	5.7±0.4	5.9±0.2	5.4±0.5	5.4±0.6	
Hematocrit (%)						
Silver Nitrate	34.9±0.7	33.6±0.8	34.5±1.0	33.0±1.0	34.2±0.7	33.9±.6
Saline	33.4±1.6	35.1±1.8	34.7±1.2	32.8±1.6	33.1±2.5	

Table 2

Blood Chemistry Analysis following Administration of 1 ml 1% Silver Nitrate or 0.9% Saline

	Mating	Drug Admin.	Time After Drug Administration			
			24 hr	48 hr	72 hr	1 month
Sodium (mEq/L)						
Silver Nitrate	145.3±1.1	146.0±0.7	145.0±1.6	145.2±0.9	144.6±0.8	146.0±0.9
Saline	148.0±0.6	145.0±1.8	149.0±1.3	145.3±0.6	146.0±1.3	
Potassium (mEq/L)						
Silver Nitrate	3.77±.16	4.02±.20	3.93±.08	3.94±.18	4.02±.17	4.03±.22
Saline	3.63±.33	4.28±.17	3.90±.24	3.68±.20	4.05±.13	
Chloride (mEq/L)						
Silver Nitrate	106.5±1.5	107.0±1.8	108.2±1.1	108.4±1.0	106.6±1.0	108.3±.8
Saline	105.7±0.7	111.0±1.9	107.3±0.9	108.0±2.7	110.0±1.3	
Glucose (mg/dl)						
Silver Nitrate	71.2±5.2	62.2±7.3	59.3±6.3	66.8±3.2	67.4±2.6	71.5±2.8
Saline	66.0±7.5	55.0±10.5	52.5±8.9	62.5±7.6	59.0±9.4	
Urea Nitrogen (mg/dl)						
Silver Nitrate	19.5±0.9	19.0±1.1	20.8±1.4	19.2±1.1	19.8±1.4	18.5±1.5
Saline	20.3±4.7	20.5±1.6	21.3±2.3	19.0±4.1	23.0±2.5	
CO_2 (mEq/L)						
Silver Nitrate	22.8±0.9	23.6±1.2	23.8±0.4	24.0±1.6	23.0±1.5	23.5±1.0
Saline	21.3±2.6	20.3±0.9	24.5±1.9	22.7±1.1	25.0±2.4	

PROJECT V. The Clinical Testing of Clinical Abortifacients

The objective of this study is to evaluate agents which have been determined by animal studies to be efficacious and free of undesirable characteristics.

Principal Investigator: Dr. M. F. Atienza

1. Azastene.

Attached is a copy of the Azastene protocol which is being utilized in this study. This drug is now available and we are recruiting patients for study.

It is anticipated that a second drug which is an inhibitor of enzymes responsible for progesterone synthesis will be available by the Fall of 1980.

FORM A

RPN. No. _____
J.H.U.M.S.

Investigator(s) _____

DO NOT WRITE IN THIS BLOCK

JOINT COMMITTEE ON CLINICAL INVESTIGATION

RESEARCH PROJECT NOTIFICATION

(New Project or Project Approved More Than Three Years Ago)

Principal Investigator: T.M. King, R.T. Burkman (must be a faculty member)

Co-Investigators: _____

TITLE OF PROJECT: The Effect of Azastene on the Course of Early Pregnancy

Yes	No	
X		Will drugs be used? If YES, and an investigational new drug is involved, append: a) summary of the preclinical data and any available clinical safety information, or an explanation of why this is unavailable; b) the IND No.; & c) the Sponsor (if applicable).
	X	Will subjects be exposed to any source of ionizing radiation (x-rays, radioisotopes)? If, YES, complete and attach the RCU Form 5 and return with this application.
X		Will research on pregnant women or fetal tissue be involved? If YES, complete and attach the Fetal Tissue Committee form with this application.
	X	Will any infectious or biohazardous agents be used: If YES, append details of containment procedures.
	X	Will the project use any device which must be reviewed by the Medical Devices Committee (either at JHH or APL)? If, YES, append the necessary information for review.
	X	Is this project being submitted for funding by the federal government (eg. DHEW, NIH, FDA, etc.)? If YES, attach a copy ONLY of the "Human Subjects" portion of the "Methods of Procedure" section from the grant application. This is the required section dealing with the clinical studies and details responsibility for patient selection and care.

Identify subjects to be used: (types of Pts, volunteers*) Reproductive age women between 18 to 40 who have elected termination of pregnancy.

Age Range of subjects: 18-40

Source of Patient Population: (JHH, OPD, Volunteers*) The Johns Hopkins Hospital, Fertility Control Unit-Fertility Control Unit

*If Volunteers are to be used, in the Research Plan, include a deposit of the recruitment procedure and any remuneration for the subjects.

No. of subjects (experimental & controls) expected to be enrolled: First year five

Total five

RESEARCH PLAN

I. Specific Research Question to be Addressed by this Protocol:

The primary objective is to determine to what extent azastene alters the hormonal and pregnancy status of women six to eight weeks pregnant. In studies with non-human primates, azastene was effective in disrupting pregnancy. The research question is "will it terminate pregnanies of six to eight weeks duration without significant untoward events.

Sections II, III, and IV, of the RPN form should be detailed in an attachment of no more than three pages and provided in consecutive order. Submission of a more elaborate description is not an acceptable fulfillment of this requirement, although supporting data, relevant publications etc. may be included as an appendix.

II. Rationale: State the problem, the state of present knowledge relevant to it, and the aims of the proposed study as relating to the research question.

III. Protocol: Briefly describe the experimental design. Identify all procedures involving research subjects, and distinguish between routine procedures for medical care and those instituted primarily for the purpose of the research.

IV. Risks: Describe major and minor risks and their expected frequency, along with any measures that will be taken to minimize and deal with them. In studies without therapeutic benefits to the subjects, both minor risks and possible late effects from the research should be included.

V. Informed Written Consent: Consent must be obtained from all participating subjects (or guardians). An original and one copy of the completed consent form (Form C) must be attached to this application.

If you believe that written consent need not be obtained for this particular study, please attach a justification.

Source of Research Support & Identifying Number:
(IF NONE, PLEASE SO INDICATE)

Title of grant or contract: The Effect of Azastene on the Course of Early Pregnancy - Sterling Winthrop Pharmaceutical Company

Awarded _____ Pending _____ (Date of Submission to Sponsor 10/19/79)

IT IS UNDERSTOOD THAT IT IS THE INVESTIGATOR'S RESPONSIBILITY TO NOTIFY THE COMMITTEE IMMEDIATELY OF ANY SIGNIFICANT UNTOWARD RESULTS OF THIS STUDY SO THAT THE MATTER MAY BE REVIEWED.

Signature of Responsible Investigator — Date 10/19/79

Signature of Departmental Chairman — Date 10/19/79

The completed, typed form with original signatures should be sent to:
Secretary, The Joint Committee on Clinical Investigation
The Johns Hopkins University School of Medicine
Room 125 Administration Bldg./720 Rutland Avenue
Baltimore, Md. 21205 Phone: 955-3005

Revision: 6/79

II. Rationale:

In the United States, approximately 1.2 million legal abortions are completed annually. Almost 80% of women seeking pregnancy terminations present by the 8th week of pregnancy and 90% are completed by suction curettage with the remainder of the patients managed by an assortment of surgical procedures. The mortality rate for operative procedures varies with the complexity of procedure which in turn is dictated by the duration of pregnancy. It would be ideal to have an orally active pharmacologic agent that would terminate early pregnancies eliminating the requirement for surgical procedures.

Azastene Win 17,625 - (17B)-4,4,17-trimethylandrosta-2,5-dieno (2,3-d) isoxazol-17-ol inhibits steroidogenesis by blocking the conversion of pregnenolone to progesterone by inhibiting the 3 beta-hydroxy-steroid dehydrogenase enzyme system. This agent is capable of causing abortion in both rats and rhesus monkeys. Induced abortions is prevented by concurrent administration of progesterone to these animals. It should be noted that this agent blocks adrenal steroidogenesis in the rat, however, in the monkey, the drug is a relatively more effective inhibitor of ovarian and placental steroidogenesis than of adrenal steroidogenesis.

The aims of the proposed study is to determine to what extent Azastene alters the hormonal and pregnancy status of women six to eight weeks pregnant. The drug would be administered to a total of five women. If results are encouraging, further studies would be designed and submitted for approval.

II. Protocol:

Five women who present to the Fertility Control Clinic for pregnancy termination who are 6 to 8 weeks pregnant as documented by pregnancy test, date of last menses, pelvic examination, and pelvic sonography will be selected for study. They will be admitted for confinement for a period of up to eight days.

The selected patients will be healthy without signs of complications of pregnancy or threatened abortion. The patient exclusion criteria include:

1) Evidence of hepatic, kidney or cardiovascular disease
2) Aberrant clinical chemistry or hematology profile
3) Need for concomitant medication
4) History of spontaneous abortion
5) History of gynecological or endocrine disorders
6) History of admission for psychiatric care or drug or alcohol abuse

Prior to entry into the study, a complete history and physical examination will be obtained. A positive urinary pregnancy test will be documented. After obtaining an informed consent, the following laboratory tests will be completed:

Serum chemistry profile
CBC
Serum progesterone, cortisol
Quantitative HCG
Routine urine analysis will also be performed
Sonographic characterization of the pregnancy sac will be determined
A pre-study 24 hour urinary collection will be obtained

The qualified admitted subjects will be given azastene 1 gram twice daily at 8:00 a.m. and 8:00 p.m. orally for five consecutive days. Plasma progesterone and cortisol will be measured at 10:00 a.m. daily. On the fifth day of the treatment schedule, ultrasound study of the pregnancy sac will be repeated, along with determination of quantitative serum HCG. At this time, chemistry profile, hematology and urine analysis will be repeated. Uterine cramps, vaginal bleeding or spotting will be documented. In the presence of these symptoms, a determination of serum prostaglandin F metabolite will be carried out within 24 hours. If abortion has not occurred by the end of the fifth day of treatment, a suction curettage will be peformed on the eight day and tissue will be submitted for histological examination to document the presence of absence of placental or decidual necrosis. If abortion occurs during the course of therapy, it's time of occurrence will be documented and tissue will be submitted to pathology to substantiate the presence of placental material. Whether the abortion is incomplete or complete will be noted and curettage will be performed within 24 to 48 hours after the onset of vaginal bleeding. The curettage material will be submitted for histological verification. Follow-up will be one month following the initiation of therapy. This will include physical examination, urine analysis, hematology and serum chemistry profile.

Attached you will find a flow sheet of relevant data to be collected.

IV. Risks:

Patients who undergo first trimester pregnancy terminations are at risk for uterine hemorrhage, infection and perforation. The requirement for transfusion in such patients ranges from 1 to 3 per 1,000 procedures. The incidence of infection is 0.3 to 0.8% and the incidence of perforation is 1 to 2 per 1,000. These complications are those that are attributed to the suction curettage.

In regards to Azastene, a rat teratology study showed no teratogenic effects at oral doses of 1 mg/kg. The effects of 5 and 25 mg/kg, largely involve fetal skeletal changes of the ribs and vertebrae, appear to be primarily a result of progesterone deficiency. Concomitant progesterone treatment ameliorated these effects.

In an initial clinical trial, Azastene was well tolerated at dosages ranging from 62.5 to 2,000 mgs given twice daily for five days to normal non-pregnant women. These subjects were receiving injections of human chorionic gonadotrophin during the luteal phase of the menstrual cycle to simulate early pregnancy. Under these conditions, the drug did not block the HCG-stimulated rise in plasma progesterone levels. In this study Azastene was noted to have a hypouricemic effect. A primate study subsequently carried out indicates a uricosuric action for Azastene.

FLOW SHEET

Procedure	Prestudy Screen	Therapy Day 1	2	3	4	5	Post Therapy 6	7	8	1 month
History	X									
Physical	X									X
Pelvic Exam	X					X			X	X
Urine pregnancy test	X									
Sonography	X					X				
Hematology	X					X			X	
Urinalysis	X					X			X	
Chemistry profile	X		X			X			X	
Plasma progesterone and cortisol	X	X	X	X	X	X	X	X		
Quantitative HCG	X					X				
Prostaglandin F metabolite			X*							
Curettage and histology			X*						X**	
24-hour urine and volume	X	X	X	X	X	X	X	X	X	

*conditional
**suction

2. Ethanol

This agent is being utilized in a total of three more women who are not pregnant and are scheduled for hysterectomy. Ethanol will be instilled at mid-cycle approximately 45 days before hysterectomy. The protocol designed for this study is attached. Early pregnancy studies will commence on completion of this non-pregnancy study.

FORM A

RPN. No. _____
J.H.U.M.S.

Investigator(s) _____

DO NOT WRITE IN THIS BLOCK

JOINT COMMITTEE ON CLINICAL INVESTIGATION

RESEARCH PROJECT NOTIFICATION

(New Project or Project Approved More Than Three Years Ago)

Principal Investigator: Theodore M. King (must be a faculty member)

Co-Investigators: T. Parmley, R. Burkman

TITLE OF PROJECT: Effect of Locally Administered Ethanol on the Reproductive Tract

Yes	No	
X		Will drugs be used? If YES, and an investigational new drug is involved, append: a) summary of the preclinical data and any available clinical safety information, or an explanation of why this is unavailable; b) the IND No.; & c) the Sponsor (if applicable). IND #13,294 – No Sponsor
	X	Will subjects be exposed to any source of ionizing radiation (x-rays, radioisotopes)? IF, YES, complete and attach the RCU Form 5 and return with this application.
X		Will research on pregnant women or fetal tissue be involved? If YES, complete and attach the Fetal Tissue Committee form with this application.
	X	Will any infectious or biohazardous agents be used: If YES, append details of containment procedures.
	X	Will the project use any device which must be reviewed by the Medical Devices Committee (either at JHH or APL)? If, YES, append the necessary information for review.
X		Is this project being submitted for funding by the federal government (e.g. DHEW, NIH, FDA, etc.)? If YES, attach a copy ONLY of the "Human Subjects" portion of the "Methods of Procedure" section from the grant application. This is the required section dealing with the clinical studies and details responsibility for patient selection and care.

Identify subjects to be used: (types of Pts, volunteers*) Females scheduled for hysterectomy and bilateral salpingoophorectomy

Age Range of subjects: 21–45

Source of Patient Population: (JHH, OPD, Volunteers*) Patients that are admitted for hysterectomy to the Johns Hopkins Hospital for reasons other than endometrial pathology

*If Volunteers are to be used, in the Research Plan, include a deposit of the recruitment procedure and any remuneration for the subjects.

RESEARCH PLAN

I. Specific Research Question to be Addressed by this Protocol:

This study is designedatd evaluates the presence or absence of side-effects of locally administered alcohol on the endometrium of the uterus and the fallopian tubes one month after instillation of ethyl alcohol.

Sections II, III, and IV, of the RPN form should be detailed in an attachment of no more than three pages and provided in consecutive order. Submission of a more elaborate description is not an acceptable fulfillment of this requirement, although supporting data, relevant publications etc. may be included as an appendix.

II. Rationale: State the problem, the state of present knowledge relevant to it, and the aims of the proposed study as relating to the research question.

III. Protocol: Briefly describe the experimental design. Identify all procedures involving research subjects, and distinguish between routine procedures for medical care and those instituted primarily for the purpose of the research.

IV. Risks: Describe major and minor risks and their expected frequency, along with any measures that will be taken to minimize and deal with them. In studies without therapeutic benefits to the subjects, both minor risks and possible late effects from the research should be included.

Informed Written Consent: Consent must be obtained from all participating subjects (or guardians). An original and one copy of the completed consent form (Form C) must be attached to this application.

If you believe that written consent need not be obtained for this particular study, please attach a justification.

Source of Research Support & Identifying Number:
(IF NONE, PLEASE SO INDICATE)

Title of grant or contract: Effect of Locally Administered Ethanol on the Reproductive Tract
Simplified Techniques of Fertility Control and Related Research
Project "Clinical Testing Of Chemical Abortifacients"

Awarded 9/29/79 Pending _____ (Date of Submission to Sponsor 7/12/79)

IT IS UNDERSTOOD THAT IT IS THE INVESTIGATOR'S RESPONSIBILITY TO NOTIFY THE COMMITTEE IMMEDIATELY OF ANY SIGNIFICANT UNTOWARD RESULTS OF THIS STUDY SO THAT THE MATTER MAY BE REVIEWED.

Signature of Responsible Investigator — Date 11/23/80

Signature of Departmental Chairman — Date 11/23/80

The completed, typed form with original signatures should be sent to:
Secretary, The Joint Committee on Clinical Investigation
The Johns Hopkins University School of Medicine
Room 125 Administration Bldg./720 Rutland Avenue
Baltimore, Md. 21205 Phone: 955-3008

Revision: 6/79

Rationale:

Increasing numbers of legal abortions are performed yearly in this country. It has been shown that the earlier the pregnancy termination is performed, the safer the procedure.

Suction curettage which is the most popular method of terminating early gestations is associated with the risks of incomplete abortion, cervical injury and uterine perforation. Menstrual extraction, while it prevents cervical injury, carries with it the risk of infection and incomplete abortion with associated hemorrhage.

A chemical method, wherein a drug is locally applied to the uterine cavity for induction of menstruation, may avoid the potential of cervical trauma and uterine injury. This will also result in reduced financial costs if surgical procedures can be avoided.

It was hypothesized that menstrual induction could be affected by introducing an agent into the uterus which would cause endometrial sloughing. Ethanol was selected for further testing after screening several compounds in pregnant rats.

A randomized control study in monkeys confirmed to be pregnant revealed that when one milliliter of 70% ethanol was instilled intracervically into the uterus, endometrial sloughing occurred within two days with termination of pregnancy. There were six successful pregnancy interruptions. All of the ethanol treated animals resumed normal menses, and three subsequently became pregnant and delivered normal monkeys.

This study in monkeys was followed by an FDA approved phase I study in humans. Three non-pregnant women scheduled for hysterectomy for reasons other than endometrial pathology, volunteered for this study. A polyethylene PE 90 catheter was threaded intracervically into the uterine cavity and 2 cc's

of 35% or 70% ethanol was injected between one and 16 hours prior to surgery. No systemic reactions were observed in these patients. Histology of the uterus showed superficial necrosis of the zona compacta of the endometrium. No damage was observed within the fallopian tubes or ovaries. Ethanol was not detectable in the plasma of the treated patients.

This study is intended to evaluate the long term effects of ethanol on the endometrium, the fallopian tubes and ovaries. Forty-five to sixty days after local instillation of ethanol into the uterus a hysterectomy and in certain instances, when indicated a bilateral salping-oophorectomy will be completed.

Protocol:

Sterile solutions of ethanol and water will be prepared by the Johns Hopkins Hospital's Department of Pharmacology Services. These will be injected into the uterus through a sterile polyethylene catheter (PE 90) previously passed through the cervix. The volume of 70% ethanol injected will not exceed 10 ml and will be stopped if the patient experiences any discomfort. The subjects will be given this ethanol treatment at the mid-cycle at least 45 days prior to hysterectomy.

The following observations will be recorded: Any subjective response of the patient at the time of the injection, as well as an interval history detailing any episodes of bleeding and cramping occurring within four weeks after the alcohol injection. Blood will be obtained before treatment for SMA 12 and serum ethanol level. The uterus and the fallopian tubes and ovaries when removed as part of the surgical specimen will be examined for gross and microscopic evidence of alcohol effects.

Risks:

Our previous monkey and human studies show that ethanol fixes only the superficial level of the endometrium and has no toxic effects on the peritoneal organs even when given in volumes that could spill out of the fallopian tubes. Alcohol has been widely studied for systemic effects which would be negligible at the dose level to be employed. Alcohol will be administered in sterile form but there is a theoretical risk of infection. The three patients which have been treated with ethyl alcohol within 24 hours prior to hysterectomy, experienced no abdominal or pelvic cramping pain. One of the three patients did note the occurrence of vaginal tingling.

CONSENT FORM

Effect of Locally Administered Ethanol on the Reproductive Tract

We are interested in developing simpler and safer techniques for inducing abortion very early in pregnancy. Through studies in animals, we have found that ethyl alcohol, the same substance that is in alcoholic beverages, is a promising drug for this purpose. Based on animal studies, as well as in a limited number of women, alcohol appears to be both safe and effective. You are being asked to participate in studies which are for the purpose of finding out what ethyl alcohol does to the lining of the uterus in women who are not pregnant. Approximately 45 days before your hysterectomy, you will have a small tube passed through the vagina into the uterus and through this tube, a small amount of ethyl alcohol will be injected. The amount will be about two teaspoons which is about equal to that in a cocktail. It is possible that this could cause some cramping discomfort and if it does, we will promptly stop and you will be given medicine to relieve the pain. There will be no change in the way your hysterectomy will be performed, but we would like to study the tissues removed which may for some women include the ovaries and tubes.

While the procedure was not harmful to animals, the effects on women is still unknown. We believe there is little or no risk to you, since the only tissues that will come in contact with the ethyl alcohol are going to be removed at the time of your surgery. There is a chance that pain and cramping could occur, but it will be relieved if it does. There is also a possibility that some vaginal bleeding or infection could occur. If this occurs, it will be treated.

I am participating in this project of my own free will and understand that I may withdraw at any time.

3. Trichosanthine.

This agent is not yet available.

PROJECT VI: Cervical Studies

The objective of this study is to assess methods of cervical dilatation for safety and efficacy.

Principal Investigator: Dr. Theodore M. King

We have commenced a random assignment study of a standard seaweed laminaria and an Indian cervical tent. Pre and post laminaria insertion cervical measurements are made. The work required for further cervical dilatation is being determined.

Monkey studies have been initiated comparing laminaria treated to the non-treated midtrimester cervix.

We have submitted for approval by the Human Studies Committee a protocol for the cervical osmatic dilator utilizing a dilator saturated with magnesium sulphate.

Publications

Manuscripts in Press:

1. Incidence of Abdominal Surgery in an Abortion Population

2. Midtrimester Induced Abortion by Hyperosmolar Urea and Prostaglandin F_2a for Patients with Previous Caesarean Section: Clinical Course and Potential for Uterine Rupture

3. Endometriosis and the Development of Tubo-Ovarian Peritoneal Fistulae After Tubal Ligation

4. Antiotensin II and Saralasin Effect on Contractile Activity and Prostaglandin Production of In-Vitro Pregnant Rat Uteri

Manuscripts in Preparation:

1. Prophylactic Cephalothin in Patients Undergoing Midtrimester Abortion Procedures

2. Effects of Laminaria on Injection Abortion Intervals of Midtrimester Abortion Patients

3. Serum Lidocaine Levels in Patients Undergoing Suction Abortion with and without Laminaria

4. Female Sterilization - Update

5. The Effect of Epsilon Amino Caporic Acid - A Fibrinolytic Inhibitor on Implantation and Fetal Viability in Pregnant Rats

6. An International Program for the Assessment of Tubal Anastomosis Using Microsurgical Technique

WORK PLAN - APRIL 1980 THROUGH SEPTEMBER 1980

In Project I, Dr. Rock will continue to complete cases that have been randomly assigned to either the optic visor loop or operating microscope. In completed cases, he will document the occurrence of pregnancy and their outcome. The paired cases will be forwarded to Dr. Kimball for statistical analysis.

Dr. Parmley in Project 2 will continue to acquire tubes that have been ligated in the past in an attempt to demonstrate the importance of the site of tubal ligation. The role of endometriosis and the development of tubal fistulae will be documented.

Dr. King in Project 3 will initiate the randomization and evacuation of pregnancies of 16 weeks duration by dilatation-evacuation and by intraamniotic urea-Prostaglandin.

In Project 4, Dr. Dubin will continue to evaluate chemical abortifacients in animals. This work will include conclusion of the pathology of the silver nitrate studies. He will initiate studies of suicide substrates and the Lh-Rh analog work.

In Project 5, Dr. Atienza will evaluate agents which have been determined to be effective in animals. This work will include the gaining of approval for clinical trials of silver nitrate and recruitment of patients into the azastene and ethanol studies.

In Project 6, Dr. King will continue the study of ceryical laminaria in first trimester cases and gain approval for the human studies of an osmotic cervical dilator. In animal work, preliminary data will be collected on the monkey cervix of midtrimester pregnancies exposed to laminaria.

"SIMPLIFIED TECHNIQUES OF FERTILITY CONTROL"

AID/DSPE-C-0054

9/28/79-9/27/80

	YEAR I Budget Award	Spent Thru 3/31/80	Projected Thru 9/27/80
Salaries	$ 123,604	$ 57,331	$ 66,273
Fringe Benefits	21,632	10,033	11,599
Equipment (materials and supplies)	30,724	17,425	13,299
Travel	300	71	229
Consultants	-0-	-0-	-0-
Other Direct Costs	47,236	10,787	36,449
Indirect Costs	105,504	30,498	75,006
Total	$ 329,000	$ 126,145	$ 202,855