

PDAAP-993

PROGRAM FOR APPLIED RESEARCH
ON FERTILITY REGULATION

S E M I - A N N U A L R E P O R T

January 1, 1984 - June 30, 1984

Submitted to: Research Division
 Office of Population
 Development Support Bureau
 Agency for International Development
 Washington, D.C. 20523

Submitted by: Program for Applied Research on
 Fertility Regulation
 Northwestern University Medical School
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In compliance with Contract AID/csd-3608,
Contract AID/DSPE-C-0035 and
Cooperative Agreement DPE-0546-A-00-1003-00

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Volume 3, Number 1, May, 1984	

REPORT SUMMARY

Project Title and Contract Number:

Program for Applied Research on Fertility Regulation
AID/csd-3608
AID/DSPE-C-0035
DPE-0546-A-00-1003-00

Principal Investigator:

John J. Sciarra, M.D., Ph.D.
Professor and Chairman
Department of Obstetrics and Gynecology
Prentice Women's Hospital and Maternity Center
333 East Superior Street
Chicago, Illinois 60611

Contractor:

Northwestern University
c/o Sponsored Projects Administration
619 Clark Street
Evanston, Illinois 60201

Contract Period:

July 1, 1975 - June 30, 1979 -- AID/csd-3608
July 1, 1979 - June 30, 1981 -- AID/DSPE-C-0035
July 1, 1981 - June 30, 1986 -- DPE-0546-A-00-1003-00

Reporting Period:

January 1, 1984 - June 30, 1984

Total Expenditures Through December 31, 1983:

AID/csd-3608	\$ 4,331,521.82
AID/DSPE-C-0035	3,370,727.26
DPE-0546-A-00-1003-00	<u>3,107,665.84</u>
TOTAL:	\$10,809,914.92

Total Expenditures January 1, 1984 Through June 30, 1984:

AID/csd-3608	\$ - 0 -
AID/DSPE-C-0035	- 0 -
DPE-0546-A-00-1003-00	<u>1,064,432.13</u>
TOTAL:	\$1,064,432.13

Commitments Through June 30, 1984: DPE-0546-A-00-1003-00 \$1,418,854.29

Additional Commitments not Reflected in Financial Reports: \$1,261,475

Total Funding Received 7/1/81-12/31/84: \$7,560,000

Total Budgeted 7/1/81-6/30/86: \$12,363,280

PROGRAM ACCOMPLISHMENTS

Scientific Summary

1. A meeting of the Scientific Advisory Committee (SAC) was held on April 11, 1984. With PARFR staff, SAC reviewed extension, formal, pilot study and informal research proposals received during the current period. Please refer to the SAC section (Program Accomplishments) and SAC Minutes (Appendix) for specific determinations.
2. Research progress was monitored by review of technical reports and site visits to projects. During this reporting period, the following research projects were visited by PARFR staff or consultants:
 - a. 1/15-17/84 -- Alfredo Goldsmith, M.D., M.P.H., San Antonio, Texas and Durango, Mexico (PARFR-341M, 341T Phase II Poly NET 90 Initiation).
 - b. 2/12-15/84 -- Alfredo Goldsmith, M.D., M.P.H., Gerald I. Zatuchni, M.D., M.Sc., Lourens J.D. Zaneveld, D.V.M., Ph.D., Donald P. Waller, Ph.D., David A. Blake, Ph.D., Edward C. Mather, D.V.M., Ph.D. and Kenneth C. Polakoski, Ph.D.; Chicago, Illinois (PARFR-338 sperm enzyme inhibitors).
 - c. 2/22/84 -- Alfredo Goldsmith, M.D., M.P.H.; Birmingham, Alabama (PARFR-341 Phase II Poly NET 90).
 - d. 3/5-7/84 -- John J. Sciarra, M.D., Ph.D. and Alfredo Goldsmith, M.D., M.P.H.; Milan, Italy (PARFR-350 Hamou Intra Tubal Device).
3. The following meetings for the purpose of project development were held and attended by PARFR staff and consultants:
 - a. 2/6-7/84 -- Alfredo Goldsmith, M.D., M.P.H.; Boston, Massachusetts (Endocon: NET Pellets).
 - b. 2/29/84 -- Lourens J.D. Zaneveld, D.V.M., Ph.D. and Donald P. Waller, Ph.D.; Silver Springs, Maryland (Shug).
 - c. 5/8-9/84 -- Alfredo Goldsmith, M.D., M.P.H. and Ricardo H. Asch, M.D.; Chicago, Illinois (Testosterone Study).
4. Dr. Alfredo Goldsmith represented PARFR at a meeting of the Society of Gynecologic Investigators held March 21-24, 1984 in San Francisco, California. PARFR also supported the attendance of Drs. Ricardo H. Asch and Jose P. Balmaceda at the Conference.
5. Drs. Alfredo Goldsmith, John Sciarra and Gerald Zatuchni represented PARFR at a meeting of the American Fertility Society held April 4-7, 1984 in New Orleans, Louisiana.

Scientific Summary (cont'd)

6. PARFR supported and helped organize a regional workshop held April 13-15, 1984 in Santo Domingo, Dominican Republic. The workshop, held by the Latin American Association of Researchers in Human Reproduction (ALIHR), was attended by 126 participants. Drs. Alfredo Goldsmith, Ricardo H. Asch and Horacio B. Croxatto participated in the workshop.
7. PARFR supported the attendance of Drs. Nancy Alexander, Deborah Anderson, Erwin Goldberg, Micheal Harper and Gerald Zatuchni, at a workhsop "Research and Development of Immunological Methods of Fertility Regulation" held April 16-18, 1984 in Bethesda, Maryland. The workshop was sponsored by NIH, PARFR and AID.
8. PARFR's 14th International Workshop "Intrauterine Contraception: Advances and Future Prospects" was held May 29 - June 1, 1984 in Chicago, Illinois. The meeting was attended by 90 participants representing 22 countries.
9. Volume 3, Number 1 of PARFR's research technical information report was produced and distributed. The issue, "The Etiology of Pelvic Inflammatory Disease," was authored by Louis Keith, M.D. and Gary S. Berger, M.D., M.S.P.H.
10. At the close of the current reporting period, the book developed from the proceedings of PARFR's 13th International Workshop was in the final stages of production. The book, "Long-Acting Contraceptive Delivery Systems" is scheduled for publication and distribution in August, 1984.
11. At the close of the current reporting period, plans for a Symposium, "Recent Advances in Contraception" were being made. The symposium will be held in Caracas, Venezuela on October 21-26, 1984 in conjunction with the FIGO meeting.
12. Planning has begun for a regional symposium, "Male Contraception," to be held in February, 1985 in Mexico City, Mexico.
13. Planning has begun for PARFR's 15th International Workshop "Male Fertility Control." The workshop will be held in Spring, 1985 in Geneva, Switzerland.

PROGRAM ACCOMPLISHMENTS

LDC Involvement

During this report period, the following subagreements in LDCs were executed:

1. PARFR-354 -- "Screening of Thai Plants for Proteins (or Lectins) as Potential Vaginal Contraceptives"
Montri Chulavatnatol, M.D., Mahidol University, Bangkok
Thailand

The following subagreements terminated during this report period:

1. PARFR-314E -- "Uterine Measurement - Clinical Comparison Study (Wing Sound II)"
Mokhtar Topozada, M.D., The University of Alexandria,
Alexandria, Egypt
2. PARFR-328B -- "An Evaluation of an Improved Needle for Transcutaneous Vas Closure"
Marcos Paulo P. de Castro, M.D., PRO-PATER, Sao Paulo, Brasil

LDC Involvement (cont'd)LDC Research Funds

As of June 30, 1984, the following funds were budgeted or expended for research in LDCs:

<u>Country & PARFR #</u>	<u>Budget (Dollars)</u>	<u>Total Expenditures To Date</u>
<u>BRASIL</u>		
PARFR-318B	\$14,982	\$14,701.49
PARFR-3283	<u>14,122</u>	<u>10,792.00</u>
TOTAL BRASIL:	\$29,104	\$25,493.49
<u>CHILE</u>		
PARFR-301C	31,476	31,476.00
PARFR-310C	8,000	8,000.00
PARFR-311C	9,000	9,000.00
PARFR-316C	27,000	27,000.00
PARFR-327C	20,880	17,870.00
PARFR-341C	<u>45,419</u>	<u>- 0 -</u>
TOTAL CHILE:	\$141,775	\$93,346.00
<u>EGYPT</u>		
PARFR-300E	11,930	11,930.00
PARFR-314E	<u>5,400</u>	<u>5,400.00</u>
TOTAL EGYPT:	\$17,330	\$17,330.00
<u>MEXICO</u>		
PARFR-300M	23,056	23,056.00
PARFR-330M	28,710	11,924.00
PARFR-341M	<u>36,135</u>	<u>3,326.00</u>
TOTAL MEXICO:	\$87,901	\$38,306.00
<u>THAILAND</u>		
PARFR-354	9,800	- 0 -
<u>VENEZUELA</u>		
PARFR-305V	24,049	21,512.10
PARFR-322V	3,600	3,600.00
PARFR-327V	<u>19,535</u>	<u>16,410.73</u>
TOTAL VENEZUELA:	\$47,184	\$41,522.83
TOTAL LDC:	\$333,094	\$215,998.32

PROGRAM ACCOMPLISHMENTS

Administrative Summary

In addition to the routine management of the program, the efforts of the PARFR Administrative Staff were chiefly directed toward:

1. Monitoring Cooperative Agreement, DPE-0546-A-00-1003-00.
2. Coordinating and mailing the agendas for the April 11, 1984 meeting of the Scientific Advisory Committee. The agenda included 7 formal proposals, 3 extension proposals, and 24 technical reports.
3. Negotiating and executing:
 - 8 New Subagreements: 341A, 353, 354, 355, 356, 357, 358, 359
 - 2 Additional Funding/Extension Amendments: 334UAB, 339
 - 1 Decrease Funding Amendment: 341A
 - 5 No Cost Extension Amendments: 340, 346, 347, 348, 349
4. Mailing 6,000 copies of Vol. 3, Number 1 in the Research Frontiers in Fertility Regulation series.
5. Coordinating and executing PARFR's 14th International Workshop "Intrauterine Contraception: Advances and Future Prospects" held May 29 through June 1, 1984 in Chicago, Illinois. The meeting was attended by 90 participants representing 22 countries.
6. Developing software for and transferring data to two micro-computers.

1/1/84 - 6/30/84

DPE-0546-A-00-1003-00

Subagreement Negotiations

PROJECT #	TITLE/INVESTIGATORS/INSTITUTION	ACTION	PERIOD	FUNDING
334UAB	"Pharmacokinetics Studies in Baboons Relating to PARFR-334SRI" Lee R. Beck, Ph.D. University of Alabama in Birmingham Birmingham, Alabama	Additional Funding/ Extension (Amendment #1)	3/1/83- 7/31/84	\$ 3,042 (\$16,741 total)
339	"Shug Device Studies" Lourens J.D. Zaneveld, D.V.M., Ph.D. Rush-Presbyterian-St. Luke's Medical Center Chicago, Illinois	Additional Funding/ Extension (Amendment #1)	7/1/83- 6/30/85	\$18,030 (\$37,746 total)
340	"Technical Assistance in NET Microcapsule Preparation" Thomas R. Tice, Ph.D. Southern Research Institute Birmingham, Alabama	No-Cost Extension (Amendment #1)	7/1/83- 1/31/84	
341A	"Phase II Poly NET 90 Injectable Study" Howard J. Tatum, M.D. Emory University Atlanta, Georgia	New Subagreement	1/1/84- 12/31/85	\$37,437
		Decrease Funding (Amendment #1)		\$ 4,532 (\$32,905 total)
346	"Development of Controlled-Release Testosterone Microcapsules for Fertility Regulation of Males" Thomas R. Tice, Ph.D. Southern Research Institute Birmingham, Alabama	No-Cost Extension (Amendment #1)	10/1/83- 8/31/84	

PROJECT #	TITLE/INVESTIGATORS/INSTITUTION	ACTION	PERIOD	FUNDING
347	"Studies on the Anovulatory Potency and Side Effects on an Inhibitory Analog of LH-RH in Cynomologous Monkeys" Ricardo H. Asch, M.D. The University of Texas Health Science Center San Antonio, Texas	No-Cost Extension (Amendment #1)	2/1/84- 1/31/86	
348	"Development of Improved Methods and Materials for Injecting Microencapsulated Steroids" Danny H. Lewis, Ph.D. Stolle Research and Development Corporation Birmingham, Alabama	No-Cost Extension (Amendment #1)	11/1/83- 11/30/84	
349	"Preparation of Fibrous Estradiol/Progesterone IUDs for Phase I Clinical Trials, Continuation of PARFR-324" Richard L. Dunn, Ph.D. Southern Research Institute Birmingham, Alabama	No-Cost Extension (Amendment #1)	11/1/83- 10/31/84	
353	"Effects of Chronic Intrauterine Release of Progesterone on Uterine Histology in Intact Rabbits" Antonio Scommegna, M.D. Michael Reese Hospital and Medical Center Chicago, Illinois	New Subagreement	3/15/84- 7/31/84	\$ 7,895
354	"Screening of Thai Plants for Proteins (or Lectins) as Potential Vaginal Contraceptives" Montri Chulavatnatol, M.D. Mahidol University Bangkok, Thailand	New Subagreement	4/1/84- 3/3 /85	\$ 9,800

PROJECT #	TITLE/INVESTIGATORS/INSTITUTION	ACTION	PERIOD	FUNDING
355	"Enhancement of the Secretary Immune Response to LDH-C ₄ " Nancy J. Alexander, Ph.D. Medical Research Foundation of Oregon Portland, Oregon	New Subagreement	4/15/84- 4/14/85	\$47,335
356	"Development of an Immunocontraceptive Vaccine: Role of 23 Kd Antigen in Immunofertility and Fertility Regulation" Rajesh K. Naz, Ph.D. Medical Research Foundation of Oregon Portland, Oregon	New Subagreement	5/1/84- 4/30/85	\$66,686
357	"Optimization of Release Profile of Norethisterone Injectable 90 Day Contraceptive" Danny H. Lewis, Ph.D. Stolle Research and Development Corporation Birmingham, Alabama	New Subagreement	5/1/84- 10/31/84	\$49,119
358	"Development of a 30 day Injectable Contraceptive" Danny H. Lewis, Ph.D. Stolle Research and Development Corporation Birmingham, Alabama	New Subagreement	5/1/84- 1/31/85	\$11,800
359	"Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins" Bonnie S. Dunbar, Ph.D. Baylor College of Medicine Houston, Texas	New Subagreement	6/1/84- 5/31/85	\$77,741

PERSONNEL

Effort and salary expenditures of PARFR personnel for this reporting period are listed below:

<u>Staff and Title</u>	<u>Effort in Man-Months</u>	<u>Salary</u>
John J. Sciarra, M.D., Ph.D. Director and Principal Investigator	.6	\$ 3,266.25
Gerald I. Zatuchni, M.D., M.Sc. Director of Technical Assistance	5.1	\$26,824.02
Alfredo Goldsmith, M.D., M.P.H. Head, Research Project Development	5.7	31,033.68
Diane Krier-Morrow, M.B.A. Director of Administration	6.0	15,499.98
Susan Dewar Project Controller	6.0	9,064.02
Kelley Osborn Publications Coordinator	0.6	3,375.00
Ruvenia Thomas Secretary II	6.0	9,848.80
Asenath Williamson Secretary I	6.0	7,009.60
Josephine Harris Secretary I	6.0	7,332.00
<u>Fringe Benefits</u>		\$ 21,136.34
<u>Indirect Costs</u>		121,554.76

SCIENTIFIC ADVISORY COMMITTEE

The membership of the Scientific Advisory Committee during this reporting period consisted of the following individuals:

John J. Sciarra, M.D., Ph.D., Chairman	Northwestern University
Andrzej Bartke, Ph.D.	The University of Texas Health Science Center at San Antonio
David A. Blake, Ph.D.	The Johns Hopkins University School of Medicine
William Droegemueller, M.D.	University of North Carolina
Uwe Goebelsmann, M.D. (deceased 6/15/84)	University of Southern California
Miriam H. Labbok, M.D., M.P.H.	The Johns Hopkins School of Hygiene and Public Health
Kamran S. Moghissi, M.D.	Wayne State University
Dean L. Moyer, M.D.	University of Southern California School of Medicine
Antonio Scornegna, M.D.	Michael Reese Hospital and Medical Center
Rochelle H. Shain Ph.D.	The University of Texas Health Science Center at San Antonio

The Committee met once during the current reporting period, on April 11, 1984 in Chicago, Illinois.

At the meeting, the Committee reviewed 7 formal and 3 extension proposals. Twenty-four technical reports were also reviewed.

Of the seven formal proposals reviewed at the meeting, four were approved. Dr. Lourens Zaneveld's proposal, "Preparation of Acrosin Inhibitors" was approved as written. Dr. Bonnie S. Dunbar's proposal, "Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins" was approved with modifications which were to be implemented by the PARFR Staff. Dr. George C. Denniston's proposal, "A Method for Pre-Testing Percutaneous Vasectomy Electrodes" was approved pending preparation of a comprehensive clinical protocol. Finally, Dr. Ricardo H. Asch's proposal, "The Effects of Testosterone Replacement in Castrated Rhesus Monkeys Using a Controlled-Release Microcapsule System" was approved according to PARFR recommendations and with the additional suggestion that tests be conducted to determine the metabolic clearance rate of testosterone and blood conversion of testosterone to DHT.

Two of the three extension proposals reviewed were also approved. Dr. Robert T. Chatterton's extension proposal for his study "Ovulation Inhibition by Anordrin" was approved in part. Dr. Lourens J.D. Zaneveld's extension proposal for his study "Toxicity Studies Required by FDA for the Development of the Shug" was approved with a request for clarification of some items in the study. This study has a component with Dr. Waller at the University of Illinois at Chicago that will be contracted separately.

Minutes of the meeting are included in the Appendix.

CONSULTANTS

The following is a list of Program Consultants, indicating their areas of expertise, contributions to the program, and payment therefore. Included in this list of consultants are members of the Scientific Advisory Committee.

<u>Consultant</u>	<u>Purpose</u>	<u>Effort</u>	<u>Fee</u>
Nancy J. Alexander, Ph.D. Reproductive Physiology	Proposal Review 2/10/84 6/12-13/84		\$ 50 100
Andrzej Bartke, Ph.D. Obstetrics and Gynecology	SAC 4/10-11/84	2 days	420
David A. Blake, Ph.D. Obstetrics and Gynecology Pharmacology	SAC 4/10-11/84 Site Visit 1/30-31/84	2 days 2 days	420 420
William Droegemueller, M.D. Obstetrics and Gynecology	SAC 4/10-11/84	2 days	420
David A. Edelman, Ph.D. Biostatistician	Project Consultation 10/25/83-12/2/83 1/26/84-3/8/84	25 days 9.5 days	5,875 2,232.50
Michael J. Free, Ph.D. Reproductive Physiology	Project Consultation 12/15-16/83	1 day	175
Uwe Goebelsmann, M.D. Reproductive Endocrinology	SAC 4/10-11/84	2 days	420
Erwin Goldberg, Ph.D. Biochemistry	RFFR: Vol. 2, No. 6		1,000
Harrith M. Hasson, M.D. Obstetrics/Gynecology and Infertility	Project Consultation 2/24-25/84	2 days	385.48
Louis G. Keith, M.D. Obstetrics/Gynecology	RFFR: Vol. 3, No. 1		600
Miriam H. Labbok, M.D., M.P.H. Epidemiology	SAC 4/10-11/84	2 days	420
Edward C. Mather, D.V.M., Ph.D. Animal Reproductive Physiology	Project Development 2/14-15/84	2 days	485.50
Dean L. Moyer, M.D. Experimental Pathology	SAC 4/10-11/84	2 days	420
Kenneth L. Polakoski, Ph.D. Andrologist	Project Development 2/14-15/84	2 days	307.70
Antonio Scommegna, M.D. Obstetrics and Gynecology	SAC 4/10-11/84	2 days	420

Consultants (cont'd)

Rochelle N. Shain, Ph.D. Medical Anthropology	SAC 4/10-11/84	2 days	\$ 420
Emil Steinberger, M.D. Endocrinologist	Proposal Review 2/14/84		50
Lourens J.D. Zaneveld, D.V.M., Ph.D. Biochemistry and Veterinary Medicine	Project Consultation 8/1/83-1/4/84	14 days	2,800
		TOTAL	<hr/> \$17,841.18

SUMMARY FINANCIAL REPORTS

This section includes:

- A. Summary of Expenditures and Commitments under AID/csd-3608, AID/DSPE-C-0035, and AID/DPE-0546-A-00-1003-00
- B. Detail of Expenditures and Commitments under AID/DPE-0546-A-00-1003-00, effective 7/1/81-6/30/86
- C. Expenditures under AID/DPE-0546-A-00-1003-00 during the period 1/1/84-6/30/84

	<u>AID/csd-3608</u> <u>(7/1/75-6/30/80)</u>	<u>AID/DSPE-C-0035</u> <u>(7/1/79-6/30/82)</u>	<u>DPE-0546-A-00-1003-00</u> <u>(7/1/81-6/30/86)</u>
Expenditures 1/1/84-6/30/84	\$ - 0 -	\$ - 0 -	\$1,064,432.13
Expenditures through 12/31/83	<u>4,331,521.82</u>	<u>3,370,727.26</u>	<u>3,107,665.84</u>
Total Expenditures	\$4,331,521.82	\$3,370,727.26	\$4,172,097.97
Total Commitments @ 6/30/84	- 0 -	- 0 -	1,418,854.29
Uncommitted Balance	<u>180,990.68</u>	<u>39,272.74</u>	<u>1,969,047.74</u>
Total Budget	<u>\$4,512,512.50</u>	<u>\$3,410,000.00</u>	<u>\$7,560,000.00</u>

COOPERATIVE AGREEMENT: AID/DPE-0546-A-00-1003-00
 EFFECTIVE DATES: 7/1/81-6/30/86
 TOTAL AWARD: \$12,363,280
 TOTAL AWARDED TO DATE: \$7,560,000

7/1/81 - 6/30/84

<u>Category</u>	<u>Budget</u>	<u>%</u>	<u>Expenditures</u>	<u>Commitments</u>	<u>Total</u>
<u>Research</u>	\$4,663,338	61.7	\$2,052,769.19	\$1,262,289.93	\$3,315,059.12
<u>Workshops and Publications</u>	451,374	6.0	266,845.34	35,243.86	302,089.20
<u>Consultants</u>	90,400	1.2	63,459.35	100.00	63,559.35
<u>Travel</u>	378,584	5.0	266,102.11	1,349.17	267,451.28
<u>Salaries and Fringe Benefits</u>	871,981	11.5	706,296.57	48,034.74	754,331.31
<u>Supplies, Communications and Rent</u>	471,694	6.2	326,236.08	11,176.03	337,412.11
<u>Equipment</u>	26,439	.4	12,511.35		12,511.35
<u>Indirect Costs</u>	606,190	8.0	477,877.98	60,660.56	538,538.54
	<u>\$7,560,000</u>		<u>\$4,172,097.97</u>	<u>\$1,418,854.29</u>	<u>\$5,590,952.26</u>

COOPERATIVE AGREEMENT: AID/DPE-0546-A-00-1003-00

EXPENDITURES FOR THE PERIOD: 1/1/84-6/30/84

<u>Category</u>	<u>Expended 7/1/81-12/31/83</u>	<u>Expended 1/1/84-6/30/84</u>	<u>Total Expended 7/1/81-6/30/84</u>
<u>Research</u>	\$1,471,932.96	\$ 580,836.23	\$2,052,769.19
<u>Workshops & Publications</u>	238,494.98	28,350.36	266,845.34
<u>Consultants</u>	42,818.17	20,641.18	63,459.35
<u>Travel</u>	165,169.92	100,932.19	266,102.11
<u>Salaries & Fringe Benefits</u>	570,909.85	135,386.72	706,296.57
<u>Supplies, Communications and Rent</u>	249,505.39	76,730.69	326,236.08
<u>Equipment</u>	12,511.35	- 0 -	12,511.35
<u>Indirect Costs</u>	356,323.22	121,554.76	477,877.98
TOTAL:	<u>\$3,107,665.84</u>	<u>\$1,064,432.13</u>	<u>\$4,172,097.97</u>

TECHNICAL REPORTS

The following technical reports were reviewed at the April 11, 1984 Scientific Advisory Committee Meeting:

<u>Project</u>	<u>Period Covered by Report</u>
PARFR-312 -- "A Clinical Evaluation of the Subdermal Contraceptive Norethindrone Pellet - Continuation of PARFR-229"	1/1/81-12/31/83 (FINAL)
PARFR-313E -- "Uterine Measure - Clinical Comparison Study (Wing Sound II)"	Through February, 1983
PARFR-320 -- "Levonorgestrel Rods Drug Release Study"	8/1/82-1/31/84 (FINAL)
PARFR-323 -- "Efficacy of Quinacrine and Tetracycline in the Primate"	Through 8/31/83 (FINAL)
PARFR-329 -- "Targeting Liposomes to the Male Reproductive Tract with Antibody LDH-C ₄ "	12/1/82-11/30/83
PARFR-330T -- "A Clinical Evaluation of the Bioabsorbable Contraceptive Norethindrone Pellet Implant (Phase II)"	10/1/83-2/29/84
PARFR-332 -- "Development of an Injectable Long-Acting Estradiol Formulation"	Through 3/23/84
PARFR-333 -- "Poly-gly NET 180 Microcapsule System"	7/7/83-1/15/84 (FINAL)
PARFR-333T -- "Poly-gly NET 180 Microcapsule System"	10/31/83-2/29/84 (FINAL)
PARFR-334SRI -- "Development of Controlled-Release Progesterone Microcapsules for the Regulation of Fertility During Lactation"	11/1/83-1/31/84
PARFR-334UAB -- "Pharmacokinetic Studies in Baboons Relating to PARFR-334SRI"	12/1/83-3/1/84
PARFR-335 -- "One-Month NET Microcapsules and Six-Months Levonorgestrel Microcapsule Preparation"	5/1/83-2/29/84 (FINAL)
PARFR-336 -- "Effects of New Synthetic Progestins on Baboon Endometrium"	6/1/83-12/31/83 (FINAL)
PARFR-337F -- "Use-Effectiveness of a Levonorgestrel-Releasing Intracervical Device"	5/1/83-1/31/84

Technical Reports (continued)

<u>Project</u>	<u>Period Covered by Report</u>
PARFR-337T -- "Intracervical Device Acceptability Study"	6/1/83-2/1/84
PARFR-343 -- "NIH/Biotech Levonorgestrel Microcapsules"	12/1/83-3/1/84
PARFR-345 -- "Effects of the Vaginal Spermicidal Barrier Contraceptive on Sperm Transport in the Human"	Through 3/21/84 (FINAL)
PARFR-346 -- "Development of Controlled-Release Testosterone Microcapsules for Fertility Regulation of Males"	
PARFR-347 -- "Studies on the Anovulatory Potency and Side Effects of an Inhibitory Analog of LH-RH in Cynomolgus Monkeys"	
PARFR-348 -- "Development of Improved Methods and Materials for Injecting Microencapsulated Steroids"	11/1/83-2/1/84
PARFR-349 -- "Preparation of Fibrous Estradiol/ Progesterone IUDs for Phase I Clinical Trials - Continuation of PARFR-324"	11/1/83-2/29/84
PARFR-350 -- "An Intra Tubal Device (ITD) for Female Sterilization"	Through 3/7/84
PARFR-351 -- "Development of Methods for Female and Male Contraception Based on LH-RH Antagonists"	1/1/84-3/12/84
PARFR-352 -- "Baboon Testing of Duration of NET from Fused Pellets"	12/1/83-3/1/84

MCA

WING SOUND II

RESEARCH

PARFR SUBAGREEMENT EXPENSE SUMMARIES

This section summarizes the expenses of PARFR subagreements active during the period January 1, 1984 to June 30, 1984. Summaries are categorized according to the following AID Contraceptive Research Areas:

- I. Female Sterilization
 - C. Transcervical
- II. Male Sterilization
 - B. Other
- III. Intrauterine Contraception
 - D. Other
- IV. Systemic Contraception
 - B. Injectables and Implants
 - C. Other
 - 1. Male Pharmacological Methods
- V. Barrier Contraception
 - B. Female
- VI. Miscellaneous

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

I. FEMALE STERILIZATION

C. TRANSCERVICAL

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
305V	Maternidad "Concepcion Palacios" Caracas, Venezuela Itic Zighelboim, M.D. Wiktor Szczedrin, M.D.	"Radio-Opaque Methylcyano- acrylate (MCA) Delivered Through Single Application (FEMCEPT) Device"	9/10/81- 9/9/83	\$ 24,049	\$ 490.31	\$21,512.10
323	University of Illinois at the Medical Center Chicago, Illinois Lourens J.D. Zaneveld, D.V.M., Ph.D.	"Efficacy of Quinacrine and Tetracycline in the Primate"	9/1/82- 8/31/83	44,100	20,324.65	44,099.78
327C	Instituto de Obstetricia y Ginecologia de Univer- sidad Austral de Chile Valdivia, Chile Rene Guzman-Serani, M.D.	"Time Interval MCA/FEMCEPT Study"	12/1/82- 11/30/84	20,880	4,524.00	17,870.00
327V	Maternidad "Concepcion Palacios" Caracas, Venezuela Itic Zighelboim, M.D. Wiktor Szczedrin, M.D.	"Time Interval MCA/FEMCEPT Study"	12/1/82- 11/30/84	19,535	8,350.31	16,410.73

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

I. FEMALE STERILIZATION (continued)

D. OTHER

<u>PARFR</u> <u>#</u>	<u>INSTITUTION &</u> <u>INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES</u> <u>THIS PERIOD</u>	<u>TOTAL</u> <u>EXPENDITURES</u>
350	Lenon Hospital Paris, France Jacques Hamou, M.D.	"An Intra Tubal Device (ITD) for Female Sterilization"	1/1/84- 6/30/85	\$ 11,000	\$ - 0 -	\$ - 0 -

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

II. MALE STERILIZATION

B. OTHER

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
328B	PRO-PATER Sao Paulo, Brasil Marcos Paulo P. de Castro, M.D.	"An Evaluation of an Improved Needle for Transcutaneous Vas Closure"	12/1/82- 6/30/84	14,122	\$ 3,732.00	\$10,792.00
339	Rush-Presbyterian-St. Luke's Medical Center Chicago, Illinois Lourens J.D. Zaneveld, D.V.M., Ph.D.	"Efficacy of Studies in Primates with the Shug in the Absence of a Tissue Wrap"	7/1/83- 6/30/85	37,746	4,085.49	4,085.49
344	Presbyterian Hospital Obstetric and Gynecological Services New York, New York Ralph M. Richart, M.D.	"Percutaneous Intra Vas Injection for Male Sterilization"	9/1/83- 8/31/83	49,668	32,315.29	32,315.29
D-32	Biologic Resources Laboratory Univ. of Illinois at Chicago	Animal maintenance - Rhesus monkeys for PARFR-317			9,845.71	11,483.56

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

III. INTRAUTERINE CONTRACEPTION

D. OTHER

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
314E	Shatby Hospital, The University of Alexandria, Egypt Mokhtar Toppozada, M.D.	"Uterine Measurement - Clinical Comparison Study (Wing Sound II)"	2/1/82-7/31/83	\$ 5,400	\$ 900.00	\$ 5,400.00
337F	University of Helsinki Helsinki, Finland Tapani Luukkainen, M.D., Ph.D.	"Use-Effectiveness of a Levonorgestrel-Releasing Intracervical Device"	5/1/83-4/30/84	49,665	27,628.92	33,647.83
337T	The University of Texas Health Science Center San Antonio, Texas Rochelle N. Shain, Ph.D.	"Intracervical Device Acceptability Study"	6/1/83-5/31/85	12,502	328.48	328.48
349	Southern Research Institute Birmingham, Alabama Richard L. Dunn, Ph.D.	"Preparation of Fibrous Estradiol/Progesterone IUDs for Phase I Clinical Trials, Continuation of PARFR-324"	11/1/83-10/31/84	49,994	40,710.65	40,710.65
353	Michael Reese Hospital and Medical Center Chicago, Illinois Antonio Scommegna, M.D.	"Effects of Chronic Intra-uterine Release of Progesterone on Uterine Histology in Intact Rabbits"	3/15/84-7/31/84	7,895	- 0 -	- 0 -
D-52	Southern Research Institute Birmingham, Alabama	Preparation of an IUD for the Fibrous E/P IUD Delivery System		5,000	- 0 -	- 0 -
D-58	Harrith M. Hason Chicago, Illinois	Postage and telephone expenses related to the Wing Sound II study		- 0 -	256.20	256.20

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

IV. SYSTEMIC CONTRACEPTION

B. INJECTABLES AND IMPLANTS

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
320	Dynatech R/D Company Cambridge, Massachusetts Donald L. Wise, Ph.D.	"Levonorgestrel Rods - Drug Release Study"	7/1/82- 3/31/84	\$ 61,723	\$ - 0 -	\$49,759.95
325	Southern Research Institute Birmingham, Alabama Richard L. Dunn, Ph.D.	"Development of a Vaginal Ring Delivery System for Norgestimate"	9/1/82- 11/15/83	57,710	4,437.40	46,712.95
330	Cornell University Medical College New York, New York Brij B. Saxena, Ph.D., D.Sc.	"A Clinical Evaluation of the Subdermal Contraceptive Norethindrone Pellet (Phase II)"	2/1/83- 6/30/84	49,995	14,814.37	40,925.19
330M	Instituto de Investigacion Cientifica Durango, Mexico Roberto Rivera, M.D.	"A Clinical Evaluation of the Subdermal Contraceptive Norethindrone Pellet (Phase II)"	4/1/83- 3/30/84	28,710	7,402.00	11,924.00
330T	The University of Texas Health Science Center San Antonio, Texas Ricardo H. Asch, M.D.	"A Clinical Evaluation of the Bioabsorbable Subdermal Norethindrone Pellet Implant (Phase II)"	3/1/83- 2/28/85	48,139	16,315.91	19,323.71
332	Stolle Research and Development Corporation Birmingham, Alabama Danny H. Lewis, Ph.D.	"Development of An Injectable Long-Acting Estradiol Formulation"	3/1/83- 2/29/84	70,000	11,884.34	70,000.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

IV. SYSTEMIC CONTRACEPTION (continued)

B. INJECTABLES AND IMPLANTS

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
333	University of Alabama in Birmingham Charles E. Flowers, Jr., M.D. and Lee R. Beck, Ph.D.	"Poly-gly NET 180 Microcapsule System"	3/1/83- 2/29/84	\$ 47,200	\$25,693.50	\$47,200.00
333T	The University of Texas Health Science Center San Antonio, Texas Jose P. Balmaceda, M.D. Ricardo H. Asch, M.D.	"Poly-gly NET 180 Microcapsule System"	3/1/83- 2/29/84	40,249	29,896.18	38,124.96
334UAB	The University of Alabama in Birmingham Lee R. Beck, Ph.D.	"Pharmacokinetics Studies in Baboons Relating to PARFR-334SRI"	3/1/83- 7/31/84	16,741	6,098.67	8,974.82
334SRI	Southern Research Institute Birmingham, Alabama Thomas R. Tice, Ph.D.	"Development of Controlled-Release Progesterone Microcapsule"	3/1/83- 2/29/84	49,500	14,145.86	49,306.35
336	University of South Alabama Mobile, Alabama Walter H. Wilborn, Ph.D.	"Effects of New Synthetic Progestins on Baboon Endometrium"	6/1/83- 12/31/83	46,061	23,308.59	44,608.59
340	Southern Research Institute Birmingham, Alabama Thomas R. Tice, Ph.D.	"Technical Assistance in NET Microcapsule Preparation"	7/1/83- 1/31/84	36,500	23,881.55	36,500.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

IV. SYSTEMIC CONTRACEPTION (continued)

B. INJECTABLES AND IMPLANTS

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
341	University of Alabama in Birmingham Charles E. Flowers, Jr., M.D. and Lee R. Beck, Ph.D.	"Phase II Poly NET 90 Injectable Study"	12/1/83- 11/30/85	\$ 79,264	\$16,234.59	\$16,234.59
341A	Emory University Atlanta, Georgia Howard J. Tatum, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84- 12/31/85	\$ 32,905	- 0 -	- 0 -
341C	Centro Nacional de la Familia Santiago, Chile Horacio B. Croxatto, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84- 12/31/85	45,419	- 0 -	- 0 -
341M	Instituto de Investiga- cion Cientifica Durango, Mexico Roberto Rivera, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84- 12/31/85	36,135	3,326.00	3,326.00
341I	Associazione per Studio della Riproduzione Umana Roma, Italy Giuseppe Denagiano, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84- 12/31/85	\$ 39,655	2,803.35	2,803.35
341T	The University of Texas Health Science Center San Antonio, Texas Jose P. Balmaceda, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84- 12/31/85	85,919	6,742.77	6,742.77

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

IV. SYSTEMIC CONTRACEPTION (continued)

B. INJECTABLES AND IMPLANTS

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
343	University of Alabama in Birmingham Lee R. Beck, Ph.D.	"NIH/Biotech Levonorgestrel Microcapsules"	11/1/83- 10/31/84	\$ 46,040	\$ 9,541.80	\$ 9,541.80
346	Southern Research Institute Birmingham, Alabama Thomas R. Tice, Ph.D.	"Development of Controlled- Release Testosterone Microcapsules for Fertility Regulation of Males"	10/1/83- 8/31/84	49,641	14,516.42	14,516.42
348	Stolle Research and Development Corporation Birmingham, Alabama Danny H. Lewis, Ph.D.	"Development of Improved Methods and Materials for Injecting Microencapsulated Steroids"	11/1/83- 11/30/84	49,995	43,802.00	43,802.00
352	University of Alabama in Birmingham Birmingham, Alabama Lee R. Beck, Ph.D.	"Baboon Testing of Duration of NET Fused Pellets"	12/1/83- 11/30/84	29,420	2,509.04	2,509.04
357	Stolle Research and Development Corp. Birmingham, Alabama Danny H. Lewis, Ph.D.	"Optimization of Release Profile of Norethisterone Injectable 90 Day Contraceptive"	5/1/84- 10/31/84	49,119	- 0 -	- 0 -
358	Stolle Research and Development Corp. Birmingham, Alabama Danny H. Lewis, Ph.D.	"Development of a 30 Day Injectable Contraceptive"	5/1/84- 1/31/85	11,800	- 0 -	- 0 -
D-41	University of Alabama in Birmingham	RIA analysis for Poly NET Studies - PARFR-300E, 300I, 300M, 300T			4,337.00	9,783.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

IV. SYSTEMIC CONTRACEPTION (continued)

B. INJECTABLES AND IMPLANTS

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
D-46	Stolle Research and Development Corp. Birmingham, Alabama	Preparation of 4 kg co-polymer of DL-Lactic and glycolic for Poly NET 90 studies		\$ - 0 -	\$15,000.00	\$15,000.00
D-53	Stolle Research and Development Corp. Birmingham, Alabama	Production of GMP Standard Operations Procedures and Rental of GMP Laboratory for Seven Days		8,500	8,500.00	8,500.00
D-55	Stolle Research and Development Corp. Birmingham, Alabama	Preparation of 194 syringes loaded with Poly NET 180 system for Phase II clinical studies		29,488	- 0 -	- 0 -
D-59	University of Alabama in Birmingham	Stability studies on recalled microcapsule doses (series PARFR-341)		- 0 -	14,540.00	14,540.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

IV. SYSTEMIC CONTRACEPTION (continued)

C. OTHER

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
309	Northwestern University Medical School Chicago, Illinois Raksha Mehta, Ph.D.	"Ovulation Inhibition by Anordrin"	1/1/82- 6/30/84	\$148,988	\$23,954.58	\$117,589.33
347	The University of Texas Health Science Center San Antonio, Texas Ricardo H. Asch, M.D.	"Studies on the Anovulatory Potency and Side Effects on an Inhibitory Analog of LH-RH in Cynomologous Monkeys"	11/1/83- 1/31/86	145,501	8,402.57	8,402.57
351	Tulane University Medical Center New Orleans, Louisiana Andrew V. Schally, Ph.D.	"Development of Methods for Female and Male Contraception Based on LH-RH Antagonist"	1/1/84- 12/31/84	65,163	11,623.98	11,623.98
356	Medical Research Founda- tion of Oregon Portland, Oregon Rajesh K. Naz, Ph.D.	"Development of an Immunocon- traceptive Vaccine: Role of 23 Kd Antigen in Immunofer- tility and Fertility Regulation"	5/1/84- 4/30/85	66,686	3,589.93	3,589.93
359	Baylor College of Medicine Houston, Texas Bonnie S. Dunbar, Ph.D.	"Active Immunization of Non- Human Primates and Rabbits with Zona Pellucida Proteins"	6/1/84- 5/31/85	77,741	- 0 -	- 0 -
D-44	Northwestern University Chicago, Illinois	Animal Purchase and Care - 12 Cynomologous Monkeys for Anordrin Study			1,158.90	3,391.81
D-51	Reproductive Endocrino- logy Laboratory Fund Chicago, Illinois	Laboratory Tests and Analysis (Ovulation Inhibition by Ancrdrin)		3,228	- 0 -	- 0 -

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

IV. SYSTEMIC CONTRACEPTION (continued)

C. OTHER

1. MALE PHARMACOLOGICAL METHODS

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
315	Northwestern University Evanston, Illinois Erwin Goldberg, Ph.D.	"Immunologic Suppression of Fertility by Synthetic Antigenic Determinants of Dehydrogenase-C ₄ - Extension of PARFR-232"	3/1/82- 6/30/84	\$122,093	\$24,919.13	\$111,875.68
329	Northwestern University Evanston, Illinois Erwin Goldberg, Ph.D.	"Targeting Liposomes to the Male Reproductive Tract with Antibody LDH-4"	12/1/82- 11/30/83	10,000	476.59	9,187.17
355	Medical Research Founda- tion of Oregon Portland, Oregon Nancy J. Alexander, Ph.D.	"Enhancement of the Secretary Immune Response to LDH-C ₄ "	4/15/84- 4/14/85	47,335	7,901.61	7,901.61
D-27	Peninsula Laboratories, Inc. San Carlos, California	Synthetic peptide for animals at BRL (PARFR-315)			5,246.00	10,261.02
D-32	Biologic Resources Laboratory, Univer- sity of Illinois	Animal maintenance, papio baboons for PARFR-315			11,200.59	61,406.41
D-42	Jim Burns Chicago, Illinois	Injection of baboons for PARFR-315		700	- 0 -	585.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

IV. SYSTEMIC CONTRACEPTION (continued)

C. OTHER

1. MALE PHARMACOLOGICAL METHODS

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
D-57	Northwestern University Evanston, Illinois	Chemicals and radioimmuno- assays - PARFR-355		\$ 15,000	\$13,050.00	\$ 13,050.00
D-61	Oregon Regional Primate Research Center Beaverton, Oregon	Sperm penetration, SI/SA and cervical mucus penetra- tion assays on serum samples from 50 baboons			3,979.82	3,979.82

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

V. BARRIER CONTRACEPTION

B. FEMALE

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
338	Rush-Presbyterian-St. Luke's Chicago, Illinois Lourens J.D. Zaneveld, D.V.M., Ph.D.	"Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives"	7/1/83- 6/30/84	\$ 49,768	\$26,233.91	\$30,720.58
345	University of Southern California Medical Center Los Angeles, California Gerald S. Bernstein, M.D., Ph.D.	"Effect of the Vaginal Spermicidal Barrier Contraceptive on Sperm Transport in the Human"	10/1/83- 3/31/84	6,375	- 0 -	- 0 -
354	Mahidol University Bangkok, Thailand Montri Chulavatnatol, M.D.	"Screening of Thai Plants for Proteins (or Lectins) as Potential Vaginal Contraceptives"	4/1/84- 3/31/85	9,800	- 0 -	- 0 -

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
 UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
 1/1/84-6/30/84

VII. MISCELLANEOUS

<u>PARFR #</u>	<u>INSTITUTION & INVESTIGATOR(S)</u>	<u>TITLE</u>	<u>DATES</u>	<u>BUDGET</u>	<u>EXPENDITURES THIS PERIOD</u>	<u>TOTAL EXPENDITURES</u>
D-60	Reproductive Endo- crinology Laboratory Chicago, Illinois	Measurement of LH and Preg- nanediol Glucuronide relating to PARFR-360		\$ 7,200	\$ - 0 -	\$ - 0 -

FOLLOWING ARE SIX MONTH TECHNICAL REPORT SUMMARIES
OF ALL PROJECTS DURING THIS PERIOD

1/1/84 - 6/30/84

Projects are listed by PARFR number and not by
"Contraceptive Research Area."

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 309

TITLE: "Ovulation Inhibition by Anordrin"

INSTITUTION: Northwestern University Medical School

PRINCIPAL INVESTIGATOR: Robert T. Chatterton, Ph.D.

FUNDING PERIOD: 1/1/82-6/30/84

AMOUNT FUNDED: \$148,988

Below is a project summary for the 1/1/84-6/30/84 period.

During this period we have completed the study of the dose-response characteristics of anordrin. An approximately linear increase in menstrual cycle length was produced by single intramuscular injections of anordrin in the range of 0.1 to 0.4 mg/kg body weight when administered within the first six days of the menstrual cycle. Luteal phase length was unaltered by the treatment and was preceded by a normal surge of estrogen. Nevertheless, there was a linear decrease in the average luteal phase pregnanediol excretion with increasing doses of anordrin. Endometrial biopsies taken in the luteal phase of treatment cycles exhibited little secretory activity.

Control cycles that followed effective treatment cycles had approximately 30% lower pregnanediol excretion than initial control cycles, but subsequent control cycles were unaffected. The length of menstrual cycles following treatment cycles was normal.

Oral availability of anordrin based on the pregnanediol response was only 4.6%.

To investigate the mechanism of action of anordrin, FSH and estrogen levels were measured in the immediate post-injection period. FSH concentrations in serum were unchanged by anordrin administration. The excretion of conjugated estrone measured in 24-hr urine collections, however, was decreased in proportion to the dose of anordrin administered. The highest dose, 0.4 mg/kg, reduced estrogen excretion to paractically zero. This is taken as evidence that anordrin acts directly on the ovary.

An LH assay for monkey serum has been validated in this laboratory, and this assay will now be used to determine the effect of anordrin on the levels of this gonadotropin in serum samples that have already been collected.

In the study of monthly administration of a dose of anordrin that delays ovulation by 3 to 4 weeks we have completed 3 months in six monkeys. Four of the monkeys have not menstruated since the first injection. Two monkeys menstruated 42 days after the first injection, but pregnanediol excretion during the 12 days before menstruation was less than 50% of that in control cycles in the same monkeys. None of the monkeys have had menstrual periods in the 2 months since the second injection, and daily pregnanediol excretion has remained at preovulatory amounts.

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Weekly vaginal smears consistently show a predominance of cornified cells with few basal cells during the months on treatment, similar to the appearance of smears taken between days 16 and 22 of the normal menstrual cycle. Since the endometrium is suppressed during treatment with anordrin, we interpret the vaginal cornification as a continuing response to low levels of estrogen in the absence of progesterone. In practical terms it appears that vaginal atrophy may not be a problem with this treatment as we had anticipated.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 315

TITLE: "Immunologic Suppression of Fertility by Synthetic Antigenic Determinants of Lactate Dehydrogenase-C₄, Extension of PARFR-232"

INSTITUTION: Northwestern University

PRINCIPAL INVESTIGATOR: Erwin Goldberg, Ph.D.

FUNDING PERIOD: 3/1/82-6/30/84

AMOUNT FUNDED: \$122,093

Below is a project summary for the 1/1/84-6/30/84 period.

A hamster ova¹ sperm, in vitro fertilization assay was evaluated as a means of assessing human fertility suppression by sera from baboons immunized with the antigenic MC5-15 peptide of LDH-C₄. Sera from immune female baboons inhibited penetration to a variable extent (31% to 100%). Pre-immune sera from three individual females and a pool of non-immune male baboon sera inhibited penetration also (76% to 100%). Therefore, this assay is not useful in assessing potential contraceptive efficacy of immunization with MC5-15 until appropriate control conditions can be developed.

Fertility studies with female baboons immunized with this peptide were begun on 2-28-84. Female baboons have continued to receive i.m. inoculations of 5.0 mg DF-MC5-15 in CGP adjuvant 7 to 14 days before ovulation. Twelve animals had received seven immunizations before their first mating, one had received five and one had received four. All have now been given at least two additional immunizations. There have been no deleterious effects on the health of the animals as a result of repeated inoculation. Antibody levels have been monitored weekly as well as immediately before and after mating. The mean antibody level at the first mating was 40.28 ng ¹²⁵I-labeled LDH-C₄ bound/ml serum (SD = 12.0). The mean level at the second mating was 41.7 ng (SD = 8.9). These means are equal to, or only slightly lower than the means of peak antibody levels following previous immunizations. While these data indicate that a consistent level of antibody specific for LDH-C₄ can be attained by serial immunization with the antigenic peptide fragment MC5-15, it is apparent that the optimum immunization protocol, especially with regard to the proper carrier and adjuvant, has not yet been achieved. Based on our previous studies with intact LDH-C₄, the antibody levels in baboons immunized with MC5-15 are only about 5 to 10% of that necessary to protect against conception. Nevertheless, there does appear to be some suppressive effect. Thus far, only 3 pregnancies have resulted from a total of 29 matings of the MC5-15 immune females. There were 10 pregnancies of 15 control animals after a total of 42 matings. At this stage of the study, the fertility of the baboons immunized with MC5-15 conjugated to diphtheria toxoid, is significantly reduced compared to the control animals.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 327C

TITLE: "Time Interval MCA/FEMCEPT Study"

INSTITUTION: Instituto de Obstetricia y Ginecologia de Universidad Austral

PRINCIPAL INVESTIGATOR: Rene Guzman-Serani, M.D.

FUNDING PERIOD: 12/1/82-11/30/84 AMOUNT FUNDED: \$20,880

Below is a project summary for the 1/1/84-6/30/84 period.

The 60 cases that this study specify, were gathered since March and August 1983. At this date all the 60 patients had their two application of MCA, one, two or three onths apart, depending of the protocol.

During January 1984 all these 60 patients finished with their follow up visit No.2 which considered x-ray examination with H.S.G. using a water-soluble radio-opaque solution.

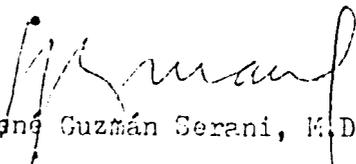
The results of this x-ray examination were:

56 cases with bilateral tubal occlusion
4 cases with only one tube occluded

During the following months, February to June 1984, all the patients have been fulfilling with their corresponding follow up visits and we have had only one problem:

Patient # 252 that had a pregnancy in spite of the HSG made on October 1983 showed bilateral tubal occlusion. This patient had an spontaneous abortion at 20 weeks of her pregnancy and now she is totally recuperated.

All the other patients are well, without menstrual disorders and without any pathological problem of the genital tract.


Rene Guzman Serani, M.D.
DIRECTOR

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

JUL 13 1984

PARFR- 327V

TITLE: "Time Interval MCA/FEMCEPT Study"

INSTITUTION: Maternidad "Concepcion Placios"

PRINCIPAL INVESTIGATOR: Itic Zighelboim, M.D.

FUNDING PERIOD: 12/1/82-11/30/84

AMOUNT FUNDED: \$19,535

Below is a project summary for the 1/1/84-6/30/84 period.

During the above mentioned period the following was accomplished:

1. H.S.G. was performed on 38 patients.
2. In 4 cases reinjections were performed, due to patency of one or both tubes as shown on H.S.G.
3. Followups were done on 11 patients, after H.S.G. showed bilateral occlusion.
4. All the protocols and the H.S.G films were mailed to BioNexus.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

JUL 3 1984

PARFR- 330

TITLE: "A Clinical Evaluation of the Subdermal Norethindrone Pellet (Phase II)"

INSTITUTION: Cornell University Medical College

PRINCIPAL INVESTIGATOR: Brij B. Saxena, Ph.D., D.Sc.

FUNDING PERIOD: 2/1/83-6/30/84

AMOUNT FUNDED: \$49,995

Below is a project summary for the 1/1/84-6/30/84 period.

Thirty women volunteers from a total of 42 have already completed the study satisfactorily and have had their pellets removed. These volunteers have been discharged from the study in good physical condition. There were six dropouts from the study, two personal reasons, one pregnancy, one mastalgia and two menstrual disturbances. The remaining six volunteers are continuing in the study satisfactorily. The data so far obtained from the women with NET-implant can be summarized as follows:

Simplification of the surgical procedure for the removal of the pellets: A less extensive procedure has been established for the removal of the pellets which is, cosmetically, highly acceptable. The procedure requires approximately 15 minutes from skin incision to the closure. 0.1 ml of 1% Lidocaine was infiltrated subcutaneously and 0.5 to 1.0 cm skin incision was applied. The fibrous capsule was identified in the fat tissue and easily dissected out. A Steritape was applied approximating skin incision.

Release rates: With three and four pellets, release rates of 174.4 ± 13.8 $\mu\text{g/day}$ and 244.2 ± 24 $\mu\text{g/day}$, respectively, were observed.

Serum NET levels: The serum NET level with three and four pellets were sustained and no initial burst effects were observed. In the initial six months, the serum NET levels were 0.4 - 0.6 ng/ml with three pellets and 0.6 - 0.7 ng/ml with four pellets. After six months the serum NET levels were 0.4 - 0.6 ng/ml with both three and four pellets. This phenomenon may be the result of fibrous capsule formation around the pellets. The drug was released from the pellets and trapped inside the capsule thus impeding the bioavailability of the drug in the body circulation. The serum samples for NET analysis from Mexico and Texas have been received and they are in the process of being evaluated. The results will be forwarded to FHI soon.

Menstrual patterns: (a) 40% of the women with three pellets and 27% of the women with four pellets had normal menstrual cycle throughout the treatment period, (b) 30% of the women with three pellets and 20% of the women with four pellets had total amenorrhea for the periods up to 4-12 months, (c) 30% of the women with three pellets and 53% of the women with four pellets experienced irregular menstrual patterns in terms of menstrual and intermenstrual bleeding and/or spotting,

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sporadic bleeding and spotting and prolonged episodes of bleeding and spotting during the entire treatment period.

Ovarian response: The ovulatory cycles were classified on the basis of the progesterone level in the luteal phase. With three pellets during 271 cycles, with four pellets during 163 cycles, 17% and 9% of the cycles were ovulatory, respectively.

Contraceptive efficacy: The contraceptive efficacy of the pellet at this point appears to be quite satisfactory. Out of 271 cycles with three pellets only two cycles in two volunteers got fertilized (one at 6 months, one at 13 months). Among the 15 volunteers with four pellets in a total of 163 cycles no incidence of pregnancy occurred up to 13 months of treatment period.

Side effects: There were no serious side effects observed during the entire treatment period. One out of 42 volunteers experienced mastalgia and dropped out in the fourth month of the study. Three women experienced transitory nausea which went away spontaneously. Two women dropped out from the study because of irregular menstrual bleeding patterns. There was no change observed in serum S/MAC and LDL-HDL ratio, PAP smear from pre- to post-implant period. There was an incidental finding of dysmenorrhea and premenstrual syndrome (PMS) being ameliorated with the use of the pellets.

Acceptability: In spite of the various side effects listed above, all of the 30 subjects who have completed the study express their desire to continue using the this method of contraception either in a continuing study or commercially if available.

General impression: Overall evaluation of IET implants as future long-term contraception may be summarized as follows. 1) The estrogen free fused Norethindrone pellets can be manufactured at low cost and be easily sterilized and stored in preloaded disposable trocars, 2) the size and shape of the pellets makes implantation and removal as an easy out-patient procedure, 3) since the pellets can be retrieved easily, this method of contraception is completely reversible at will, 4) lack of serious side effects and convenience in use render these pellets highly acceptable contraceptive for extended periods, 5) the advantages of this mode of contraception outweigh the menstrual irregularity and minor transitory side effects, 6) it should, however, be pointed out that although these pellets are chemically biodegradable, they nevertheless induce formation of a fibrous capsule around them during the implant period. It has been observed that the drug is constantly released from the pellet and is trapped in the fibrous capsule which acts as a barrier and thus seriously effects the bioavailability of the drug in the serum, 7) Finally, modification of these pellets in a manner which will retard the formation of fibrous capsule around the pellets could provide a potential simple, safe and long-acting contraceptive.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 330M

TITLE: "A Clinical Evaluation of the Subdermal Contraceptive Norethindrone Pellet (Phase II)"

INSTITUTION: Instituto de Investigacion Cientifica, Durango, Mexico

PRINCIPAL INVESTIGATOR: Roberto Rivera, M.D.

FUNDING PERIOD: 4/1/83 - 9/30/84

AMOUNT FUNDED: \$28,710

Below is a project summary for the 1/1/84-6/30/84 period.

Nineteen of the 20 planned cases have been enrolled in the study. Eighteen of them terminated their participation in the study within this period. No problems were observed at the site of the insertion. One subject complained of mastalgia, and one more presented cystitis. Nine of the 18 cases that terminated in this period presented intermenstrual bleeding or spotting, or prolonged bleeding. The remaining 9 cases showed normal menstrual patterns. No instance of amenorrhea was observed. Two of the subjects complained of the bleeding problems associated with the use of the implant. In the 6 cases with weekly determinations of estradiol and progesterone, ovulation inhibition was observed in only 2 of the cases. In two cases the progesterone determinations suggest the presence of ovulation in all the cycles. The other 2 cases showed progesterone values suggestive of ovulation in only one and two of the observed cycles, respectively. The 6 subjects showed ovulatory progesterone values after removal of the implant. One pregnancy occurred in the 17th week after insertion, terminating in a spontaneous abortion.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 330T

TITLE: "A Clinical Evaluation of the Bioabsorbable Subdermal
Contraceptive Norethindrone Pellet Implant (Phase II)"

INSTITUTION: The University of Texas Health Science Center at
San Antonio

PRINCIPAL INVESTIGATOR: Ricardo H. Asch, M.D.

FUNDING PERIOD: 3/1/83-2/28/85

AMOUNT FUNDED: \$48,139

Below is a project summary for the 1/1/84-6/30/84 period.

The purpose of this study is to identify the lowest dose of Norethindrone and duration that it is contraceptively effective using implants of 3 or 4 NET pellets.

To date fifteen patients have completed the study having had the pellets removed without complications. The pellets were mailed to Dr. Saxena for residual NET determinations. Tissue that surrounded the pellets was sent to Dr. Saxena for histological examination by experienced pathologists for evidence of fibrosis, inflammation or neoplastic-type changes.

Analysis of blood samples collected during the study period show no change in SMAC or lipoprotein profiles and an absence of ovulation in most patients as determined by serial progesterone levels. Serum NET levels are not available to us as of yet.

Patient acceptability was excellent. Serious side effects were observed in only one patient who experienced nausea, vomiting and membranous dysmenorrhea with passage of an endometrial cast. Symptoms ceased immediately after the removal of the pellets. One patient experienced amenorrhea during the entire study (monthly pregnancy tests were negative).

None of the patients became pregnant. Menstrual bleeding calendars have been collected on all subjects who have completed the study.

Four patients retained their pellets and are continuing with the study for a period to total one year.

Rough data has been mailed to International Fertility Research Program for further statistical analysis. We plan to send the results of the study for publication and to present them in scientific meetings as soon as the analysis of the data in all patients is completed.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

JUL 17 1984

For the Period January 1, 1984 - June 30, 1984

PARFR-332

TITLE: "Development of an Injectable Long-Acting Estradiol Formulation"

INSTITUTION: Stolle Research and Development Corporation

PRINCIPAL INVESTIGATOR: Danny H. Lewis, Ph.D.

FUNDING PERIOD: 3/1/83-2/29/84

AMOUNT FUNDED: \$70,000

Below is a project summary for the 1/1/84-6/30/84 period.

The major objective of this research program is to develop a controlled-release microsphere formulation that will efficaciously deliver an estrogen for a duration of 3 to 6 months. The microspheres will be a free-flowing powder consisting of spherical particles less than 200 μm in diameter. The microspheres will contain ethynylestradiol encapsulated in poly(DL-lactide-co-glycolide), a biocompatible, biodegradable polyester.

We have prepared ethynylestradiol (EE) microspheres with core loadings ranging from 5 to 50 wt % drug. These microspheres were prepared by a patented Stolle process. After preparation, we thoroughly characterized each batch of microspheres. This characterization included observation of the surface morphology and determination of the drug content. We also determined the in vitro release of ethynylestradiol from the microspheres.

Four different microsphere formulations have been tested in baboons. Initially a group of animals was treated with 5 mg of ethynylestradiol by means of microspheres 20 to 70 μm in diameter and 25% loaded. We found that this dose was much too high. Consequently, three formulations containing either 9.5, 24.3, or 43% by weight of EE were tested at a dose of approximately 0.5 mg EE.

All three baboons treated with 9.5% loaded microspheres had at least two apparently normal cycles following treatment (eggs were recovered nonsurgically from four of the six cycles), and then experienced prolonged turgescence assumedly related to ethynylestradiol treatment. Deturgescence started approximately 112 to 127 days post-treatment (PT).

Animals, treated with 24% loaded microspheres, have

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exhibited variable response. One baboon ovulated in the treatment cycle, then underwent a period of prolonged turgescence from days 38 to 65 PT, and initiated an ovulatory cycle on day 85 PT. The second baboon maintained maximum turgescence from days 6 to 54 PT, deturgesced, and appears to be starting a new cycle on day 72 PT. The third baboon in this group has exhibited maximum turgescence for the entire 120-day interval since treatment.

The three baboons, treated with the 43% loaded microspheres, had all completely deturgesced by 55 to 57 days PT, and new cycles started 66 to 70 days PT in the three baboons.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

JUL 17 1984

For the Period January 1, 1984 - June 30, 1984

PARFR-333

TITLE: "Poly-gly NET 180 Microcapsule System" (Phase I)

INSTITUTION: University of Alabama in Birmingham

PRINCIPAL INVESTIGATOR: Charles E. Flowers, Jr., M.D.

FUNDING PERIOD: 3/1/83-2/29/84

AMOUNT FUNDED: \$47,200

Below is a project summary for the 1/1/84-6/30/84 period.

Pharmacokinetics and pharmacodynamics of a new long-acting injectable microcapsule contraceptive delivery system were tested in 10 women. Microcapsules made from a biocompatible, biodegradable polymer poly(D,L-lactide-co-glycolide) which range in diameter from 125 to 212 microns and contain 22% by weight norethindrone (NET) were prepared by a solvent evaporation process. Two doses of the microcapsule formulation (75 and 150 mg NET) were administered by intramuscular injection of Day 5 of the menstrual cycle. Serum samples obtained biweekly during the post-treatment period were analyzed by RIA for NET, progesterone and estrogen. Menstrual diary cards were used to keep daily records of menstrual bleeding, and uterine biopsies were obtained before, during, and after the treatment. High density lipoproteins were analyzed before and after treatment. Treatment suppressed ovarian function and inhibited ovulation in all subjects for 4 months. Serum NET levels could be detected up to 180 days posttreatment. Norethindrone levels in subjects that received the higher dose (150 mg NET) were proportionately greater than in those who received the lower dose (75 mg NET). Following injection there was a rapid rise in the serum NET levels followed by a gradual decline until 15 to 17 weeks. Between 15 and 17 weeks posttreatment there was a secondary rise and fall in the serum NET levels. The biphasic NET release profile occurred in all subjects completing the study and is characteristic of this formulation. The treatment caused spotting and/or irregular menstrual cycles in all subjects. The degree of bleeding irregularities decreased during the last half of the treatment. Normal bleeding patterns resumed in all subjects within 8 months after injection.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-334SRI

TITLE: "Development of Controlled-Release Progesterone Microcapsule"

INSTITUTION: Southern Research Institute

PRINCIPAL INVESTIGATOR: Thomas R. Tice, Ph.D.

FUNDING PERIOD: 3/1/83-4/30/84

AMOUNT FUNDED: \$49,500

Below is a project summary for the 1/1/84-6/30/84 period.

An injectable biodegradable delivery system for the controlled release of progesterone would minimize the deficiencies present in current contraceptive methods used by lactating women. Because progesterone is not active by the oral route, any progesterone present in the mother's milk would not affect the child as could synthetic contraceptive steroids. It is, therefore, desirable to develop a system that can be easily administered as a single injection that will provide contraceptive protection for about 3 months.

To meet this goal, a highly loaded progesterone microcapsule formulation has been fabricated by using a solvent-evaporation microencapsulation process. The microcapsules consist of 65 wt % progesterone and 35 wt % poly(DL-lactide-co-glycolide), a biocompatible polyester that biodegrades to lactic acid and glycolic in about 6 months. The microcapsule product consist of spherical particles (63 to 125 μ m in diameter) with continuous polymeric film coatings and no evidence of unencapsulated drug. After intramuscular injection into baboons, the microcapsules release progesterone at a controlled rate, maintaining a serum level of 3 to 5 ng/mL for 3 months.

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 334UAB

TITLE: "Pharmacokinetic Studies in Baboons Relating to PARFR-334SRI"

INSTITUTION: The University of Alabama in Birmingham

PRINCIPAL INVESTIGATOR: Lee R. Beck, Ph.D.

FUNDING PERIOD: 3/1/83-2/29/84

AMOUNT FUNDED: \$13,699

Below is a project summary for the 1/1/84-6/30/84 period.

Two additional formulations of progesterone - releasing microcapsules were evaluated in pharmacokinetic studies in baboons. Two groups (C and D) of 3 baboons each were treated with 250 to 265 mg progesterone 56% (Group C) to 60% (Group D) loaded. Two intact baboons in group C initiated sex skin turgescence suggestive of a return to cyclicity on days 94 and 105 posttreatment. Serum progesterone levels peaked at up to 10 ng/ml 2 wks posttreatment, and presented a biphasic pattern, with a decline to 0.28 to 0.51 ng/ml 56 to 77 days posttreatment followed by a secondary rise to 2.24 to 2.79 ng/ml on day 77 up to 105 posttreatment. Group D is in progress, currently entering the third month posttreatment.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-337F

TITLE: "Use Effectiveness of a Levonorgestrel-Releasing
Intracervical Device"

INSTITUTION: University of Helsinki; Helsinki, FINLAND

PRINCIPAL INVESTIGATOR: Tapani Luukkainen, M.D., Ph.D.

FUNDING PERIOD: 5/1/82-4/30/86

AMOUNT FUNDED: \$97,537

Below is a project summary for the 1/1/84-6/30/84 period.

Group 1. In this study group altogether 200 women will be enrolled for the insertion of a levonorgestrel-releasing intracervical contraceptive device. The first one hundred women will participate the acceptance study conducted by Dr. Rochelle Shain. Altogether, 97 insertions have been performed, for one patient repeatedly after an expulsion. No accidental pregnancies have occurred. At present, 34 woman have been followed more than one year. The total experience in the present study is 764 woman-months. Five expulsions have occurred with the present device, which is only half of the rate with the prototype device (6 vs. 14 %). Two expulsions were experienced by the same woman who had the total length of cervix and uterine cavity of only 40 mm by sound. This decrease in expulsions may account for the spring-like vertical arms of the device. Three infections have occurred. One case was a salpingo-oophoritis caused by gonorrhea. In two other cases an endometritis was clinically diagnosed within 2 weeks after insertion.

No side-effects were reported by 41 % and the most common side-effect was the bleeding disturbance, as reported by 16 %. However, only four women wanted the device to be removed because of bleeding disturbances.

The enrollement of volunteers using levonorgestrel-IUD or copper IUD, as controls for the acceptance study, is continuing. The questionnaire forms have been forwarded to Dr. Rochelle Shain for computer analysis.

The enrollement of patients for the acceptability study will be completed this month but it continues for the controls. Therefore, no results are expected to be available within next 6-12 months. The clinical performance will be analyzed every 3 months and reported to PARFR. Our results so far support our expectations.

During the first week of June the enrollement of patients has continued. In the acceptance study 25 interviews have been performed.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-337T

TITLE: "Intracervical Device Acceptability Study"

INSTITUTION: The University of Texas Health Science Center at
San Antonio

PRINCIPAL INVESTIGATOR: Rochelle N. Shain, Ph.D.

FUNDING PERIOD: 6/1/83-5/31/85 AMOUNT FUNDED: \$12,502

Below is a project summary for the 1/1/84-6/30/84 period.

A total of 260 questionnaires have been received from Finland. These include 137 initial interviews (92 - ICD; 21 - NOVA T; and 24 - LNG IUD); 100 3 month follow-ups (majority are ICD) and 23 12-month follow-ups (ICD). To date (June 27) we are in the process of coding the last batch of questionnaires. As soon as this is accomplished the data will be entered into the computer so that preliminary analyses can be conducted. However, it should be noted that meaningful results will be forthcoming only after a sufficient number of 3-month follow-up questionnaires from control patients have been received.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

JUL 9 1984

PARFR-338

TITLE: "Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives"

INSTITUTION: Rush-Presbyterian-St. Luke's Medical Center

PRINCIPAL INVESTIGATOR: Lourens J.D. Zaneveld, D.V.M., Ph.D.

FUNDING PERIOD: 7/1/83-6/30/84 AMOUNT FUNDED: \$49,768

Below is a project summary for the 1/1/84-6/30/84 period.

During this period, one of our efforts was aimed at preparing a formulation that could be applied vaginally, would give adequate distribution of the inhibitors and could ultimately be used clinically. A number of aqueous and non-aqueous formulations were investigated as possible delivery systems. The solubilizing agents selected were all FDA-approved for use in vaginal suppositories and jellies. Among these were the various molecular weight polyethylene glycols (PEG's) and a new dispersing agent, pluronic F-127. Although the inhibitors were found to be stable in these preparations, none of them were entirely satisfactory and other vehicles are presently being investigated.

Another effort was directed at assessing the sperm membrane penetrating capacity of the spermatozoa. On incubation with human sperm, 10^{-5} M of the inhibitors caused 90-95% inhibition of total acrosin activity. Also, proacrosin activation to acrosin was completely inhibited. Thus, it appears that the inhibitors rapidly penetrate sperm membranes at low concentrations and bind to acrosin and proacrosin.

Finally, subacute toxicology studies (50 day application) were concluded with a pathological evaluation of some major organs. None of the animals died on vaginal application of the compounds and, as compared to the controls, no significant pathological alterations were found in any of the organs. Vaginal irritation appeared to be minimal or absent with the inhibitors. On intraperitoneal injection, several of the inhibitors proved to be much less toxic than nonoxynol-9, the most widely used vaginal contraceptive on the market. Also, pathological changes in the tissues occurred much more rarely with these inhibitors. From the present data as well as those obtained previously, it can be concluded that several of the inhibitors are much less toxic and much more contraceptive than nonoxynol-9.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

JUL 9 1984

PARFR-339

TITLE: "Efficacy of Studies in Primates with the Shug in the Absence of a Tissue Wrap"

INSTITUTION: Rush-Presbyterian-St. Luke's Medical Center

PRINCIPAL INVESTIGATOR: Lourens J.D. Zaneveld, D.V.M., Ph.D.

FUNDING PERIOD: 7/1/83-6/30/84 AMOUNT FUNDED: \$19,446

Below is a project summary for the 1/1/84-6/30/84 period.

Shug device modifications were made to allow easier implantation. A stainless steel tip, covered with silicone, was added to the end of each silicone plug. The tip is bullet pointed although not sharp. This allows for easy insertion of the device through the pinhole opening made in the vas deferens.

Shugs had been implanted into 5 primates (rhesus macaques). Only one animal ejaculated sperm after implantation. Exploratory surgery showed that the Shug had not been implanted correctly on one side. After correction, this animal also became sterile. During the present period (following 6-7 months of implantation), the devices were removed from three of the five animals. The vasa of the other two animals were excised for histological studies. All three primates ejaculated spermatozoa within two ejaculates after Shug removal. The last 5 ejaculates of the primates averaged approximately the same number of spermatozoa as found in the preinsertion specimen and the spermatozoa possessed the same motility. One animal was an exception because its values were slightly lower although they were still normal. These data confirm the results obtained during a previous study in which also a 100% reversal of sperm output was observed after device removal.

An IDE was prepared for the FDA so that Phase I clinical trials can be initiated. In general, approval for the study was obtained providing that the questions raised concerning the toxicity of the medical grade silicone can be answered satisfactory and a rat study is performed to assess the toxicity of the silicone in the vas.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

JUL 17 1984

For the Period January 1, 1984 - June 30, 1984

PARFR-341

TITLE: "Phase II Poly NET 90 Injectable Study"

INSTITUTION: The University of Alabama in Birmingham

PRINCIPAL INVESTIGATOR: Charles E. Flowers, Jr., M.D.

FUNDING PERIOD: 12/1/83-11/30/85

AMOUNT FUNDED: \$79,264

Below is a project summary for the 1/1/84-6/30/84 period.

A baboon pharmacokinetic study was completed on the master batch of NET microcapsules made for the Phase II clinical study of the Poly NET 90 injectable contraceptive. The baboons were given the same dose intended for women, to ensure the duration of NET release could be accurately determined. Serum levels of NET gradually increased until approximately 20 days PT, at which time levels became high enough to inhibit ovulation. Levels remained fairly constant until day 50, after which an abrupt increase in serum NET occurred in response to rapid biodegradation of the microcapsules. NET levels declined to undetectable levels 140 days posttreatment.

A similar pattern of NET release is occurring in the 17 women treated with this formulation. Due to the larger body size and blood volume, serum NET levels were undetectable in the women for up to 2 months posttreatment, when a substantial increase in serum NET occurred similar to that observed in the baboons. Due to the low levels of NET in the early posttreatment period, the Phase II studies have been discontinued while additional microcapsule batches are being evaluated to further refine and control the rate and duration of NET release.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-341A

TITLE: "Phase II Poly NET 90 Injectable Study"

INSTITUTION: Emory University

PRINCIPAL INVESTIGATOR: Howard J. Tatum, M.D.

FUNDING PERIOD: 1/1/84-12/31/85

AMOUNT FUNDED: \$32,905

Below is a project summary for the 1/1/84-6/30/84 period.

Project delayed due to reformulation requirements for the NET Microspheres.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 341C

TITLE: "Phase II Poly NET 90 Injectable Study"

INSTITUTION: Centro Nacional de la Familia

PRINCIPAL INVESTIGATOR: Horacio B. Croxatto, M.D.

FUNDING PERIOD: 1/1/84-12/31/85

AMOUNT FUNDED: \$45,419

Below is a project summary for the 1/1/84-6/30/84 period.

Four women were enrolled in the study. Blood samples were drawn during one pretreatment cycle in 2 subjects. Posttreatment samples were obtained weekly in 2 women during month 1, in 1 women during month 2 and in all women during months 3, 4 and 5. An aliquot of each serum sample was lyophilized and send to Dr. Lee Beck in Alabama for NET determination. The rest of each sample was frozen for measuring E_2 and Progesterone. These assays are pending.

Women were protected with IUD or spermicides according to the instructions received. Bleeding irregularities were observed in the 4 women. No other problems were detected.

Clinical follow up and blood sampling will continue until the first normal cycle is detected.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 3411

TITLE: "Phase II Poly NET 90 Injectable Study"

INSTITUTION: Associazione per Studio della Riproduzione Umana

PRINCIPAL INVESTIGATOR: Giuseppe Benagiano, M.D.

FUNDING PERIOD: 1/1/84-12/31/85 AMOUNT FUNDED: \$39,655

Below is a project summary for the 1/1/84-6/30/84 period.

The project was started during January 1984. Five subjects were recruited during the first month, and were followed during one control cycle. However, in view of the instructions received from PARFR, they were not injected.

During the month of February no additional patient was recruited, waiting for the results from animal experiments carried out in Birmingham. Two additional subjects were recruited during the month of March, and they were followed during a control cycle.

On 21 March we received a telex from PARFR informing us that the release rates of the NET-90 injectable formulation that we had received were too low to insure contraceptive efficacy. PARFR had decided not to initiate the Phase 2 study. Therefore, we informed our seven patients that they would not be injected and that they were free from any further engagement with reference to this trial.

We were instructed to send back to Atlanta all syringes and vials in our possession for this study. Samples were shipped to Birmingham via air freight and on 23 May Professor Lee Beck confirmed receipt of all materials from our center.

In accordance with the instructions received, we are waiting for the new studies to be conducted in Birmingham on a new batch of NET polymer.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 341M

TITLE: "Phase II Poly NET 90 Injectable Study"

INSTITUTION: Instituto de Investigacion Cientifica,
Juarez University of Durango

PRINCIPAL INVESTIGATOR: Roberto Rivera, M.D.

FUNDING PERIOD: 1/1/84-12/31/85

AMOUNT FUNDED: \$36,135

Below is a project summary for the 1/1/84-6/30/84 period.

The syringes containing the NET microspheres were received. A special container to store safely these syringes was made. It will ensure that only Dr. Rivera will have access to the syringes. The protocol was translated into Spanish to be used by the local staff. Various meetings were held with the local participating staff to instruct them in their functions, responsibilities and the purpose of the study. All the administrative arrangements necessary to initiate the study were made with PARFR. The identification of volunteers was initiated. Before the first subject was admitted notice was received that the study was being canceled because of technical problems with the preparation.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 341T

TITLE: "Phase II Poly NET 90 Injectable Study"

INSTITUTION: The University of Texas Health Science Center
at San Antonio

PRINCIPAL INVESTIGATOR: Jose P. Balmaceda, M.D.

FUNDING PERIOD: 1/1/84-12/31/85 AMOUNT FUNDED: \$82,487

Below is a project summary for the 1/1/84-6/30/84 period.

Project delayed due to reformulation requirements for the NET Microspheres.

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JUL 17 1984

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-343

TITLE: "NIH/Biotech Levonorgestrel Microcapsules"

INSTITUTION: The University of Alabama in Birmingham

PRINCIPAL INVESTIGATOR: Lee R. Beck, Ph.D.

FUNDING PERIOD: 11/1/83-10/31/84

AMOUNT FUNDED: \$46,040

Below is a project summary for the 1/1/84-6/30/84 period.

Ten baboons have been treated with levonorgestrel microcapsules provided by BIOTEK, Inc, containing 10 or 20 mg drug (5 baboons each dose). Blood samples are being obtained and assayed for estradiol, progesterone and levonorgestrel with split samples provided to NIH. It appears that only the higher dose is effective at inhibiting cyclicity as the posttreatment interval increases. At least two of the baboons on the lower dose have had progesterone (P₄) levels greater than 3 ng/ml. Sex skin turgescence has occurred in four of the five baboons treated with 10 mg norgestrel, in a regular cyclic pattern in the two baboons with ovulatory P₄ levels. Cyclicity has been completely suppressed in the group treated with 20 mg LN. After norgestrel levels up to 6 (10 mg dose) to 13 (20 mg dose) ng/ml in the first month, posttreatment values have gradually declined in the two groups, with 4 month posttreatment norgestrel levels over 0.5 ng/ml in the 10 mg group and over 1.5 ng/ml in the 20 mg group.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 344

TITLE: "Percutaneous Intra Vas Injection for Male Sterilization"

INSTITUTION: Presbyterian Hospital, Obstetrics and Gynecological Services

PRINCIPAL INVESTIGATOR: Ralph M. Richart, M.D.

FUNDING PERIOD: 9/1/83-8/31/84

AMOUNT FUNDED: \$49,668

Below is a project summary for the 1/1/84-6/30/84 period.

This study is an evaluation of chemical agents applied intravasally in 24 mongrel dogs to produce vasal closure. The compounds which were studied include BioNexus MCA, BioNexus MCA with .05% trifluoroacetic acid (TFA), BioNexus MCA with .025% TFA, and an iodide compound with gum tragacanth. All the agents were radio-opaque. The dogs were observed clinically with sperm counts and by histological examination of the treated segment of the vas as well as histological examination of the epididymus, testis, and portions of untreated vas. Animals were sacrificed on the average, 30, 50, and 60 days following application of the vas-closing agent. All the compounds studied were effective in producing vasal closure. At 30 days the vasa were filled with necrotic material with wide-spread epithelial necrosis and a low closure rate; by 50 days all the iodide compound-treated vasa were closed; and by 60 days 5 of the 7 BioNexus MCA-treated vasa were closed - the other two had extensive epithelial damage and would probably have closed subsequently. There were no clinical sequelae noted in the animals treated under this protocol, and there were no histological changes in the epididymus, testis, or untreated portions of the vas. It was concluded that the chemicals which were studied will produce vasal closure with the iodide mixture closing more rapidly, more completely, and with fewer local histological changes. It is probable that a percutaneous method can be developed to deliver these agents to the vas in humans and that it would be highly effective clinically.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-345

TITLE: "Effect of the Vaginal Spermicidal Barrier Contraceptive
on Sperm Transport in the Human"

INSTITUTION: Professional Staff Association, Los Angeles County,
University of Southern California Medical Center

PRINCIPAL INVESTIGATOR: Gerald S. Bernstein, M.D., Ph.D.

FUNDING PERIOD: 10/1/83-3/31/84

AMOUNT FUNDED: \$6,375

Below is a project summary for the 1/1/84-6/30/84 period.

Dr. Bernstein neglected to submit a report.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-346

TITLE: "Development of Controlled-Released Testosterone Microcapsules for Fertility Regulation of Males"

INSTITUTION: Southern Research Institute

PRINCIPAL INVESTIGATOR: Thomas R. Tice, Ph.D.

FUNDING PERIOD: 10/1/83-8/31/84

AMOUNT FUNDED: \$49,641

Below is a project summary for the 1/1/84-6/30/84 period.

The objective of this research program is to develop an injectable, biodegradable, controlled-release testosterone microcapsule formulation. With a single administration, the ideal formulation should deliver about 2 to 6 mg of drug per day to maintain a serum testosterone level of about 2 to 4 ng/mL for a duration of 90 days. This testosterone microcapsule formulation should be useful for the suppression of gonadotropins for male contraception or as a supplemental testosterone treatment used in conjunction with leuteinizing hormone-releasing hormone based male contraceptives.

Microcapsules containing 44 wt % testosterone and 56 wt % of 85:15 poly(DL-lactide-co-glycolide), prepared during the last quarter of 1983, were tested in castrated rhesus monkeys during the first quarter of 1984. The results of this pharmacokinetics study indicated that testosterone was released from the microcapsules for a duration of at least 90 days. The rate of release of testosterone from the microcapsules, however, was not as constant as desired, where the rate of testosterone released during the first 50 days of treatment was nearly three times the rate during the period of Day 50 to 90.

As a result, much of our work during the first two quarters of 1984 has been to even out the release of testosterone from the microcapsules. We also wanted to increase the core loading of the microcapsules to further minimize the quantity of microcapsules injected into a patient. To achieve these goals we studied various microencapsulation process variables (type and amount of solvent, amount of surfactant, evaporation time, and polymer composition). We also attempted to prepare microcapsules with higher core loadings, looked at the release kinetics of microcapsules of different sizes, and considered the aseptic preparation of microcapsules and lower doses of gamma radiation to achieve microcapsule sterility without compromising testosterone release kinetics. With the results of this work in hand, we now plan to prepare microcapsules for a second pharmacokinetics study in monkeys.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-347

TITLE: "Studies on the Anovulatory Potency and Side Effects of an Inhibitory Analog of LH-RH in Cynomologous Monkeys"

INSTITUTION: The University of Texas Health Science Center at San Antonio

PRINCIPAL INVESTIGATOR: Ricardo H. Asch, M.D.

FUNDING PERIOD: 11/1/83-10/31/85

AMOUNT FUNDED: \$145,501

Below is a project summary for the 1/1/84-6/30/84 period.

Due to an unexpected delay in receiving approval from the Laboratory Animal Resources Committee we just began the study in mid February, 1984.

Animals were ordered from local vendors and purchased, and according to the original schedule placed in quarantine.

Animals were treated so far with LH-RH antagonist at the dose of 100 and 1,000ug (Groups 1 and 4) per day for 60 consecutive days. Then, they were challenged with progesterone in oil (100mg intramuscularly).

Blood samples were drawn according to the original protocol and submitted for assays of FSH, LH, Estradiol and Progesterone by RIA.

As of yet none of the results are back from the laboratory in order to ascertain the effects of treatment in ovulation, luteal function and/or hormonal levels in blood.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

JUL 17 1984

PARFR- 348

TITLE: "Development of Improved Methods and Materials for Injecting Microencapsulated Steroids"

INSTITUTION: Stolle Research and Development Corporation

PRINCIPAL INVESTIGATOR: Danny H. Lewis, Ph.D.

FUNDING PERIOD: 11/1/83-6/30/84

AMOUNT FUNDED: \$49,555

Below is a project summary for the 1/1/84-6/30/84 period.

The objective of this project is the development of improved methods and materials for injecting contraceptive microspheres. Of particular interest is a system suitable for the administration of the 90-day norethisterone (NET) formulation currently under evaluation by PARFR. Results from Phase I clinical studies indicated that injection efficiencies were quite variable and were highly operator dependent. The standard injection vehicle has been sterile water containing 2% of carboxymethylcellulose and 1% of Tween 20. This vehicle was selected early in the program after preliminary screening of suitable materials.

In the current project, independent research programs aimed at this problem are being conducted at two university laboratories with recognized expertise in this technical field. Laboratory experimentation is being carried out on NET microspheres between 25 and 90 microns in diameter and loaded with either 25% or 50% of drug by weight. Numerous formulations with different combinations of various suspending agents and surface agents have been studied. Several formulations show promise of improvement over the current vehicle. The vehicles are being evaluated with respect to their ability to wet and disperse the microspheres, the degree or rate of sedimentation, and the amount of microspheres which remains as a residue in the syringe after expulsion. Discussions were held with two leading syringe manufacturers concerning the production of a modified design for a syringe to improve the injectability of the microspheres. An experimental syringe has been procured from one of the companies for this aspect of our research.

Lyophilization studies were conducted to determine the feasibility of that approach. Lyophilization studies are underway on microspheres dispersed only in water and

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reconstituted in vehicle, microspheres dispersed in an aqueous solution of wetting agent and reconstituted in a suspending vehicle, and microspheres dispersed in the total vehicle and reconstituted in water. It was found that lower concentrations of wetting and suspending agents can be used with this approach. There appear to be some advantages with this method and it is still under investigation.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 349

TITLE: "Preparation of Fibrous Estradiol/Progesterone IUDs for Phase I Clinical Trials, Continuation of PARFR-324"

INSTITUTION: Southern Research Institute

PRINCIPAL INVESTIGATOR: Richard L. Dunn, Ph.D.

FUNDING PERIOD: 11/1/83-4/30/84 AMOUNT FUNDED: \$49,994

Below is a project summary for the 1/1/84-6/30/84 period.

Most of our effort during this six-month period has been directed toward identifying an alternative sheath polymer for the coaxial fibers. As described in our First Technical Report (5428-I, SoRI-EAS-84-254) on Project 5428 (PARFR-349), Exxon Chemicals discontinued production of the LD-600 polyethylene (PE) that we had been using as the sheath material for both the progesterone- and estradiol-releasing fibers. Therefore, we contacted several manufacturers to identify other materials that would be suited to this application.

Five candidate materials were identified. These include three polyethylenes--LL 6101 (Exxon Chemicals, Houston, TX), NA 202 (US Industrial Chemicals [USI], Charlotte, NC), and Chemplex 1017X (Chemplex, Rolling Meadows, IL)--and two ethylene-vinyl acetate (EVA) copolymers--Ultrathene (UE) 633 and 635 (USI Chemicals). Progesterone- and estradiol-loaded coaxial fibers have been prepared with each sheath material except for the Chemplex 1017X, which is in progress. No significant problems were encountered in the fiber spinning.

In vitro release studies were then conducted to evaluate the release of the steroids from fibers having a sheath of either LL 6101, NA 202, or UE 633. Also, the in vitro release from fiber-wrapped IUDs was determined. These results are summarized in the following table.

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Steroid	Sheath polymer	Geometry	Overall diam, mm	$r_o:r_i$	Average release rate, $\mu\text{g}/\text{cm}/\text{day}$
P_4	LL 6101	Fiber	0.463	1.695	1.62
P_4	LL 6101	IUD	0.463	1.695	2.34
E_2^β	LL 6101	Fiber	0.409	1.414	0.015
E_2^β	LL 6101	IUD	0.409	1.414	0.057
P_4	NA 202	Fiber	0.476	1.729	2.90
P_4	NA 202	IUD	0.476	1.729	3.53
E_2^β	NA 202	Fiber	0.362	1.427	0.044
E_2^β	NA 202	IUD	0.362	1.427	0.060
P_4	UE 633	Fiber	0.518	1.790	58.65
P_4	UE 633	IUD	0.518	1.790	56.54
E_2^β	UE 633	Fiber	0.470	1.728	3.75
E_2^β	UE 633	IUD	0.470	1.728	3.58

These data show that the permeability of progesterone in the LL 6101 and NA 202 polyethylenes is higher than in LD 600. Consequently, the rate of release is slightly higher than the target values of 1.0-1.5 $\mu\text{g}/\text{cm}/\text{day}$. Also, the rate of release of progesterone from the fiber-wrapped IUDs is higher than for the individual fibers. This trend is consistent with previous results. A similar pattern is seen for the estradiol-loaded fibers having PE sheaths.

The data for fibers prepared with an EVA sheath (UE 633) show that the permeability of the steroids in the polymer is too high for this application. However, these results do illustrate that fibers prepared from an elastomeric sheath material exhibit the same release profile when wrapped around an IUD as for the individual fibers.

When we complete our evaluation of the Chemplex 1017X PE, we will select the best PE for use as the sheath for the fibers. We are hopeful that the Chemplex 1017X will perform as well as the other materials because a Drug Master File is on record with the Food and Drug Administration, which should reduce the effort required for obtaining approval for Phase I Clinical Trials. Also, USI has recently expressed some reluctance toward allowing the use of their materials for any IUD application.

The remainder of our effort during this period involved preliminary development of an analytical method for determining residual dichloromethane (DCM) in the steroid/polymer blends. We developed a gas-chromatographic procedure for detection of residual DCM. This method provides sensitivity down to about 5 to 10 ppm. We anticipate that only a few refinements in the procedure will be required to support the IND application.

SoRI-EAS-84-668
 Project 5428-II
 July 12, 1984
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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 350

TITLE: "An Intra Tubal Device (ITD) for Female Sterilization"

INSTITUTION: Tenon Hospital, University of Paris

PRINCIPAL INVESTIGATOR: Jacques Hamou, M.D. 2 Chaussée de La Muette, PARIS 75016
tel: 283.02.85.

FUNDING PERIOD: 1/1/84-6/30/85

AMOUNT FUNDED: \$11,000

Below is a project summary for the 1/1/84-6/30/84 period.

From december 9th 1983 and June 21st, 1984, 43 volunteers were selected for participation in our program for applied research in female sterilization. All the patients met the criterias submitted in the protocol.

An Intra Tubal Device (ITD) made of radio opaque flexible nylon was used. Bilateral insertion during the same Microhysteroscopic procedure was performed through a 1,7 mm soft catheter.

All the procedures were performed in an office setting without any premedication or anesthesia except for 1 patient who was elected for general anesthesia and 2 others had local anesthesia because of pelvic pain.

Both devices were inserted during the first attempt in one case with an irreductible cervical atresia, and one with an obscured right ostium. 2 patients not included in the 43 patients did not have the procedure started because of instrumentation difficulty (insufflator).

Pelvic pain was reported in 2 cases during the procedure and one lasted few hours.

Early follow up performed by a repeat microhysteroscopy a month later shows 2 unilateral refection of an ITD but still present in the uterine cavity, and in one case both ITD were expelled during next retarded menstruation. 2 congestive endometritis were noted.

No major complications occurred, especially not pelvic inflammation or heavy abnormal bleeding occurred.

One patient complaints of intermittent plevic pain for one menstrual cycle. One patient with past history of peritonitis had the ITD removed one month later following plevic pain, antibiotics were given but laparoscopy did not show evidence of infection.

No pregnancy is reported

All patients who have past history of bleeding or infection with IUD did not have any complications.

Experience reveals that minor changes should be made to the ITD (softness, distal end) and to the technique of insufflation.

This first study assess the inocuity and the non invasive aspect of the method, longer term follow up will be necessary to assess the exact reliability and reversibility of the procedure.

Spencer

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-351

TITLE: "Development of Methods for Female and Male Contraception Based on LH-RH Antagonist"

INSTITUTION: The Tulane University School of Medicine

PRINCIPAL INVESTIGATOR: Andrew V. Schally, Ph.D.

FUNDING PERIOD: 1/1/84-12/31/84

AMOUNT FUNDED: \$65,153

Below is a project summary for the 1/1/84-6/30/84 period.

Objectives:

To investigate the contraceptive effects of antagonistic analog (N-Ac-D-p-Cl-Phe^{1,2}, D-Trp³, D-Arg⁶, D-Ala¹⁰)-LH-RH in male and female rats. Fertility of the animals, genetic abnormalities in the offspring and effects on blood pressure in rats are also being tested.

Male Rats

120 male rats were divided according to the following groups:

48 rats were injected with the vehicle solution (0.5% gelatin plus 5% mannitol) at a volume of 0.2 ml/rat sc.

12 rats were injected with ORG 30276 at a daily dosage of 10 µg/kg sc.

12 rats were injected with ORG 30276 at a daily dosage of 100 µg/kg sc.

48 rats were injected with ORG 30276 at a daily dosage of 1000 µg/kg sc.

(A) The treatment lasted 60 days. The day after the last injection, 12 animals per each group were killed by decapitation, and the weights of the anterior pituitary glands, testes, seminal vesicles and ventral prostates were recorded. Blood was collected, sera were separated and kept frozen until assayed. Anterior pituitaries and hypothalami were also kept frozen until assayed.

The treatment with the highest dose of the antagonist brought about a significant decrease in the weights of the anterior pituitaries, testes, seminal vesicles and ventral prostates. The intermediate dose of the antagonist only affected seminal vesicle weights.

The intermediate and highest dose of the analog induced a significant decrease of serum prolactin levels, and only the highest dose significantly decreased serum and pituitary LH concentrations. The histology of the testes from rats treated with the highest dose showed spermatogenesis markedly depressed, not beyond the stage of spermatocytes I; the interstitium showed cell with fibroblastic appearance. The testes of the rats injected with the lowest and the intermediate dose of the analog were similar to the control rats.

(B) Twenty days after stopping treatment with the analog, 10 control and 10 rats treated with the highest dose of the analog were killed by decapitation and the study proceeded as described previously. The rats injected with the antagonist showed a marked recovery of the weight of the testes, seminal vesicles and ventral prostates, which were still, however, lower than in the control rats.

(C) 60 days after stopping the treatment with the analog, 12 additional rats from control and from the rats treated with the highest dose of the antagonist

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were killed by decapitation. We proceeded as described above.

These rats showed anterior pituitary, testes, seminal vesicle and ventral prostate weights very similar to those of control animals, evidencing a practically complete recovery of these organs after the withdrawal of the treatment with the analog.

Female Rats

160 female rats (Charles River Breeding Labs), weighing 160-180 g, have been injected either with the vehicle or the antagonist. Four groups of 40 rats each were injected as follows:

Control: injected with the gelatin-mannitol vehicle.

1. injected with ORG 30276 at a daily dosage of 10 $\mu\text{g}/\text{kg}$ sc.
2. injected with ORG 30276 at a daily dosage of 100 $\mu\text{g}/\text{kg}$ sc.
3. injected with ORG 30276 at a daily dosage of 1000 $\mu\text{g}/\text{kg}$ sc.

The rats were injected every day, for 14 days.

Vaginal smears were checked daily. At the end of the treatment, 10 rats of each group were killed by decapitation. Body weights were recorded. Vaginal smears were checked on the day of sacrifice. Anterior pituitaries, ovaries, and uteri were dissected free and weighed. One half of each pituitary gland was frozen, to be assayed for LH, FSH and prolactin, the other half was fixed in Bouin's fluid to be studied histologically. Hypothalami were also removed, some were frozen for LH-RH assays and some others were fixed for histological studies. Ovaries were immersed in Bouin's fluid, for histological studies. Blood was collected from the trunk, sera were separated and kept frozen until assayed for LH, FSH, prolactin, estradiol and progesterone.

The treatment with ORG 30276 did not modify body weight. Anterior pituitary weight was significantly decreased in the group of rats treated with the highest dose of the antagonist. Ovarian weight decreased significantly, showing a dose-response relationship. Uterine weights were significantly reduced in the rats treated with the highest dose of ORG 30276, but were found to be higher in the rats treated with the medium dose.

Conclusions

In male as well as in female rats the highest dose of the antagonist was the most effective in suppressing gonadal function. The long term treatment in male rats brought about a marked depression of spermatogenesis. It is also evident that the gonadal function recovers completely after a period of time without any further treatment. This is evidence that the gonadal suppressive effects of the antagonistic analog of LHRH are completely reversible after the withdrawal of its administration.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

JUL 17 1984

For the Period January 1, 1984 - June 30, 1984

PARFR- 352

TITLE: "Baboon Testing of Duration of NET from Fused Pellets"

INSTITUTION: The Board of Trustees of the University of Alabama for
the University of Alabama in Birmingham

PRINCIPAL INVESTIGATOR: Lee R. Beck, Ph.D.

FUNDING PERIOD: 12/1/83-11/30/84

AMOUNT FUNDED: \$29,420

Below is a project summary for the 1/1/84-6/30/84 period.

Five baboons were implanted by Dr. Brij Saxena with cholesterol fused norethindrone (NET) pellets to determine the rate and duration of steroid release in vivo. Two to four pellets per baboon were implanted subcutaneously lateral to the spine, and blood samples are being obtained weekly for determination of serum NET by radioimmunoassay. Additional samples will be acquired if necessary should cyclic ovarian activity be suspected due to changes in sex skin turgescence. One baboon is being maintained on depo-estradiol (5 mg/3 weeks) to alleviate dysmenorrhea and concomitant anorexia experienced in the early posttreatment interval. The baboons are currently 170 days posttreatment, with no evidence of cyclicity in the remaining four baboons. NET levels ranged from 0.3 to 1.1 ng/ml in the baboon treated with 2 pellets, from 0.4 to 1.59 ng/ml in 2 baboons treated with 3 pellets, and from 0.63 to 2.66 ng/ml in the 2 baboons treated with 4 pellets.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 353

TITLE: "Effect of Chronic Intrauterine Release of Estradiol and Progesterone on Uterine Histology in Intact Rabbits"

INSTITUTION: Michael Reese Hospital and Medical Center

PRINCIPAL INVESTIGATOR: Antonio Scommegna, M.D.

FUNDING PERIOD: 3/15/84-7/31/84

AMOUNT FUNDED: \$7,895

Below is a project summary for the 1/1/84-6/30/84 period.

Since the start of this project three objectives were accomplished.

1. Twenty female New Zealand white rabbits were obtained at an average weight of 7 lbs.
2. Control fiber bundles, estradiol loaded bundles, and progesterone loaded bundles were obtained from Southern Research Institute, PARFR purchase order #37001953.
3. All 20 rabbits had laparotomy under general anesthesia and uterine horn was incised longitudinally in two areas, one close to the tubal end and the second about 0.5 inches from the cervix. IUD's were inserted as per study design with no modification. The rabbits recovered well from the operation.

One of the rabbits died on 5/1/84, 3 weeks after surgery due to bowel obstruction with a hair ball.

Our objectives in the study did not change from those stated in the research grant proposal.

FUTURE PLAN

Rabbits termination 3 months after fiber bundle insertion, and histologic determination of the endometrial changes.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR-354

TITLE: "Screening of Thai Plants for Proteins (or Lectins) as Potential Vaginal Contraceptives"

INSTITUTION: Faculty of Science, Mahidol University

PRINCIPAL INVESTIGATOR: Montri Chulavatnatol, Ph.D.

FUNDING PERIOD: 4/1/84-3/31/85 AMOUNT FUNDED: \$9,800

Below is a project summary for the 1/1/84-6/30/84 period.

This project commenced on April 1, 1984 and this summary is for the period of 3 months (4/1/84 to 6/30/84).

The objective of this project is to search for new proteins or lectins from Thai plant extracts that can agglutinate human sperm and/or inhibit human sperm motility.

Several volunteers were recruited at the beginning of the project to deliver semen samples to the laboratory within 2 hours after collection. The samples were screened under a phase-contrast microscope and selected for use only those with high sperm count ($\leq 50 \times 10^6/\text{ml}$), good motility ($\leq 50\%$) and free from bacterial contamination. Various Thai fruits were purchased and seeds were collected by helpers in the laboratory. Fresh seeds were normally used to make extracts. However, some unused seeds were sun-dried and kept in sealed plastic bags in a freezer at -20°C for future test. Extraction of 25 gm of seed was made in 75 ml of ice-cold phosphate-buffered saline (PBS) using a tissue homogenizer. After removing insoluble material by centrifugation at 10,000 g for 30 min at 4°C , the supernatant fluid was made 80% saturation with ammonium sulfate by adding the solid salt. Precipitated protein was collected by centrifugation at 10,000 g for 30 min at 4°C re-dissolved in PBS and dialyzed against a large volume of PBS overnight at 4°C to remove ammonium sulfate and other small molecules. The dialyzed fraction was then used to test for human sperm agglutination and/or sperm motility inhibition as outlined in the proposal.

Results of the pre-liminary study can be summarized as follows.

1. Among extracts from different seeds, protein concentration varied widely from 0.01 to 1.0 mg/ml. Concentrating the extract by lyophilization was often necessary.
2. Washed human sperm seemed to be better agglutinated than unwashed whole semen.

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3. Among the extracts tested sofar, those having sperm-agglutinating activities (Table 1) also inhibited sperm motility. So, sperm-agglutination test was generally preferred in the initial screening of the extracts.

4. Table 1 lists the seed extracts giving partial to total agglutination of human sperm, washed or unwashed (whole semen). The active agents in some extracts were heat-labile, suggestive of protein nature. Several extracts which showed no sperm-agglutination effect (and no sperm motility inhibition) are not listed but will be tested again. For an extract to be of use as a future vaginal contraceptive, it should be effective to the whole semen. From Table 1, agents from Jack fruit and red kidney bean were most promising at this stage.

Table 1 Agglutination of human sperm by seed extracts at 0.5 mg protein/ml. (n=3) The agglutination was rated from zero (no effect) to 4 (total agglutination).

<u>Seed extract</u>	<u>Whole semen</u>	<u>Washed sperm</u>
Jack fruit (<i>Artocarpus heterophyllus</i>)	4 ± 0*	4 ± 0*
Red kidney bean (<i>Phaseolus vulgaris</i>)	4 ± 0	4 ± 0
Broad bean (<i>Vicia faba</i>)	3.3 ± 0.6*	4 ± 0*
Garden pea (<i>Phaseolus vulgaris</i>)	3.0 ± 1.0*	4 ± 0*
Sataw (<i>Parkia speciosa</i>)	1.0 ± 0.6	2.6 ± 1.3*
Yard-long bean (<i>Vigna unguiculata</i>)	0.6 ± 0.3	1.6 ± 0.8*
Green gram or mungbean (<i>Vigna radiata</i>)	1.6 ± 0.8	3.0 ± 1.0
Soy bean (<i>Glycine max</i>)	0	0.6 ± 0.3*
Pea nut (<i>Arachis hypogaea</i>)	0.3 ± 0.3	2.3 ± 1.2*

After heating the extract at 80°C for 15 min, sperm-agglutinating activity was lost from the extract marked with an asterisk indicating heat-labile active agent.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PROJECT NUMBER: PARFR-355
TITLE: Enhancement of the Secretary Immune Response to LDH-C₄
INSTITUTION: Medical Research Foundation of Oregon
PRINCIPAL INVESTIGATOR: Nancy J. Alexander, Ph.D.
FUNDING PERIOD: 4/15/84 - 4/14/85
AMOUNT FUNDED: \$47,335

PROJECT SUMMARY:

Sixteen cycling female rhesus of known fertility were assigned to the BALT/GALT study. Each of the sixteen animals received a primary immunization of 2.0 mg of LDH-C₄ plus Bordetella pertussis, administered subcutaneously at several sites. Eight of the monkeys were given a secondary immunization by the BALT route, which consisted of administering 5.0 mg of LDH-C₄ to the lungs via a nebulizer, a procedure done with the monkey under general anesthesia. The other eight monkeys were given a secondary immunization via the GALT route. For these immunizations, each animal was made to swallow a capsule enterically coated with hydroxypropyl methylcellulose phthalate containing 5.0 mg of LDH-C₄. The capsules were prepared shortly before administration to the animal. Three or four days after the secondary immunization every animal received an agarose vaginal plug containing 2.5 mg of the antigen plus concanavalin A. The stitches holding the vaginal plug in place were removed several days later, at which time the animal was skin tested for IgE antibodies by giving intradermal injections of the antigen, as well as B. pertussis and saline as a control. The skin test results were read at 4, 24, and 48 hours. On the ninth or tenth day after the secondary immunization, four animals (2 BALT and 2 GALT) were laparotomized in order to collect oviductal fluid. At the time of the primary and secondary immunizations of the vaginal implant, of the skin testing, of the laparotomy (if done), and at days 14, 21, and 42 after the secondary immunization, the following samples were collected and frozen: serum, oral lavage with saline, and vaginal lavage with saline. Table 1 lists the dates on which these samples were actually collected from each of the animals. The dates vary because we tried to schedule the immunizations such that samples collected shortly after the secondary immunization would fall midcycle when the secretions would most likely contain immunoglobulin. Two shipments of the frozen samples have so far been sent to Dr. Goldberg for evaluation of levels of antibodies to LDH-C₄.

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Table 1. BALT/GALT Study Sampling Dates

	1°	2°	Vaginal implant Day 3/4	Remove vaginal stitches & skin test Day 6/7/8	Day 9/10 ^a	Day 14	Day 21	Day 42
<u>BALT</u>								
7251	4-16	4-23	4-27	5-1		5-7	5-14	6-4
7698	4-13	4-20	4-23	4-26		5-14	5-11	6-1
7736	5-22	5-29	6-1	6-4	6-7	6-12	6-19	[7-10] ^b
8016	5-22	5-29	6-1	6-4	6-7	6-12	6-19	[7-10] ^b
8029	4-13	4-20	4-23	4-26		5-14	5-11	6-1
8983	4-16	4-23	4-27	5-1		5-7	5-14	6-4
9140	4-20	4-27	4-30	5-3		5-11	5-18	6-8
9296	5-22	5-29	6-1	6-4		6-12	6-19	[7-10] ^b
<u>GALT</u>								
6813	4-13	4-20	4-23	4-26		5-14	5-11	6-1
7220	4-27	5-4	5-7	5-10	5-14	5-18	5-25	6-15
7820	4-13	4-20	4-23	4-26		5-14	5-11	6-1
7954	5-4	5-11	5-14	5-17		5-25	6-1	6-22
8022	5-1	5-8	5-11	5-14	5-18	5-22	5-29	6-19
9030	4-20	4-27	4-30	5-3		5-11	5-18	6-8
9129	4-13	4-20	4-23	4-26		5-14	5-11	6-1
9141	4-13	4-20	4-23	4-26		5-14	5-11	6-1

^aLaparotomy date to sample oviductal fluid.

^bDates in brackets not yet done.

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PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

For the Period January 1, 1984 - June 30, 1984

PARFR- 356

TITLE: "Development of an Immunocontraceptive Vaccine: Role of 23-Kd Antigen in Immunoinfertility and Fertility Regulation"

INSTITUTION: Medical Research Foundation of Oregon

PRINCIPAL INVESTIGATOR: Rajesh K. Naz, Ph.D.

FUNDING PERIOD: 5/1/84-4/30/85

AMOUNT FUNDED: \$66,686

Below is a project summary for the 1/1/84-6/30/84 period.

The general objective of this project is to investigate the use of the 23-Kd glycoprotein (FA-1), isolated by using monoclonal antisperm antibody MA-24, as an effective immunocontraceptive. MA-24 developed against a human sperm membrane glycoprotein belongs to the IgG_{2a} subclass and is germ-cell specific. It cross-reacts with mouse, rabbit, rhesus, and human sperm. It blocks human sperm penetration of zona-free hamster ova and also inhibits in vitro fertilization of mouse ova by murine sperm. The inhibition is by mechanism(s) other than agglutination and immobilization of sperm. This monoclonal antibody is against a membrane glycoprotein of 23 Kd which is localized on the postacrosome, midpiece, and tail of sperm. FA-1 has been isolated from human sperm and testis by using an MA-24-IgG-Sepharose-4B immunoaffinity column and shows a single band on SDS-polyacrylamide gel electrophoresis (PAGE). This purified testicular glycoprotein shows a significant reaction with sera from immunologically infertile couples in an enzyme-linked immunosorbent assay (ELISA) (Science, in press). We have now isolated this antigen from mouse testes using the same immunoaffinity column. The antigen isolated from murine germ cells again shows a single band on SDS-PAGE even with an ultrasensitive silver stain and occurs mainly as a dimer of 46-Kd molecular mass. The possibility for isolation of the antigen from bovine testes which will be a less expensive source is being investigated. Presently we are engaged in producing large amounts of ascites to construct more immunoaffinity columns for isolation of FA-1 in sufficient quantity required for active immunization studies. Before starting active immunization studies, we are checking the effect of MA-24 on the fertilization and fertility in vivo. Recently we artificially inseminated female rabbits with sperm treated with IgG purified from MA-24 ascites. Preliminary results show an inhibition of fertility at 9 days post insemination as expressed by percentage of implants vs corpora lutea ratio. These experiments show that these antibodies are effective in reducing fertility in in vivo conditions. These data will also indicate the amounts of antibody required in local secretions for inhibition vs complete block of fertilization and fertility.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

JUL 17 1984

For the Period January 1, 1984 - June 30, 1984

PARFR- 357

TITLE: "Optimization of Release Profile of Norethisterone
Injectable 90-Day Contraceptive"

INSTITUTION: Stolle Research and Development Corporation

PRINCIPAL INVESTIGATOR: Danny H. Lewis, Ph.D.

FUNDING PERIOD: 5/1/84-10/31/84

AMOUNT FUNDED: \$49,119

Below is a project summary for the 1/1/84-6/30/84 period.

In a recent Phase II clinical trial with the 90-day NET microsphere formulations serum NET did not reach efficacious levels until several weeks posttreatment in that study. This was primarily due to an improvement in the microsphere quality after we switched to an improved microencapsulation process. We have now demonstrated that faster releasing formulations can be achieved by increasing the NET content of the microspheres. An obvious advantage also gained is the reduced total mass of material to be administered to the patient. In vitro release profiles and preliminary data from baboon evaluations indicate that we have made substantial progress in optimizing the NET release profile. During this reporting period, we found that microspheres loaded with 40 to 50% by weight of NET appear to be the most promising. We observed that when we attempted to load higher amounts (65%) into the polymeric excipient, the quality of the formulations was compromised.

Photomicrographs taken by scanning electron microscopy (SEM) show no or very little evidence of unencapsulated NET at loadings of 40 to 50%. The samples look very much like the 25%-loaded formulations tested in the Phase II trial. In vitro data clearly show that we have significantly increased the rate of NET release. We now have a complete data base of in vitro-in vivo correlations to guide our selections; therefore, we have a high level of confidence that the higher loaded microspheres will be useful as a 90-day injectable. Baboon data are now being generated on three of the higher loaded samples. The critical question yet to be answered is how long will the higher-loaded formulations release NET in the baboon.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTHS TECHNICAL REPORT SUMMARY

JUL 17 1984

For the Period January 1, 1984 - June 30, 1984

PARFR-358

TITLE: "Development of a 30-Day Injectable Contraceptive"

INSTITUTION: Stolle Research and Development Corporation

PRINCIPAL INVESTIGATOR: Danny H. Lewis, Ph.D.

FUNDING PERIOD: 5/1/84-1/31/85 AMOUNT FUNDED: \$11,800

Below is a project summary for the 1/1/84-6/30/84 period.

During this reporting period, we have conducted several microencapsulation runs with NET and lactide:glycolide copolymers of different comonomer ratios. We have determined that NET can be successfully loaded at levels up to 50% in each of the polymeric excipients by means of the patented Stolle microencapsulation procedure. We have successfully produced microspheres ranging from less than 25 to 90 microns in diameter from 50:50, 70:30, and 85:15 lactide:glycolide copolymers. The microsphere formulations are presently being characterized in vitro as to their release profiles. Samples prepared from the 50:50 and 70:30 materials have recently been loaded into syringes and are currently being sterilized by gamma irradiation. We anticipate that baboon evaluations on those unique formulations will begin in July. We are presently evaluating in baboons a sample prepared from the 85:15 copolymer. This sample contains 40% by weight of NET and the microspheres are 25 to 45 micron in diameter. It is probable that this specific formulation will release NET over a period much greater than 30 days. The faster degrading formulations should be more ideal for a one-month injectable.

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JUL 24 1984

Program for Applied Research on Fertility Regulation

6 Month Technical Report
January 1, 1984-June 30, 1984

Grant Title: "Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins."

Institution: Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030

Principal Investigator: Bonnie S. Dunbar, Ph.D.

Funding Amount Awarded: June 1, 1984 to May 31, 1985: \$77,481

Project Summary:

The objectives of the proposed studies are to:

1. Determine whether primates develop antibodies against porcine zona pellucida (ZP) antigens which cross-react with their own zona pellucida.
2. To characterize these antigens using established radioimmunoassay, enzyme-linked immunoassay and 2D-PAGE western blotting methods. To determine whether these antibodies interfere with ovarian function as well as fertility.

Progress Made for June 1 to June 30, 1984.

During the first month of this progress we have isolated 30 mg of porcine zona pellucida protein. 12 mg of porcine ZP protein I have been purified using electroelution from 2D-PAGE gels. These samples have been lyophilized and are ready for delivery to Dr. Shoba Segal in India.

A letter, along with the final proposal and first year time table was sent via Dr. Gabriel Bialy's office to Dr. Segal during the first part of May. As of July 22, we have received no answer. We are holding the ZP protein until we make contact.

PARFR SCIENTIFIC ADVISORY COMMITTEE

MEETING XXXXIV

Wednesday, April 11, 1984

O'HARE HILTON
Rosemont, Illinois
(312) 686-8000

MINUTES

VOTING SAC MEMBERS PRESENT

John J. Sciarra, M.D., Ph.D.
Andrzej Bartke, Ph.D.
David A. Blake, Ph.D.
William Droegemueller, M.D.
Uwe Goebelsmann, M.D.
Miriam H. Lobbok, M.D., M.P.H.
Dean L. Moyer, M.D.
Antonio Scommegna, M.D.
Rochelle H. Shain, Ph.D.

VOTING SAC MEMBER ABSENT

Kamran S. Moghissi, M.D.

PARFR STAFF PRESENT

Alfredo Goldsmith, M.D., M.P.H.
Diane Krier-Morrow, M.B.A.
Gerald I. Zatuchni, M.D., M.Sc.

USAID STAFF PRESENT

Jeffrey M. Spieler, M.Sc.

I. ANNOUNCEMENTS

- A. Next SAC Meeting is Monday, July 30, 1984 at the Hyatt Arlington in Arlington (Roslyn), Virginia (National Airport).
- B. PARFR's International Workshop on Intrauterine Contraception: Advances and Future Prospects will be held in Chicago, Illinois, May 29 - June 1, 1984.
- C. Dr. Sciarra informed SAC that the following were invited by the organizing committee, at not cost to PARFR, to participate in the V European Seminar on Fertility Control in Genoa, Italy: Drs. Ricardo Asch, A. Goldsmith, K. Moghissi, J. Sciarra and A. Scommegna.
- D. PARFR assisted with support and in the organization of the Latin American Association of Researchers in Human Reproduction (ALHR) regional workshop held in the Dominican Republic. One-hundred twenty-six participants attended, 2 of which were from Puerto Rico and 18 were 3rd year residents. Drs. Asch, Croxatto and Goldsmith participated.
- E. PARFR will organize the symposium on "Recent Advances in Contraception" to be held in Caracas on October 21-26, 1984 in conjunction with the FIGO meeting. Drs. Asch, Goldsmith, Moghissi, Scommegna and Zatuchni will participate. An audience of over 200 is expected.

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I. ANNOUNCEMENTS (continued)

- F. With PARFR support, Drs. N. Alexander, D. Anderson, I. Goldberg, M. Harper and G. Zatuchni will attend the April 16-18, 1984 NIH/PARFR Workshop on "Research and Development of Immunological Methods of Fertility Regulation" in Bethesda, Maryland.
- G. PARFR will support a one day workshop on Male Immunocontraception to be held in conjunction with the International Andrology Meeting to take place in Boston in April, 1985.
- H. The topic of "Male Fertility Control" was chosen for the PARFR Spring, 1985 Workshop, which is expected to be held in Mexico City.

II. NEW BUSINESS

A. FORMAL PROPOSALS

John C.M. Tsibris, Ph.D., University of Illinois, Chicago
"Inter- and Intra-Cycle Variation of Genital Peroxidases"
Funding Requested: \$77,013 Length of Project: Eight Months

The revised proposal was not approved as presented. The Committee requested PARFR staff to work with the principal investigator in order to prepare a simple proposal in which guaiacol peroxidase levels, obtained from cervical mucus only will be correlated to indirect ovulation indicators such as LH and pregnanediol in urine. Budget should not exceed \$30,000.

Tapani Luukkainen, M.D., Ph.D., University of Helsinki, Finland
"Tailless IUD: The IUD and Cervical Bacteriology"
Funding Requested: \$48,650 Length of Project: Three Years

The Committee felt that this proposal should not be approved because the objectives that the PI intended to study cannot be solved with bacteriological studies of the cervix and vagina. The number of patients proposed was too low to assess any relationship between an IUD tail and PID.

Lourens J.D. Zaneveld, D.V.M., Ph.D., Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois
"Preparation of Acrosin Inhibitors (Arylguanidinobenzoates) For Phase I Clinical Trials"
Funding Requested: \$204,467 (Excluding indirect costs at the University of Illinois)
Length of Project: Two Years

The 1/31/84 and 2/14/84 site visit reports were reviewed and SAC members concur with PARFR staff that all technical recommendations are included in the revised proposal. Therefore, the proposal was approved as written.

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A. FORMAL PROPOSALS (continued)

Ricardo H. Asch, M.D., The University of Texas Health Science Center
at San Antonio

"The Effects of Testosterone Replacement in Castrated Rhesus
Monkeys Using a Controlled-Release Microcapsule System"

Funding Requested: \$78,942 Length of Project: One Year

PARFR-346 -- Thomas R. Tice, Ph.D., Southern Research Institute,
Birmingham, Alabama (Technical Report)

"Development of Controlled-Release Testosterone Microcapsules
for Fertility Regulation of Males"

The Committee reviewed the proposal, and the independent reviews and PARFR staff recommendation. The Committee approved PARFR's staff recommendations and suggested that, in addition, metabolic clearance rate of testosterone and blood conversion of testosterone to DHT should be conducted.

Duane L. Venton, Ph.D., University of Illinois, Chicago and
Robert T. Chatterton, Ph.D., Northwestern University Medical School

"Inhibition of Ovulation by Specific Antagonists of Prostaglandin F_{2a}"

Funding Requested: \$29,400 -- University of Illinois (Excluding indirect cost)
\$41,887 -- Northwestern University Medical School

Length of Project: Two Years

The proposal was not approved as presented. Dr. Venton will be requested to submit acute toxicology data on both compounds and other detailed information.

Bonnie S. Dunbar, Ph.D., Baylor College of Medicine, Houston, Texas

"Active Immunization of Non-human Primates and Rabbits with Zona
Pellucida Proteins"

Funding Requested: \$163,117 Length of Project: Three Years

The Committee was briefed on the Indo-USA Scientific Agreement, the investment of large amounts of funds to immunocontraception by the government of India and the potential role of PARFR/NIH/AID in this area. As a result of this agreement, one of the 3 proposals reviewed and approved by NIH was sent to PARFR for funding. PARFR-SAC approved the proposal with some modification that will be implemented by PARFR staff.

George C. Denniston, M.D., Population Dynamics, Seattle, Washington

"A Method for Pre-Testing Percutaneous Vasectomy Electrodes"

Funding Requested: \$20,700 Length of Project: One Year

Previous PARFR activities in this area were reviewed and the proposed clinical project was approved (in principle) pending preparation of a full, comprehensive clinical protocol.

B. EXTENSION PROPOSALS

PARFR-344 -- Ralph M. Richart, M.D., Presbyterian Hospital, Obstetrical and Gynecological Services, New York, New York

"A Rapid and Effective Percutaneous Intra Vas Injection for Male Sterilization"

Funding Requested: \$59,329

Length of Project: One Year

The Committee reviewed the technical report of PARFR-344 and recommended not to approve the extension request because this technique necessitates the introduction of the sclerosing material into the vas lumen and the extension request deals mainly with equipment development. The budget is almost entirely allocated to salary of the Chinese investigator who is now in Columbia University.

PARFR-309 -- Robert T. Chatterton, Ph.D., Northwestern University Medical School, Chicago, Illinois

"Ovulation Inhibition by Anordrin"

Funding Requested: \$38,888

Length of Project: One Year

PARFR staff presented the status of the PARFR/WHO negotiations in relation to the upcoming Phase I clinical trials. PARFR staff reminded the Committee that all necessary FDA studies will be conducted once AID approves them. The Committee approved part of the extension proposal and recommended that the transcutaneous route of delivery should not be conducted at this time and the study on the effects on the endometrium should be done using the Beck/Wilborn design.

PARFR-339 -- Lourens J.D. Zaneveld, D.V.M., Ph.D., Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois

"Toxicity Studies Required by FDA for the Development of the Shug"

Funding Requested: \$27,375 -- Rush-Presbyterian-St. Luke's Medical Center

\$48,504 -- University of Illinois (excluding indirect cost)

Length of Project: One Year

PARFR staff presented to the Committee the recent FDA requirements to grant an IDE for the Shug device. The Committee approved the proposal and requested that Dr. Zaneveld should clarify the following: (1) size of the silicon rods to be used in rats; (2) how the rod will be retained in its original position; (3) how food intake will be measured; and (4) how will the behavioral changes be described.

C. TECHNICAL REPORT REVIEW

The following technical reports were reviewed:

1. PHASE II POLY NET 90 STUDY

- a. Dr. Goldsmith presented the results of the March 20, 1984 meeting in Washington, D.C.
- b. PARFR-348 -- Danny H. Lewis, Ph.D., Stolle Research and Development Corporation, Birmingham, Alabama (Technical Report)
"Development of Improved Methods and Materials for Injecting Microencapsulated Steroids"

The enrollment of patients in the Phase II clinical study was stopped due to lower release rates of NET than the rates found in the Phase I trials. The entire program was reviewed in a joint meeting attended by representatives of AID, PARFR, Ortho, UAB, Stolle and FHI. The problem was identified as due to the lack of outside contamination with NET which is due to the improved, large batch two-step procedure.

New formulations will be prepared, tested in vitro and in the baboon model and once a formulation is acceptable, a Phase I study will be conducted in Alabama. Once the Phase I study is underway and satisfactory NET rates are obtained, the Phase II multi-center study will be reinitiated. Stolle will submit a proposal for the new formulation and baboon studies.

2. PHASE I POLY NET 180 STUDY

- a. PARFR-333 (FINAL) -- Charles E. Flowers, Jr., M.D. and Lee P. Beck, Ph.D., The University of Alabama in Birmingham
"Poly-gly NET 180 Microcapsule System"
- b. PARFR-333T (FINAL) -- Jose P. Balmaceda, M.D., The University of Texas Health Science Center at San Antonio
"Poly-gly NET 180 Microcapsule System"

The final technical report was reviewed and NET release rates provide a satisfactory level for contraception up to around day 150. Phase II clinical trials will not be initiated until Stolle modifies the formulation to allow contraceptive protection for up to 185 days.

3. PHASE I NET FUSED PELLETT (2 PELLETT) STUDY

- a. PARFR-312 (FINAL) -- Brij B. Saxena, Ph.D., D.Sc., Cornell University Medical College, New York, New York
"A Clinical Evaluation of the Subdermal Contraceptive Norethindrone Pellet - Continuation of PARFR-229"

The Phase I final technical report was reviewed and publication will be prepared by PARFR staff.

C. TECHNICAL REPORT REVIEW (continued)

4. PHASE II NET FUSED PELLETT STUDY

- a. PARFR-330T -- Ricardo H. Asch, M.D., The University of Texas Health Science Center at San Antonio
"A Clinical Evaluation of the Bio-absorbable Subdermal Contraceptive Norethindrone Pellet Implant (Phase II)"
- b. David A. Edelman, Ph.D. -- Site Visit Report
- c. Family Health International Computer Report
- d. PARFR-352 -- Lee R. Beck, Ph.D., The University of Alabama in Birmingham
"Baboon Testing of Duration of NET from Fused Pellets"

The ongoing Phase II clinical trial was reviewed. No further funding is anticipated until the problems related to ownership of the patent between Endocor, Population Council and Cornell are solved.

5. PARFR-332 -- Danny H. Lewis, Ph.D., Stolle Research and Development Corporation, Birmingham, Alabama
"Development of an Injectable Long-Acting Estradiol Formulation"

The baboon release rates were much higher than anticipated. Dr. Lewis will work with Dr. Flowers in developing an IMD for Phase I clinical trials once appropriate release rates are obtained.

6. PARFR-335 -- Danny H. Lewis, Ph.D., Stolle Research and Development Corporation, Birmingham, Alabama

- a. Six-Month Levonorgestrel Microcapsules (FINAL)

Appropriate release rates were obtained for a six month duration; however, no further spending is expected until PARFR completes the evaluation of the NIH/Biotech levonorgestrel system.

- b. One-Month NET Microcapsule Preparation (FINAL)

Release rates in baboons were of larger duration than expected, + 65 days. Stolle will prepare a new formulation at no cost to PARFR which will be tested in the baboon model.

C. TECHNICAL REPORT REVIEW (continued)

7. PARFR-343 -- Lee R. Beck, Ph.D., The University of Alabama in Birmingham
"NIH/Biotech Levonorgestrel Microcapsules"

SEE 6a.

8. PARFR-336 (FINAL) -- Walter H. Wilborn, Ph.D., University of South Alabama, Mobile
"Effects of New Synthetic Progestins on Baboon Endometrium"

A final technical report was reviewed. No conclusion can be made until the slides will be read by an independent pathologist chosen by NIH.

9. Methylcyanoacrylate/FEMCEPT Studies for Non-Surgical Female Sterilization -- Family Health International Computer Report

Data of the ongoing study was reviewed. No further spending is anticipated until BioNexus shares the toxicology and carcinogenic data with PARFR and identifies the "final" radio-opaque MCA formulation.

10. PARFR-345 -- Gerald S. Bernstein, M.D., Ph.D., University of Southern California, Los Angeles
"Effects of the Vaginal Spermicidal Barrier Contraceptive on Sperm Transport in the Human"

The data was reviewed and recommendations to terminate the study was made due to poor results in the post-coital tests.

11. PARFR-323 (FINAL) -- Lourens J.D. Zaneveld, D.V.M, Ph.D., University of Illinois at the Medical Center, Chicago
"Efficacy of Quinacrine and Tetracycline in the Primate"

Data was reviewed and, as expected, neither the pig nor the monkey were good animal models for quinacrine and tetracycline pellets. No further funding is expected in this area.

12. PARFR-349 -- Richard L. Dunn, Ph.D., Southern Research Institute, Birmingham, Alabama
"Preparation of Fibrous Estradiol/Progesterone IUDs for Phase I Clinical Trials - Continuation of PARFR-324"

Phase I protocol is written. Final IDE is being compiled and will be submitted to the FDA when Dr. Scornegna's (PARFR-353) rabbit data is completed.

C. TECHNICAL REPORT REVIEW (continued)

13. PARFR-329 (FINAL) -- Erwin Goldberg, Ph.D., Northwestern University, Evanston, Illinois
"Targeting Liposomes to the Male Reproductive Tract with Antibody LDH-C₄"

Antibody to LDH-C₄ was conjugated to liposomes with the bifunctional reagent, SPDP. These liposomes did not bind to mouse spermatozoa, apparently because of insufficient amounts of antibody.

14. WING SOUND II

- a. Drs. Harrieth M. Hasson and David A. Edelman's Site Visit Report
b. Family Health International Computer Report
c. PARFR-313E -- Robert Snowden, Ph.D., Institute of Population Studies, University of Exeter, England
"Uterine Measure - Clinical Comparison Study (Wing Sound II)"

Data being assembled at FHI. Preliminary analysis shows no relation between uterine length and IUD events.

15. PARFR-347 -- Ricardo H. Asch, M.D., The University of Texas Health Science Center at San Antonio (MANUSCRIPT)
"Studies on the Involuntary Potency and Side Effects of an Inhibitory Analog of LH-RH in Cynomolgous Monkeys"

Study recently initiated.

16. PARFR-351 -- Andrew V. Schally, Ph.D., Tulane University, New Orleans, Louisiana
"Development of Methods for Female and Male Contraception Based on LH-RH Antagonists"

Study recently initiated.

17. PARFR-320 (FINAL) -- Donald L. Wise, Ph.D., Dynatech R/D Company, Cambridge, Massachusetts
"Levonorgestrel Rods Drug Release Study"

Final technical report reviewed. No further funding in this area.

18. PARFR-350 -- Jacques Hamou, M.D., Tenon Hospital, Paris, France
"An Intra Tubal Device (ITD) for Female Sterilization"

Site Visit by Drs. Sciarra and Goldsmith, March, 1984 was reviewed.

C. TECHNICAL REPORT REVIEW (continued)

19. PARFR-334SRI -- Thomas R. Tice, Ph.D., Southern Research Institute, Birmingham, Alabama
"Development of Controlled-Release Progesterone Microcapsules"

The progress report was reviewed and PARFR staff will identify a U.S. center to conduct the Phase I study in conjunction with Dr. Croxatto in Chile. IND is being prepared.

20. PARFR-334UAB -- Lee R. Beck, Ph.D., The University of Alabama in Birmingham
"Pharmacokinetic Studies in Baboons Relating to PARFR-334SRI"

SEE 19.

21. PARFR-337F -- Tapani Luukkainen, M.D., Ph.D., University of Helsinki, Finland
"Use Effectiveness of a Levonorgestrel-Releasing Intracervical Device"

The preliminary technical report was reviewed and implementation of the ICD post-partum component was not approved.

22. PARFR-337T -- Rochelle N. Shain, Ph.D., The University of Texas Health Science Center at San Antonio
"Intracervical Device Acceptability Study"

Dr. Shain reported on the preliminary data collection flow between the center and computer facility.

III. MISCELLANEOUS

- A. The following subagreements are executed on projects reviewed and approved at the December 14, 1983 SAC meeting:

1. PARFR-354 -- Montri Chulavatnatol, Ph.D., Faculty of Science, Mahidol University, Bangkok, Thailand
"Screening of Thai Plants for Proteins (or Lectins) as Potential Vaginal Contraceptives"

Funding Period: 4/1/84-3/31/85 Amount Funded: \$9,800

2. PARFR-355 -- Nancy J. Alexander, Ph.D., Medical Research Foundation of Oregon, Beaverton, Oregon
"Enhancement of the Secretary Response to LDH-C₄"

Funding Period: 4/15/84-4/14/85 Amount Funded: \$47,335

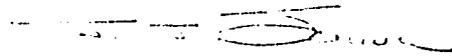
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III. MISCELLANEOUS (continued)

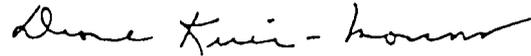
3. PARFR-356 -- Rajesh K. Naz, Ph.D. and Nancy J. Alexander, Ph.D.,
Medical Research Foundation of Oregon, Beaverton, Oregon
"Development of an Immunocontraceptive Vaccine: Role of 23-Kd
Antigen in Immunoinfertility and Fertility Regulation"
Funding Period: 5/1/84-4/30/85 Amount Funded: \$66,686

There being no further business, the meeting adjourned at 3:00 P.M.

Respectfully submitted,



John J. Sciarra, M.D., Ph.D.
Program Director, PARFR
Chairman, Scientific Advisory Committee



Diane Krier-Morrow, M.B.A.
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RESEARCH FRONTIERS IN FERTILITY REGULATION

THE ETIOLOGY OF PELVIC INFLAMMATORY DISEASE

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Pelvic inflammatory disease (PID) is a major medical problem worldwide. Its treatment and sequelae absorb a significant part of the health resources of many countries (33). The term PID refers either to an acute or a chronic inflammatory response involving the upper female genital tract (endomyometrium, tubes, ovaries, and supporting structures). The term is nonspecific and the condition may result from a variety of infections in a variety of organs, e.g. gonococcal salpingitis, chlamydial parametritis, and bacteroides tubo-ovarian or pelvic abscesses. Unless otherwise indicated, pelvic infections related to pregnancy or gynecologic surgery, or secondary to other gynecologic conditions such as cancer, and their treatment have not been considered in this report because they are traditionally given diagnostic terms other than PID.

Although inflammation of the upper genital tract can result from diverse causes such as endometriosis or extension of an intra-abdominal infection, in most cases, infectious PID is considered a sexually derived disease (SDD) resulting from sexually transmitted pathogens (STP) (46). PID is rarely diagnosed in women who do not engage in heterosexual vaginal intercourse. The pathogenic microorganisms responsible for PID are not always transmitted from the woman's partner, however; they may be part of the resident flora of her lower genital tract, and they may not become pathogenic until they gain access to her upper genital tract (41).

There are no uniformly accepted criteria for the diagnosis of PID and there is a significant degree of imprecision in this diagnosis. As early as 1928, Farr and Findlay reported that salpingitis was correctly diagnosed preoperatively in only 70% of 545 women admitted to a hospital

and operated upon with this diagnosis (17). Forty-one years later, Jacobsen and Westrom reported a similar rate of correct diagnosis (65%) of acute salpingitis, compared with the findings at laparoscopy (34).

INCIDENCE

According to Westrom, the annual incidence of PID in modern Western society is estimated to be 1% among women aged 15 to 34 (88). In the high risk age group, 15 to 24, the annual incidence is 2%. In the United States, the annual incidence of PID is at least 2% among fecund, sexually active women aged 13 to 44.

There is no national reporting system for PID in the United States. From a sample of discharge records at 7900 short-stay civilian hospitals, there were an estimated average of 213,000 hospitalizations for PID per year from 1970 to 1975 (36). PID was the primary diagnosis in 65% and the secondary diagnosis in the other 35% of cases. Hysterectomies were performed in 23% of the women admitted in whom PID was the principal diagnosis. Admissions for PID accounted for about 897,000 hospital bed days each year from 1970 to 1975 (74). Among non-pregnant women over 14 years of age, about 1 in every 100 hospital admissions was for PID (74).

Based on the National Ambulatory Medical Care Survey for the period 1973 to 1977, there were about 1.89 million patient visits for PID per year to office-based private practicing physicians (73). An estimated one-half million physician hours were required to see and treat these patients. One of every 17 of these office visits resulted in hospital admission, accounting for about 55% of all hospital admissions for PID during 1973 to 1977. In addition,

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there were an estimated 680,000 patient visits to clinics and hospital emergency rooms for PID each year (10).

Including only those cases in which the principal diagnosis was PID, there are at least three-quarters of a million cases of PID each year in the United States, with a resulting hospitalization rate of nearly 20%. Curran estimated the direct costs of PID and associated ectopic pregnancy in 1979 to be \$700 million (10). This figure does not include the costs of treating infertility problems resulting from PID. There is no accurate way to measure the total economic consequences of PID, either in the United States or worldwide.

MEDICAL CONSEQUENCES OF PID

The medical consequences of PID are well known and include repeated episodes of PID, ectopic pregnancy, infertility, and chronic pelvic pain. Westrom reported a greater than 6 fold higher rate of ectopic pregnancy and nearly a 4-fold higher rate of chronic pelvic pain among women with laparoscopically confirmed PID who were followed up for 6 to 14 years, compared to a group of women who did not have PID (87). In the same study, the proportions of women who were unable to become pregnant were 13%, 36% and 75% after one, two and three or more episodes of acute PID, respectively. Excluding women who elected not to become pregnant, only 69% of the women who had acute PID became pregnant, compared to 96% of the control group. In a later study by the same group of investigators (75), infertility rates among women with laparoscopically verified PID were 22% after the first episode of PID and 46% after subsequent episodes of PID. The data from both of these studies probably underestimate the true risk of infertility following acute PID, since the studies included only women with clinically evident disease who were treated medically; it is probable that many cases of PID are subclinical.

CAUSATIVE AGENTS

The microbiology of the female genital tract is extraordinarily complex and knowledge of it is limited by the microbiological methods available at present. This subject has aptly been described as a microbiologist's nightmare (13). Despite the significant advances in microbiological techniques that have been made during the past 10 to 15 years, an accurate understanding of the microbiology of PID is only beginning to emerge.

It is now recognized that *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma hominis* and a variety of other aerobic and anaerobic micro-organisms are causal in most cases of upper genital tract infection in the female (46, 77). The relative contribution of each of these organisms to the development of PID depends upon a variety of factors, including the prevalence of each organ-

ism in a given population, the state of health of each woman, and her prior disease history. Until recently, it was thought that *N. gonorrhoeae* initiated infection in nearly all cases of PID. Studies performed over the past 15 years have dispelled this notion, however, and the importance of other micro-organisms in the etiology of PID is now commonly acknowledged (9, 16).

In the United States, gonorrheal infections are associated with 30% to 80% of the PID cases, and 10% to 17% of the women with gonorrhea subsequently develop clinically diagnosed PID (31). Epidemiologic studies from other countries do not always agree on the relative importance of gonorrhea and other sexually transmitted pathogenic organisms in the development of PID. Reports from the United States as well as England and Wales have found PID rates to parallel gonorrhea rates (1, 36, 64). A decline in the prevalence of gonorrhea in Sweden was accompanied by a decline in the prevalence of gonococcal PID; at the same time, however, a concomitant increase in the prevalence of all cases of PID was noted (88). These data imply that control of gonorrheal infections alone cannot be relied upon to materially decrease the prevalence of PID.

Our understanding of the bacterial pathogenesis of PID has improved with the advent of reliable methods for the growth and identification of anaerobic bacteria (41). Some of the major microbiological advances of the past decade have included a rapid tissue culture method for the diagnosis of *Chlamydia trachomatis* (26) and a microimmunofluorescent technique to identify *C. trachomatis* antibodies in sera (85). Unfortunately, these techniques are not uniformly available to clinicians, and the importance of *C. trachomatis* in the etiology of PID is only recently becoming recognized among clinicians (42, 46, 77). According to Treharne and co-workers, over 50% of the PID in women under 25 years of age in one city in Sweden was caused by infection with *C. trachomatis* (83). In contrast, investigators in the United States generally have reported lower isolation rates (from 5% to 20% using endocervical cultures) of *C. trachomatis* from women with PID (35). Perhaps of greater importance is the finding of Jones and co-workers that antichlamydial antibodies were present in the serum of 35% of a group of 172 infertile women (35); these findings are indicative of prior chlamydial infections. No doubt, the clinical relevance of chlamydia and other organisms, such as ureaplasma, will become clearer as additional improvements are made in the methods of bacterial isolation. Of course, it is important to keep in mind that even with rapid methods of specimen transport and accurate methods of bacterial culture, results obtained from endocervical cultures do not always reflect the microbial picture present at a higher level in the genital tract, even when a lower genital infection accompanies an upper genital tract infection.

As noted above, clinical opinion until recent years was that gonococcal infection paved the way for invasion by secondary pathogens at a later date. In this regard, studies in which laparoscopy was used to obtain tubal culture and biopsies from women with acute salpingitis have demonstrated two important points. The first is that even at the outset, tubal infections are polymicrobial and often include *N. gonorrhoeae*, as well as anaerobes, aerobes, and *chlamydia* (14). The second is that fewer organisms are recovered in patients with longer durations of symptoms, even when meticulous attention is paid to the culture methods (14).

EPIDEMIOLOGICAL STUDIES

Over the past decade, numerous epidemiological studies have identified risk factors associated with PID. Although many such factors have been identified, it is crucial to remember that these associations do not necessarily imply a cause-and-effect relationship between the presence of the risk factor(s) and the occurrence of PID.

Case Control Studies. Prior to 1974, clinical impressions and the medical literature strongly supported an association between sexual activity and the occurrence of PID (50). Since 1974, the association between contraceptive usage, in particular the use of IUDs, and PID has become a focus of attention and has been studied extensively. Table 1 lists 18 case control studies (or studies

Thaler and co-workers, 1978 (80)	Prior abdominal/pelvic operations	11.0
	Prior abortion	2.7
Flesh and co-workers, 1979 (19)	Race: black	4.0
	Previous PID	3.1
	> 1 sexual partner	5.2
	Current use of IUD	2.2
Osser and co-workers, 1980 (54)	Current use of IUD	2.4
	Current OC use	0.3
	Prior pregnancy	2.2
Paavonen & Vesterinen, 1980 (56)	Current use of IUD	3.0
	Current OC use	0.3
Potterat and co-workers, 1980 (61)	Married	0.5
	Current OC use	0.4
	Race: black, Hispanic	2.5
Burkman, 1981 (6)	Age < 25 years	1.8
	Education < high school	1.7
	> 1 sexual partner in last 6 months	1.6
	Prior PID	3.2
	Prior gonorrhea	2.5
	Current use of IUD	1.6
	Current contraceptive use other than IUD	0.3
	Race: non white	1.3
	Single	1.3
Kelaghan and co-workers, 1982 (39)	Current use of barrier contraceptives	0.6
Rosenfeld and co-workers, 1983 (65)	Prior use of IUD	7.2
	Prior OC use	0.4
	Prior use of mechanical contraceptive method	0.3
	History of acute PID	54.6
Wolner Hanssen and co-workers, 1983 (92)	Current use of IUD	3.3

*Higher RR for earlier age at first intercourse. RR cannot be estimated from these data

Table 1. Estimated relative risks (RR) for factors associated with either an increased or decreased risk of PID.

that can be analyzed as case control studies) published since 1973 that have identified factors associated with either an increased or decreased risk of PID. The list is not all-inclusive, but represents major contributions to the identification of risk factors for PID. For each factor identified, an estimate of the relative risk (RR) of PID was computed for women with the risk factor compared to women without the risk factor. An RR value greater than 1 indicates the factor is associated with a significantly greater ($p < 0.05$) risk of PID, and a value less than 1 indicates the factor is associated with a significantly lower ($p < 0.05$) risk of PID. If estimates of the relative risks were not provided by the authors, they were derived using standard methods for the calculation of the crude odds ratios. If necessary, the original estimates were recalculated, depending upon the methods used by the authors.

The statistical computations in the present report were performed by David A. Edelman, Ph.D., Medical Research Consultants, Inc., Chapel Hill, North Carolina

Reference	Risk Factor	Est. RR
Noonan & Adams, 1974 (51)	Current use of IUD	7.8
	Current OC use	0.4
Targum & Wright, 1974 (78)	Current use of IUD	9.3
	Prior induced abortion	2.3
Phaosavasdi and co-workers, 1975 (58)	Past current use of IUD	12.2
Brown & Cruickshank, 1976 (5)	Married	0.5
	> 1 sex partner in previous 6 months	3.2
	Age at first intercourse	*
	Previous PID	5.3
Faulkner & Ory, 1976 (18)	Current use of IUD	5.0
	Febrile cases	2.6
	Afebrile cases	3.9
Westrom and co-workers, 1976 (90)	Current use of IUD	3.0
	Age < 26, nulligravid, IUD user	7.6
Eschenbach and co-workers, 1977 (15)	Current use of IUD	2.4
	Intercourse > 2 week	1.4
	Prior pregnancy	1.5
	Intercourses avoided with menses	2.1
	Race: non white	1.6
	Previous gonorrhea	1.7
	> 2 sexual partners in past 6 months	1.6
	Current OC use	0.5
	Sterilization	0.3

Table 1 lists only those factors associated with either an increased or decreased risk of PID. In some studies (5, 54, 78) various factors (e.g., age, marital status, parity) could not be evaluated, since cases (women with PID) and controls (women without PID) were matched on these factors. Also, factors identified in one study as being associated with either a higher or lower risk of PID were not necessarily identified in other studies. For example, frequency of intercourse was identified as a risk factor by Eschenbach and co workers (15) but not by others (5, 6). Table 1 does not include those factors not found to be associated with a higher or lower risk of PID. There are undoubtedly many more factors than those listed in Table 1 that might have a direct and possibly causal association with the development of PID, e.g., the bacterial flora in the patients' vaginas or the presence of urethritis/prostatitis in the patients' sexual partners.

Other Studies. Studies that have evaluated women with PID without any reference to a control group have also identified factors associated with increased risks of PID. A wide variety of findings have been reported.

Wright and Laemmle found that PID rates in an indigent population were increased in women aged 20 to 24 who were neither currently married nor ever married, and among IUD users (93). Jones and co workers reported higher PID rates for divorced or separated women, non-white women, and women aged 15 to 30 (36). Potterat and associates implied that the health seeking behavior patterns of women with gonococcal PID and their male contacts differed from those of women with uncomplicated gonorrhea and their contacts (61). Qvistad and associates found an association between the risk of PID post-abortion and prior positive cervical culture for *C. trachomatis* (63). Gump and co workers investigated the relationship between serum antibodies to *C. trachomatis*, prior to confirmed diagnosis of PID and prior IUD use (28). These investigators found that the proportion of women with prior PID was higher among women who had previously used the IUDs compared to those who had not (28), it was also higher among women with higher antibody titres to *C. trachomatis*, regardless of IUD usage.

The four major categories of risk factors for PID noted in Table 1 are sexual activity, sociodemographic characteristics, contraceptive method used, and other medical conditions. The role of each of these factors is discussed in the following sections.

RISK FACTORS

SEXUAL ACTIVITY

PID is predominately a sexually derived disease (STD). Exceptions include those cases resulting from tubercule infections, extension of appendiceal or other inflamma-

tions of the bowel, pelvic surgery, and gynecologic operations such as dilatation and curettage, abortion, insertion of an IUD, and uterine biopsy, to mention a few. Unfortunately, the literature on PID contains few studies that specifically attempt to relate sexual activity, PID, and specific bacteriologic agents. Of the 16 studies listed in Table 1, only 4 (5, 6, 15, 19) identified some aspect of sexual activity as a risk factor for PID. Nevertheless, all studies found factors in which sexual activity was implicated, e.g., use of a contraceptive method or prior pregnancy.

Only three aspects of sexual activity have been studied and identified as risk factors for the development of PID. They are: frequency of intercourse, number of sex partners, and age at first intercourse. Even so, all studies listed in Table 1 that evaluated these factors (5, 18, 61, 65, 78) were not in agreement as to their importance. This is not surprising for two reasons. First, in the reported studies, the information on sexual activity obtained by having the patients complete questionnaires or by direct questioning has generally not been validated by any other source to assess its reliability. Second, and perhaps more important, only superficial information on sexual activity has been obtained in studies reported to date. Specific aspects of sexual activity that have not been evaluated among women with PID include the following: frequency of homosexual relations or heterosexual contact with bisexual partners, practice of oral and anal sex, and the sexual exposure and habits of the women's partners). Although a complete evaluation of sexual variables would be a most difficult task, a true understanding of the relationship between sexual activity and the development of PID requires that this be done. In the past, analyses have generally evaluated only one aspect of sexual activity at a time and have not considered the importance of their interrelationships. For example, coital frequency and the number of partners have been evaluated separately in most studies. In terms of the risks of acquiring a sexually transmitted disease (STD), a high coital frequency with one partner clearly is not the same as either a high or low coital frequency with many partners. Moreover, the number of partners a woman has might be associated with different risks of PID, depending on her marital status, age, and other factors, regardless of coital frequency.

The role of the complex variables included in the term "sexual activity" and the interrelationship with sociodemographic factors, contraceptive choices, and other medical conditions is just now beginning to gain the attention of investigators. The prevailing view is that coital frequency and number of sexual partners provide measures of the frequency of exposure to sexually transmitted pathogens (STP). The third variable dealing with sexual activity, namely, age at first intercourse, may be only an indirect measure of later sexual behavior with

respect to the exposure to a variety of sexual partners (88).

SOCIODEMOGRAPHIC CHARACTERISTICS

Sociodemographic factors, such as marital status and age, probably are indicators of the sexual behavior that determine the risks of a woman acquiring PID. For example, sexually active separated or divorced women have been shown to be at a higher risk of acquiring PID than married women of the same age (36). This association may reflect a higher number of sexual partners among nonmarried women and/or the multiplicity of sexual contacts among the women's partners and a concomitantly higher risk of being exposed to an STP. In addition, it seems reasonable to hypothesize that other sociodemographic characteristics might reflect different sexual preferences and lifestyles. To our knowledge, these interrelationships have not been specifically investigated.

Variables such as race that have been associated with an increased risk of PID may only reflect different prevalence rates of STDs, such as *N. gonorrhoeae* or *Chlamydia trachomatis* in various racial/ethnic groups. The possibility also exists that there are differences in the host response to sexually transmitted infections that either increase or decrease the risks of developing PID. It is also necessary to consider that the health-seeking behaviors of different population groups may differ in such a way as to place them at differential risks of PID. From a public health point of view, appropriate educational programs may address behavioral issues such as the number of sexual partners and type of contraceptive used. They can do nothing, however, to alter demographic factors such as a woman's age, race, and prior pregnancy history.

CONTRACEPTIVE METHODS

Barrier Methods. Both clinical and epidemiological studies have demonstrated that use of barrier (both physical and chemical) methods of contraception reduces a woman's risk of PID (8, 67, 68, 69). Since the bacteriocidal properties of spermicidal preparations protect women from many lower genital tract infections and presumably their subsequent ascent into the upper genital tract, the reduced risk of PID most likely is due to use of the barrier contraceptives per se. However, it may also be due to other factors, since the choice of a contraceptive method may be different for different groups of women and be associated with sexual activity factors.

Women who use sterilization as their method of contraception are also at a reduced risk of PID (32). It is commonly thought that contraceptive sterilization protects against PID by preventing pathogens from gaining access to large portions of the tubes, as well as the

ovaries and the peritoneal cavity. This condition of limited access may be especially important during ovulation and menstruation, when a greater susceptibility to PID exists (15). An additional explanation for the lower risk of PID observed among sterilized women is that these women are usually in the demographic subgroups known to have lower risks of PID, e.g., married women over 30 years of age. These women may live in stable relationships, and their sexual lifestyles and preferences may place them at a lower risk of exposure to externally acquired pathogens that may initiate an infectious process.

Oral Contraceptives (OCs). Two reasons have been suggested for the reduced risk of PID among women using oral contraceptives (OCs) (15, 66) that has been found in case control studies (see *Table 1*). The first is that OC use tends to reduce menstrual flow. This not only reduces the time during which there is a more favorable environment for bacterial growth, but also reduces the need for the prolonged use of tampons or sanitary napkins. The second is that OCs change the characteristics of the cervical mucus. Since OC use inhibits the preovulatory estrogen surge there is an absence of receptive mid cycle cervical mucus that promotes sperm access to the upper female genital tract.

Intrauterine Devices. The IUD has been the most extensively studied contraceptive method in terms of the risks of PID, and it has been identified as a risk factor for PID in many studies (*Table 1*) published in the late 1960s and 1970s (11, 12). In 1968, Wright and Laemmle first reported that IUD users had a higher risk of PID compared to users of oral contraceptives (relative risk, 4.9) and compared to women using contraceptive foam (relative risk, 2.6) (93).

The principal difficulty in trying to assess whether or not the use of IUDs increases the risk of PID relates to the selection of an appropriate comparison group. Although non-contraceptors appear to constitute an appropriate comparison group, this group in reality includes several subgroups with different risks of PID and therefore may be an inadequate comparison group.

The question of whether the higher reported risk of PID among IUD users represents higher risk due to the use of IUDs per se or represents a higher risk as a result of the choice of comparison groups against which IUD users are evaluated has not yet been clearly answered. Since the use of barrier or oral contraceptives as well as sterilization protects women against PID, the relative risk of PID to IUD users compared to users of these methods might only reflect the protective effect of the latter methods, rather than a truly enhanced risk of PID among IUD users. As previously stated, it is inappropriate to compare the risks of PID to IUD users with those of sterilized women who are inherently at a lower risk of

PID. It is equally inappropriate to select non-contraceptors (including women using rhythm, coitus interruptus and any of the so-called natural family planning methods) as a comparison group, since the group of non-contraceptors includes the following subgroups, who probably have different risks of acquiring PID:

1. Sexually inactive women.
2. Sexually active women desiring pregnancy. (These women may be at a minimal risk of PID, since they generally have stable monogamous sexual relationships.
3. Sexually active women not desiring pregnancy but who elect not to use contraceptive methods. (This group includes women who have occasional coitus at times when they do not think they can become pregnant. These women, as well as others in the non-contraceptor group, may terminate their pregnancies by abortion).

Sexually active women who do not wish to become pregnant but who are not using any contraceptive method represent a unique group. If these women are as sexually active as women who use contraceptive methods, they will be repeatedly exposed to the risks of pregnancy as well as PID. These women have a far greater risk of becoming pregnant before contracting PID, since the monthly probability of pregnancy is about 5% to 6% and that of contracting PID is about 0.1% to 0.2%.

Sexually active non-contracepting women may also be an inappropriate comparison group, because women who become pregnant unintentionally are also exposed to the risk of upper genital tract infections associated with the termination of pregnancy, either at term or by abortion. This group of women may include subgroups who are at a high risk of PID. For example, Qvigstad and co-workers found that 1.6% of the women with negative serologic cultures for *C. trachomatis* developed PID within 1 month of abortion by dilatation and vacuum aspiration (63). In sharp contrast, the rate among women with positive cultures was 20%. A similar finding has been reported by others (55). Women who have incomplete septic abortions or upper genital tract infections following induced abortion are not usually classified as having PID, even though the effects of these infections in terms of future fertility may be similar to those of non-pregnant women who acquire any form of PID.

It is possible that sexually active, non-contracepting women who do not wish to become pregnant are probably at a different risk of PID compared to sexually active, non-contracepting women who wish to become pregnant. To our knowledge, studies that have evaluated the relative risks of PID associated with contraception have not distinguished between these two sexually active non-contracepting groups of women. The two groups may also differ with respect to numerous other important

factors, including their health attitudes and habits. In comparing the relative risks of PID among women using different contraceptive methods, several important factors need to be considered, including the sexual activity of the women and their frequency of exposure to STDs. These factors may be quite different for women in the different contraceptive groups. For example, Valenti recently reported that the desire for more frequent coitus was greater among IUD users compared to OC users in the first 3 months after initiation of the method (84).

There are two temporal aspects to the risk of PID among IUD users. The first is the risk of PID resulting from the insertion procedure per se, and the second is the risk of PID due to factors associated with the IUD and the user. The higher risk of PID during the initial month of IUD use (Table 4.3 of 11, 12, 43) is thought to result from the transmission of bacteria from the endocervix to the uterus at the time of the insertion procedure. This observation follows from the study of Mishell and co-workers showing that bacterial contamination of the uterus followed IUD insertions; the bacteria were eliminated over a period of weeks (48). Although the prevailing clinical opinion is that the uterine cavity is normally able to maintain sterility (70), several studies have shown bacteria to be present in the uterine cavities of a significant proportion of asymptomatic women who were not using IUDs (71). Some of these studies, however, may include false positive culture results due to the sampling methods used (71).

In a recent case control study reported by Lee and associates in which women were excluded if they were sexually inactive, amenorrheic, sterile, or had recently been pregnant (all factors known to influence a woman's risk of PID), the estimated relative risk of PID to IUD users compared to women using no contraceptive methods was 3.1 for women who used their IUDs for less than 5 months, whereas it was only 1.1 (a value not significantly different from 1, $p > 0.05$) for women who used their IUDs for over 4 months (43). These data imply an elevated risk of PID following the IUD insertion procedure itself, but no increased risk due to continuing IUD use. This finding is contrary to the commonly accepted opinion that IUD use is associated with an increased risk of PID at any time following insertion. Since this study did not ascertain whether the IUD users and users of no contraceptive methods were different with respect to other known risk factors for PID, the reported estimated relative risk of 1.1 may represent either an under or overestimate of the actual risk (43). Moreover, since the nonuser group included women who were at a reduced risk of PID for various reasons, the estimated relative risk value may represent an overestimate of the true value. A similar point of view has been expressed by Luukkainen and associates, who suggested that the higher rate of PID for IUD users found in case control studies might only

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reflect the risk of PID attributable to the insertion procedure (44).

A major area of concern regarding IUD use has been the presence of tail strings. In the early 1960s, many clinicians suggested that the use of strings might put women at an increased risk of infection by providing a pathway for the ascent of bacteria from the vagina to the uterus. At the same time, however, it was recognized that the tail facilitated removal of the IUD and avoided complications that might occur at the time of removal if the IUDs were tailless. The consensus of experts was that the benefits of adding a tail string outweighed the risks.

Two decades later, the question of whether or not the IUD tail per se places women at a higher risk of PID remains unanswered. Adequate clinical studies to evaluate the role of the IUD tail in the etiology of PID have not been conducted. Four of five studies that have compared tailed and tailless IUDs found no increased risk of PID to users of tailed IUDs (11). These studies, however, were not designed specifically to evaluate the role of the IUD tail. In one on-going study in which 1100 women were randomly assigned to use a Copper T IUD either with or without a tail, no statistically significant differences in the PID rates have been found between the two groups of women who have been followed for up to 1 year (7).

Since 1975, the question of whether the risk of acquiring PID is different for IUDs using different types of tail strings also has been debated widely. Today, most, if not all, IUD tail strings consist of one or two strands of nylon. In the past, some IUDs have used tails made from a bundle of fine filaments (multifilamental tails) e.g., Antigon F, Birnberg bow, Dalkon Shield, Latex Leaf, Majzlin spring). None of these IUDs is used in clinical practice in the United States today. Comprehensive evaluations of the PID rates for different types of IUDs, regardless of the type of tail string used, have not found any one type of IUD to be consistently associated with higher rates of PID (11, 12).

The controversy over the type of IUD tail was widely published by Tatum and co-workers, who demonstrated in a series of *in vitro* experiments that live *E. coli* could move up the Dalkon Shield tail along with fluid under certain laboratory conditions, near the upper knot at the base of the IUD (79). These investigators also reported the presence of anaerobic and aerobic bacteria obtained from Dalkon Shield tail strings removed from asymptomatic users (79). The authors suggested that a multifilament tail could provide a means for bacteria to enter the uterus. However, this hypothesis does not explain the clinical observations of the occurrence of PID in women wearing IUDs with monofilament tail strings or IUDs with solid plastic tail configurations (Margulies spiral).

In one *in vitro* investigation (62), bacteria appeared to migrate through a layer of cervical mucous coating the outside of multifilamental and monofilamental IUD tails. In a later study by the same group of investigators (72), the apparent migration of bacteria along the IUD tail was confirmed indirectly in IUD users undergoing hysterectomies. Aerobic and anaerobic cultures of the uterine cavities demonstrated bacteria in the uteri of 12 of 14 women using IUDs with monofilamental tails and in the uteri of 3 women using IUDs with multifilamental tails. Most bacteria cultured in this study were commensals, such as *Gardnerella vaginalis* and *Corynebacterium* sp., but potentially pathogenic organisms such as *Escherichia coli* and *Streptococcus faecalis* were also found. The types of bacteria cultured from the extirpated uteri were similar to the types of bacteria present in the vaginas of these same women. These authors concluded that the IUD tail in some way interfered with the protective mechanisms of the cervix, thus permitting bacteria to enter the uterine cavity. These two studies (62, 72) suggest that there is no significant difference between monofilamental and multifilamental tails with respect to their ability to facilitate the transfer of bacteria from the cervix and vagina to the uterine cavity. However, one of the studies (72) included only a few subjects, and all of them had gynecologic conditions requiring surgical treatment. For this reason, they may not be representative of all asymptomatic IUD users.

The purported ability of bacteria to migrate along the outside or the inside of IUD tails has not been related to the risk of PID for IUD users compared to the risk for nonusers. Clearly, the presence of bacteria on either the inside or the outside of the IUD string does not indicate the presence of a pelvic infection. Bank and associates performed scanning electron microscopy studies of Dalkon Shield tails removed from asymptomatic women who had used their IUDs for 2 or more years (2). These IUDs had intact tail strings that were found to contain bacteria, both above and below the double knot at the base of the IUD. The study did not determine how long the bacteria had been present within the strings. The Dalkon Shield tails evaluated by Tatum and associates came from IUDs that were removed electively from women who did not have any symptoms of PID at the time their IUDs were removed (79).

With prolonged use, almost all IUDs undergo physical changes, including distortion of their shape, discoloration, and deposition of mineral salts (especially calcium) on their surface. The deposits on IUDs appear to build up over time (27). The significance of these changes, if any, in terms of increasing the user's risk of acquiring PID or developing other significant complications is not known. Recent research efforts have been directed at further evaluation of the surface deposits found on IUDs (Lippes Loop, Saf-T-Coil, Dalkon Shield, Copper 7,

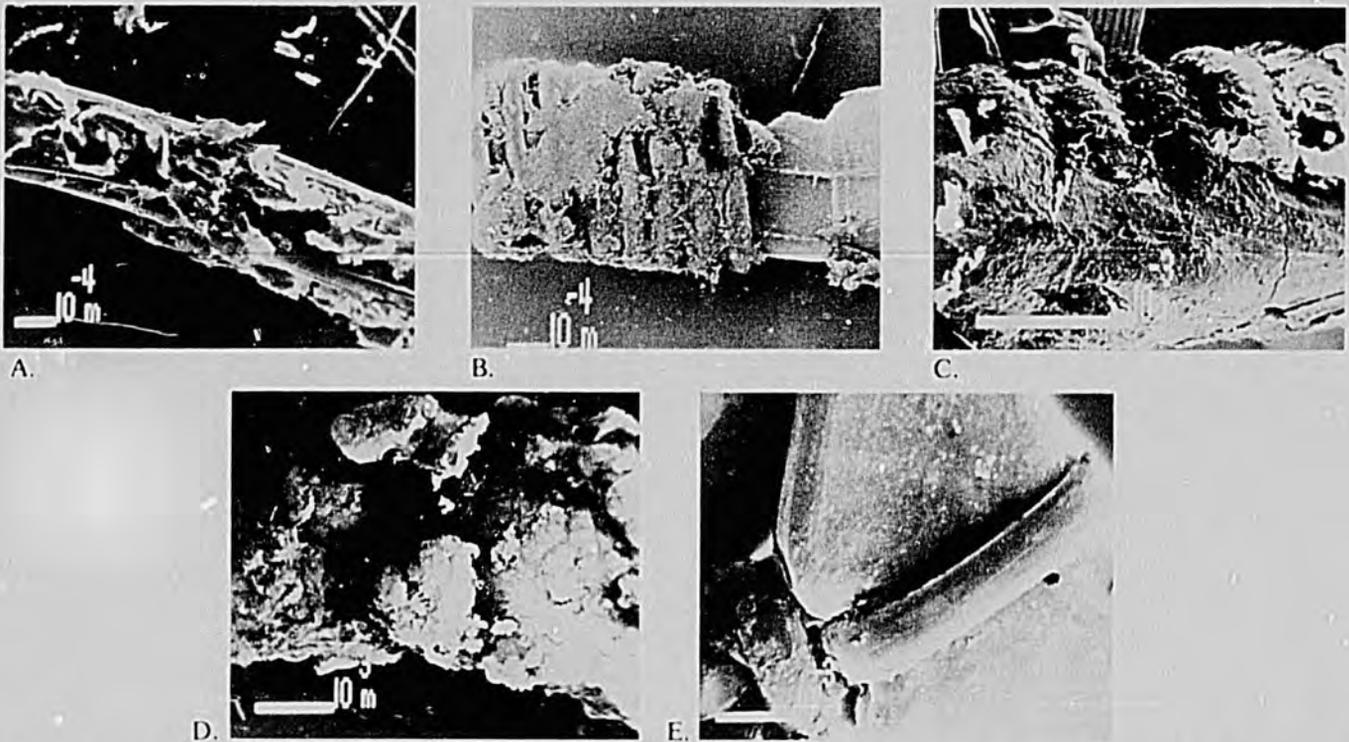


Figure 1. Scanning electron microscopy of various IUDs after removal. A. Lippes Loop, 100x. Flaking encrustations on tail string. B. Copper-7, 10x, lower margin of copper wire and IUD body. C. Copper T, 40x, side view of copper wire covered with hard surface encrustation with crevices and irregular topography. D. Saf-T-Coil, 20x,

irregular granular surface encrustation and bulbous nodular formation. E. Dalkon Shield, 20x, area of lower portion of IUD body and string interconnection, with plaque-like encrustation on topographical areas of the string.

Copper T) that have been used for prolonged periods (4 to 15 years) (38). Figure 1 shows scanning electron micrographs (SEMs) of the surfaces of sections of some of these IUDs and their tails. The deposits on the surfaces of these IUDs were further evaluated by energy dispersive spectrometry and x-ray diffraction methods. These studies identified several minerals and salts on the surfaces and tails of the IUDs (Figures 2 and 3). Of particular interest is the identification of calcium apatite crystals on the surface of a Lippes Loop. This salt is a common component of dental calculus and results from the mineralization of plaque. Similarities between the calculus found on teeth and deposits found on IUDs may be due to a similarity between the bacterial inhabitants of the mouth and vagina, such as anaerobic streptococcus, viellonella and other organisms involved in plaque formation. It should be recognized, however, that calcium may precipitate on the surface of IUDs in the absence of bacteria on the IUD surface. No evidence has been presented to indicate that the formation of deposits on IUDs varies with the specific type of IUD; it appears to be generic to all types of IUDs (38).

Marrie and Costerton have described transmission electron microscopic studies of IUDs (Copper-T, Copper-7, Saf-T-Coil) electively removed; they found microcolonies of bacteria adherent to specific areas of the surfaces of

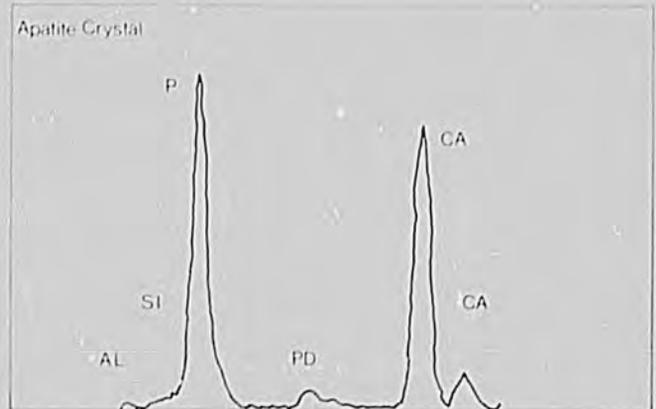


Figure 2. Artist rendition of energy-dispersive spectrometry of calcium apatite crystal from Lippes Loop IUD.

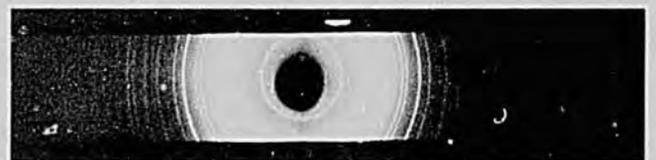


Figure 3. X-ray diffraction pattern of calcium apatite crystal shown in Figure 2.

these IUDs (47). These investigators thought the bacteria were not contaminants from the cervix and vagina at the time of IUD removal, but had been on the surface of

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the IUDs for a long time. From this study and other similar types of studies it cannot be determined whether the bacteria adherent to the IUD surfaces were deposited on the IUDs during the insertion procedure, or "migrated" to the uterus along the IUD string, or came to be there by passive transport, such as by attachment to sperm or other motile organisms.

Pelvic actinomycosis is an uncommon form of pelvic infection. Until the mid-1970s only about 300 cases had been reported in the literature (12). Beginning in the early 1970s, reports began to appear that associated IUD usage with pelvic actinomycosis. Since this time, about 100 such cases have been documented (12, 29). The risk of actinomycosis appears to be generic to all types of IUDs and appears to increase with increasing duration of IUD use (12). In 1976, Gupta and co-workers first documented the recognition of *Actinomyces* in cervical Fast smears stained with Papanicolaou stain (30). This observation has been confirmed by several other investigators (12). Some reports have indicated the presence of *Actinomyces*-like organisms only in cervicovaginal smears. In some studies, the prevalence among IUD users was over 40%. Gupta noted that in cervicovaginal smears, *Actinomyces* needs to be distinguished from a number of biologically active and inert substances (29). O'Brien and associates have suggested that the prolonged exposure of IUDs to body fluids may result in dissociation of material from the surface of IUDs that is identified as a sulfur granule, which in turn is associated with the presence of *Actinomyces* (52). Gupta concluded that less than 10% of IUD users may have *Actinomyces* organisms detected on Pap smears (29).

In summary, the data suggest that IUD use increases a woman's risk of acquiring PID during the first weeks or months following the IUD insertion. This higher risk is probably attributable to the insertion procedure rather than to the IUD use per se. The higher risk of PID among IUD users subsequent to the first month of use that has been reported in the literature appears to be based largely on the lower risk of PID among women included in the group(s) against which the risks of PID among IUD users have been compared.

OTHER MEDICAL FACTORS

Since about the beginning of the 20th century, the vagina has been known to contain a variety of bacterial species. During the past decade, however, with the development of improved culture techniques for anaerobic bacteria, investigators have reported on the extensive aerobic and anaerobic flora of the lower female genital tract in healthy premenopausal women (41). A summary of the more common aerobic and anaerobic species found in studies published since 1970 are listed in Table 2. Although a wide range in the prevalence of the various organisms has

been reported, Larsen noted that about ten aerobic and anaerobic species are frequently cultured simultaneously from the vagina and cervix, and that the average number of species per culture is 5 (41). The "normal" flora of the vagina and cervix includes bacteria that may be pathogenic when they gain access to the upper genital tract. These bacteria are frequently implicated in serious pelvic infections.

In addition to the bacterial species listed in Table 2, *Trichomonas vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Candida albicans* and other yeasts and *Chlamydia trachomatis* are frequently isolated from the lower genital tracts of symptomatic and asymptomatic women. In a group of healthy women attending a health department, Persson and co-workers reported the following rates of positive cultures (57): *M. hominis*, 19%; *U. urealyticum*, 60%; *C. trachomatis*, 5%; yeasts, 22%. The precise role of these organisms in the causation of PID is not clear since they exist so frequently in the presence of other pathogens. Persson and co-workers suggested that since the above organisms frequently occurred with *N. gonorrhoeae*, they might have epidemiologic characteristics similar to that of *N. gonorrhoeae* (57).

It is generally thought that lower genital tract infection (LGTI) precedes upper genital tract infection or PID. This may or may not always be the case, however, depending on the bacterial etiology of the PID and the mechanisms responsible for the transmission of the bacteria to the upper genital tract. Mardh and associates noted that among women with PID not attributable to either a gynecologic procedure or infection in an adjacent organ, a LGTI probably acquired by means of sexual

Aerobes	Anaerobes
Lactobacillus	Lactobacillus
Diphtheroids	Propionibacterium
Staphylococcus	Eubacterium
aureus, epidermidis	Bifidobacterium
Streptococcus	Clostridium
A,B,C,D + enterococcus	perfringens, other
E. coli	Peptococcus
Klebsiella + enterobacter	prevotii, asacharolyticus,
Proteus	magnus, other
Pseudomonas	Peptostreptococcus
	intermedius, productus,
	micros, anaerobius
	Gaffrya
	Fusobacterium
	Bacteroides
	fragilis, melaninogenicus,
	bivius, other
	Veillonella

Source: Larsen B: Normal Genital Microflora. In Keith L, Berger GS, Edelman DA (eds): Infections in Reproductive Health. Lancaster, England, MTP Press (In press).

Table 2. Aerobic and anaerobic bacteria isolated from asymptomatic, premenopausal women.

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intercourse was nearly always present (46). Obviously, the presence of potentially pathogenic bacteria in the lower genital tract does not necessarily constitute an infection, either in the lower or upper genital tract. A major difficulty in assessing the importance of LGTIs in the etiology of PID is that they are not uniformly defined, just as there is no uniformly accepted definition for PID.

Even though there is an extensive literature on PID as well as on LGTIs, it is unfortunate that with the exception of *N. gonorrhoeae*, studies do not relate the two in a meaningful way. Various investigators report that approximately 10% to 20% of women with cervical gonorrhea go on to develop an upper genital tract infection. If most LGTIs result from sexually transmitted pathogens, then the risks of these infections also need to be studied in relation to their propensity to cause upper genital tract infections.

The interrelationships between the use of various contraceptive methods and the presence of various micro-organisms in the vagina, as well as LGTIs, need to be evaluated. The literature on this subject is difficult to interpret for a number of reasons. Many studies have evaluated women attending venereal disease clinics; some had symptoms of an LGTI, others did not. Some women had positive cultures for pathogenic organisms; others did not. In some studies, control groups were included that provided a basis for comparison; other studies did not include control or comparison groups. In the following paragraphs some of the conflicting results obtained from recent studies are summarized.

In a study of women attending a family planning clinic, diaphragm users had a significantly higher isolation rate of fungi from the cervical os or vaginal fornix compared to users of other contraceptive methods (24). In another study by the same group (86), vaginal cultures indicated that the prevalence of *Bacteroides* spp. was significantly higher among IUD users, compared to women using other contraceptive methods; IUD users compared to diaphragm users had a higher prevalence of anaerobic cocci; the prevalence of coliforms was higher among diaphragm users; and finally, the prevalence of lactobacilli was increased in the OC group. The prevalence rate of each bacterial group was unrelated to age, parity, or social class of the women.

According to Osborne and co-workers, the relative risk (about 0.5 to 0.6) of symptomatic vulvovaginitis was similar for IUD, barrier, and OC users, compared to women using other contraceptive methods or no method (53). In contrast, Piot found the risk of "nonspecific" vaginitis to be four times higher for IUD users compared to OC users (59).

Among women attending a sexually transmitted disease clinic, OC users compared to non-users of OCs had

higher isolation rates of chlamydia, but lower isolation rates of trichomonads (40). In the same clinic OC users compared to non-users had higher yeast infection rates (40). Svensson and associates found the relative risk of cervical infection with chlamydia for OC and IUD users compared to users of other or no contraceptive methods was 2.4 and 0.8, respectively (76). The same study indicated an elevated risk of infection for women who had been pregnant and who had prior PID.

In a gonorrhea screening program of women attending a family planning clinic, rates of positive cultures were associated with the contraceptive methods used (3). The prevalence rates were similar for OC users (11.5%) and IUD users (9.9%) and were significantly higher compared to women who were sterilized (3.3%) or women who used barrier contraceptive methods (4.2%).

The question of whether users of different methods of contraception have different susceptibilities to LGTIs has not yet been adequately answered. There is some evidence that yeast infections may be more prevalent among OC users (4), and possibly among users of barrier contraceptives. Spermicides provide women with some protection against some micro-organisms as a result of their bacteriocidal effects (68, 69).

Prior PID and gonorrhea have both proven to be risk factors for PID. Two explanations for this seem plausible. First, the tubal epithelium damaged by an infection may lose some of its ability to maintain host resistance and thus become more susceptible to subsequent infections. Second, the lifestyles of women who have had a prior episode of PID probably place them at an increased risk of repeated episodes of PID.

Prior abortion or pregnancy, as a risk factor for PID, may simply reflect the increased risk of clinical and subclinical infections due to the abortion procedure or delivery and/or sexual exposure without the protective effects of certain contraceptive methods.

The well-known clinical observation of the relation between menstruation and the onset of clinical symptomatology of PID has been subject to little study. Menstruation usually precedes the onset of PID in about 50% of the cases. This observation is consistent with:

1. The presence of pathogenic organisms in the cervix and/or vagina;
2. The decline in the relative proportion of aerobic organisms in the cervix in the week immediately preceding menses;
3. The lack of cervical mucus protection during menses;
4. The physiological widening of the cervical and endocervical canal at the time of menses.

All these factors and possibly others could explain the clinical picture of post-menstruation PID.

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CLINICAL CONSIDERATIONS

The classical concept of the pathogenesis of PID (not related to pregnancy, surgery, gynecologic procedures or extensions of a gastrointestinal tract infection) is that lower genital tract infections ascend directly to the upper genital tract along the endocervical mucosa (37, 46). Lymphatic drainage provides an alternative pathway (37, 46). The spread of the infectious process then may pave the way for secondary invasion of the upper genital tract by bacteria normally found in the vagina (46). This concept of PID has been described as "woefully inadequate" (89). A principal omission of this concept is any direct reference to sexual activity preceding the lower genital tract infection.

Little doubt exists that genital tract infections can spread through the lymphatics (46). The pelvic lymphatic system is extensive (60), surrounding the entire uterus and providing interconnecting bridges between the cervix and corpus. The lymphatic network also spreads laterally to the tubes and ovaries. Figure 4 illustrates the lymphatic drainage from the posterior cervix laterally to the pelvic side walls through the broad ligament, inferiorly to the rectum and superiorly along the entire surface of the uterus to the level of the fallopian tubes and ovaries.

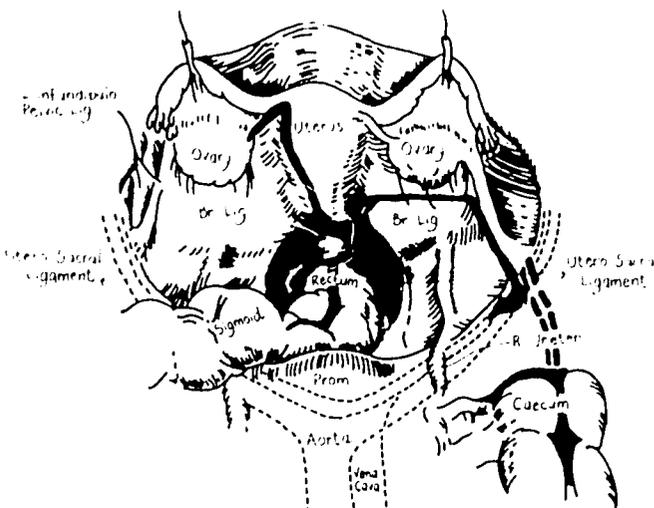


Figure 4. Schematic illustration of lymphatic pathways alongside uterus to tube and ovary (left) and to broad ligament (right). Redrawn with permission after original experiments by Dr. Edward Eichner.

Recently, researchers have investigated the mechanisms of the spread of infection from the lower to the upper genital tract in laboratory animals. Moller and associates inoculated monkeys with *C. trachomatis* or *M. hominis* (49). The route of spread of these two bacteria apparently differed somewhat. The lymphatics appeared to be more involved in the spread of *M. hominis* and less with *C. trachomatis*. Mardh and associates have reported

that in the human, gonococcal infections spread from the lower genital tract canalicularly over the uterine mucosa to the tubes, whereas chlamydial salpingitis derives from infections of the lymphatics and blood vessels of the parametria and broad ligaments where parametritis is first produced (46). This observation is compatible with the recent findings of Gibson and associates that chlamydial infection in the human is more likely to result in parametrial disease including severe adhesions and distal hydrosalpinx formation (22).

The classical theory of the pathogenesis of PID does not consider other possible mechanisms that might promote the ascension of pathogenic bacteria into the upper genital tract. These include:

1. The male factor, in terms of the bacterial flora of the seminal fluid;
2. Passive transport of bacteria from the lower female genital tract to the upper genital tract;
3. The transport of bacteria into the upper female genital tract by their attachment to spermatozoa or trichomonads.

Some aspects of these mechanisms in the pathogenesis of PID are discussed in the following sections.

THE MALE FACTOR

Although there have been numerous publications on the bacteriology of the seminal fluid, the role of seminal fluid in the etiology of PID generally has been considered only recently. Toth evaluated the bacterial flora of the seminal fluid from groups of fertile men, infertile men without a history of genital tract infection, and men with a history of genital tract infections (81). Isolates of the bacterial flora (aerobic and facultative, as well as anaerobic) were similar for fertile and infertile men without a history of infection. A significantly higher number of bacterial isolates were obtained from the semen of males with prior genital infections compared to either fertile or infertile men without a history of infection. Four observations of Toth are noteworthy (81). First, most men with bacteriospermia were without symptoms. Second, the severity of the bacteriospermia was related to the number of prior sexual partners. Third, women married to men with high bacterial counts in their seminal fluid had a greater chance of developing a pelvic infection. Fourth, wives of azoospermic men rarely developed PID.

The incidence of bacteriospermia in men reflects, among other things, their sexual lifestyles, prior genital tract infections and the treatment of these infections, as well as their use of systemic antibiotics for other conditions. During intercourse any bacterial component of the seminal fluid is added to that component otherwise present in the vagina. The extent to which the addition of seminal bacteria disrupts the normal bacteriologic state of the

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vagina is not known. It is likely that the greater the frequency of intercourse with bacteric spermic males, the greater the risk of an LGTI to the female. Also, vaginal and cervical bacteria may become adherent to sperm following their ejaculation. These events might increase the risk of acquiring an upper genital tract infection. Clearly, further research is required to evaluate possible interactions between the bacteriology of the lower genital tract of the female and that of the seminal fluid, and the presence of lower and upper genital tract disease.

PASSIVE TRANSPORT OF BACTERIA

Since the early 1930s it has been known that particulate matter can be rapidly transported from the vagina to the uterus and into the fallopian tubes (37). In several animal species, experiments have shown that dead sperm can be transported to the fallopian tubes following artificial insemination (37). The exact mechanisms for the passive transport of sperm and particulate matter from the lower to the upper genital tract are not known, either in humans or in lower animals. One possible explanation is that pressure differentials between the peritoneal cavity and the vagina are created by normal respiratory movements of the diaphragm and/or uterine contractions. Whether the same mechanisms that transport particulate matter and sperm can also transport bacteria to the upper genital tract remains to be studied.

BACTERIAL ATTACHMENT

The attachment of bacteria to spermatozoa has been described by several investigators. Specifically, the attachment of *N. gonorrhoeae*, *U. urealyticum*, *E. coli* and *C. trachomatis* elementary bodies has been demonstrated (20, 23, 25, 91). Figure 5 shows an example of the



Figure 5. Transmission electron microscopy of sperm with *E. coli* attached to tail. Original magnification 5400x. Magnification of inset, 23,000x.

in vitro attachment of *E. coli* to sperm. Probably, there are variations in different strains of bacteria of the same species in their ability to attach to sperm. For example, it has been found that certain isolates of *E. coli* have only a slight capability to bind to sperm, while others have marked binding abilities (21).

The *in vivo* (20) and *in vitro* (37) studies that show bacterial attachment to sperm have not yet clarified the role of sperm as a mechanism for the transport of bacteria from the seminal fluid or the lower female genital tract to the upper genital tract. This can only be established by further studies designed to investigate that specific question. Evidence that sperm can provide a mechanism for the transport of bacteria comes from the *in vitro* studies of Toth and co-workers (82). These investigators demonstrated that, in the presence of sperm, aerobic and anaerobic bacteria migrated through a column of ovulatory-phase cervical mucus (82). In the absence of sperm, however, bacteria were not observed to move through the cervical mucus. These investigators also noted that no sperm penetration or bacterial migration occurred when cervical mucus was used from either pregnant women or from the luteal phase of the menstrual cycle. Toth and co-workers did not determine whether bacterial migration was by the attachment of the bacteria to the sperm; they only observed bacterial migration in the presence of sperm (82). Also, they noted that PID was rare among the wives of azoospermic men. Indirectly, this finding implicates spermatozoa in the etiology of PID. However, other factors also need to be considered, such as the sexual lifestyles of couples and their exposure to pathogens that might place them a higher risk of acquiring PID.

The experiments of Toth and co-workers unify much of the information currently available concerning the pathogenesis of PID (82). In our opinion, the presence of pathogens in the seminal fluid, cervix, or vagina act to increase the likelihood of a woman acquiring PID. A high frequency of sexual intercourse may only increase the risk of acquiring PID if there is a significant bacterial contamination of the seminal fluid, sperm, or vagina. The lower risk of PID to OC users may be due in part to the absence of ovulatory phase cervical mucus favorable to sperm penetration and possibly bacterial migration. The higher risk of PID to IUD users compared to OC users may reflect the lack of any inhibitory effect of IUDs on ovulatory (estrogenic) cervical mucus.

Trichomonads also have been found in the upper genital tract (including the fallopian tubes) of women with serious pelvic infections (45). Trichomonads may thus be causative agents for PID if they act as carriers of other micro-organisms. A recent *in vitro* experiment has demonstrated that *E. coli* may attach to trichomonads (37).

COMMENT

Improved microbiological methods of the past decade have led to significant progress in our understanding of the bacteriologic etiology of PID. In contrast, there has been little significant advance in the understanding of the epidemiology of PID, although some progress has been made toward understanding the mechanism through which women acquire PID. The following summarizes our thinking on the development of PID.

In general, PID is a sexually derived disease, but those specific aspects of sexual activity that place a woman at an increased risk of acquiring PID have not been adequately studied. Micro-organisms are transmitted to the lower genital tract during sexual intercourse by direct contact and/or the seminal fluid/sperm. These micro-organisms are added to those already present in the vagina and cervix.

Some LGTIs progress to an upper genital tract infection. The risk of this occurring is dependent on numerous factors, including the particular micro-organisms responsible for the LGTI. However, the conditions under which a LGTI progresses to PID are not well understood. It is questionable whether the risks of LGTIs are different for different methods of contraception, except for a reduced risk to women using spermicides that have bacteriocidal effects (68, 69).

Micro-organisms may be transmitted from the lower genital tract to the upper genital tract through the cervix, lymphatics, by passive transport, by attachment to sperm, in the case of IUD users by migration along the IUD tail string, or by direct extension following any gynecologic procedure in which an instrument is passed through the cervical canal and into the uterus. The relative importance of each of these different routes of transmission is unknown and more than one route may be operative concomitantly in the same woman.

There are physiologic reasons why the use of oral contraceptives, barrier contraceptives, spermicidal preparations, and contraceptive sterilization protect women against PID. For other reasons, women who use natural family planning methods of contraception may also be at a reduced risk of PID. On the other hand, the use of IUDs does not protect women from PID. Compared to a group of non-contraceptors with a similar risk of exposure to STPs, IUD users probably do not have a significantly higher risk of acquiring PID, except for the risk of PID resulting from the insertion procedure.

Much of the epidemiologic work to evaluate and identify risk factors associated with PID has been superficial and provides only minimally useful information for either clinical or public health decision makers. For the most part epidemiological studies have identified only broad cate-

gories of individuals who are at an increased risk of acquiring PID, e.g., women who are divorced/separated, black, or who have multiple sex partners. This knowledge is of limited clinical value since modifying these factors is difficult, if not impossible.

With the increasing spread of sexually transmitted diseases worldwide, it would be a mistake to erroneously attribute PID to the use of contraceptive methods that provide no protection against infection to the upper genital tract. The widespread use of methods that protect against sexually acquired pelvic infection will have a significant impact on the prevalence of PID, especially if these methods are used by women who are at a high risk of acquiring sexually transmitted pelvic infections.

CONCLUSIONS

Future research on the pathogenesis of PID should include the following:

1. Evaluation of the interrelationships between bacteriosperma and lower and upper genital tract infections;
2. Investigation of the mechanisms responsible for the presence of infections in the fallopian tubes, adnexa, and ovaries but not in the uterus;
3. Evaluation of the relationships between the use of different contraceptive methods and the risks of lower and upper genital tract infections;
4. Determination of the importance of bacterial transmission by sperm and trichomonads from the lower to the upper genital tract in the etiology of PID;
5. Determination of those specific aspects of sexual activity that are related to increased risks of acquiring lower or upper genital tract infections;
6. Epidemiologic investigations to identify the interrelationships of risk factors for acquiring PID;
7. Evaluation of the physiological and bacteriological events occurring at menstruation that predispose to the onset of PID;
8. Clarification of the difference between so-called primary and secondary bacteriologic pathogens as they relate to the female genital tract;
9. Evaluation of the effect of different contraceptive methods on host resistance as it relates to the onset of PID.

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