PD-AAT-056

44221

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SEMI-ANNUAL REPORT

January 1, 1985 - June 30, 1985

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
Suite 1525
875 N. Michigan Avenue
Chicago, Illinois 60611
(312) 664-0085

In compliance with
Cooperative Agreement DPE-0546-A-00-1003-00

TABLE OF CONTENTS

		Page
I.	REPORT SUMMARY	1
II.	PROGRAM ACCOMPLISHMENTS	2
	Scientific Summary	2
	LDC Involvement/Research Funds	5
	Administrative Summary	7
	Subagreement Negotiations	8
	Personnel	11
	Scientific Advisory Committee	12
	Consultants	14
III.	FINANCIAL	
	Detail of Expenditures and Commitments Under Current Agreement	16
	Expenditures for the Period 1/1/85-6/30/85 Under Current Agreement	17
IV.	RESEARCH	
	Technical Reports Received	18
	Summaries of Active Subagreements by AID Contraceptive Research Area	20
	Six Month Technical Report Summaries	36
V.	APPENDIX	
	Scientific Advisory Committee Minutes April 12, 1985	
	Research Frontiers in Fertility Regulation Volume 3, Number 3, February, 1985	
	Volume 3, Number 4, April, 1985	

REPORT SUMMARY

Project Title and Contract Number:

Program for Applied Research on Fertility Regulation
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, 1981 - June 30, 1986

Reporting Period: January 1, 1985 - June 30, 1985

Total Expenditures Through December 31, 1984: $5,063,595.00

Total Expenditures January 1, 1985 Through June 30, 1985: $1,337,527.21

Commitments Through June 30, 1985: $1,489,915.29

Total Appropriation 7/1/81-9/30/85: $10,060,000

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

PROGRAM ACCOMPLISHMENTS

Scientific Summary

1. Meeting of the Scientific Advisory Committee (SAC) was held on April 12, 1985. With PARFR staff, SAC reviewed extension, formal, informal, and pilot study 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 project development visits. During this reporting period, the following project development visits were made by PARFR staff:

   a. 1/6-8/85 -- Gerald I. Zatuchni, M.D., M.Sc., Bernhard Cinader, Ph.D., Vernon C. Stevens, Ph.D., Kenneth S.K. Tung, M.D.; at PARFR, Chicago, Illinois (PARFR-315 Goldberg LDH-C_4).

   b. 1/16/85 -- Alfredo Goldsmith, M.D., M.P.H., Gerald I. Zatuchni, M.D., M.Sc., David A. Blake, Ph.D., Gary D. Hodgen, Ph.D.; at PARFR, Chicago, Illinois (PARFR-309 Chatterton Anordrin Study).

   c. 3/7-9/85 -- John J. Sciarra, M.D., Ph.D. and Alfredo Goldsmith, M.D., M.P.H. visited Jacques Hamou, M.D. in Paris, France to discuss his research on the Intracervical Device (PARFR-350).

   d. 3/21/85 -- Alfredo Goldsmith, M.D., M.P.H. and Diane Krier-Morrow, M.B.A. visited Stolle Research and Development Corporation in Cincinnati, Ohio.

   e. 5/5-6/85 -- Alfredo Goldsmith, M.D., M.P.H., Diane Krier-Morrow, M.B.A., Robert T. Chatterton, Ph.D., Joyce Shelton, Ph.D.; Baylor College of Medicine, Houston, Texas. (PARFR-359 -- Principal Investigator - Bonnie S. Dunbar, Ph.D., Co-Investigator - Vernon C. Stevens, Ph.D., Ohio State University).

   f. 5/13-14/85 -- Alfredo Goldsmith, M.D., M.P.H., Andrzej Bartke, Ph.D., Danny H. Lewis, Ph.D.; The University of Texas Health Science Center at San Antonio, Texas. (PARFR-361 - Ricardo H. Asch, M.D.)

   g. 6/17/85 -- Alfredo Goldsmith, M.D., M.P.H., Diane Krier-Morrow, M.B.A., Lourens J.D. Zaneveld, D.V.M., Ph.D., Gerald I. Zatuchni, M.D., M.Sc.; at PARFR, Chicago, Illinois. (PARFR-369 and PARFR-370 - Milos Chvapil, M.D., Ph.D., D.Sc.)

3. Dr. Gerald Zatuchni presented a post-graduate course on Contraception at the Georgia Baptist Medical Center on January 7-8, 1985 in Atlanta, Georgia.

Scientific Summary (cont'd)

4. Dr. Gerald Zatuchni attended the Ortho Pharmaceutical Conference on Contraception on January 11-12, 1985 in New York, New York.

5. Drs. Alfredo Goldsmith and Gerald Zatuchni represented PARFR at an AID Poly NET 90 meeting on February 20, 1985 in Washington, D.C.

6. Dr. Gerald Zatuchni attended the G.E. Searle-sponsored symposium on Contraception on March 7-10, 1985 in the Bahamas.

7. Drs. Alfredo Goldsmith and John J. Sciarra represented PARFR at a Collaborating Agency meeting on March 11-12, 1985 at WHO in Geneva, Switzerland.

8. Dr. Gerald Zatuchni presented a post-graduate course on Contraception at Evanston Hospital on March 21, 1985 in Evanston, Illinois.

9. Dr. Gerald Zatuchni participated in the University of Utah Drug Delivery System conference on March 23-27, 1985 in Salt Lake City, Utah.

10. Dr. Gerald Zatuchni gave a presentation titled: "Update on Oral Contraceptive Steroids" at Northwestern University on April 16, 1986 in Chicago, Illinois.

11. Dr. Alfredo Goldsmith represented PARFR at the International Congress of the Society for Andrology meeting on April 28 - May 1, 1985, in Boston, Massachusettes.

12. Drs. Alfredo Goldsmith and Gerald Zatuchni and Diane Krier-Morrow attended an AID meeting on May 17, 1985 in Washington, D.C.

13. Dr. Alfredo Goldsmith represented PARFR at a WHO Steering Committee on Long-Acting Steroidal Contraception in Geneva, Switzerland on June 2-6, 1985.

14. Drs. Gerald Zatuchni, Nancy Alexander, Deborah Anderson, Bonnie Dunbar and Erwin Goldberg attended the PARFR-supported Immunocontraception Workshop at NIH on June 4-7, 1985 in Bethesda, Maryland.

15. Dr. Gerald Zatuchni participated in a post-graduate course on Intrauterine Devices and Barrier Contraception on June 10, 1985 in Chicago, Illinois.

Scientific Summary (cont'd)

16. PARFR 15th International Workshop, "Male Contraception: Advances and Future Prospects", was held May 28-31, 1985 in Geneva, Switzerland. The meeting was attended by 62 participants representing 13 countries.

17. During the current reporting period, the book, "Intrauterine Contraception", developed from the proceedings of PARFR's 14th International Workshop, was published and distributed.

18. Volume 3, Number 3 and Volume 3, Number 4 of PARFR's research technical information report were produced and distributed. The issue, Volume 3, Number 3, "Immunologic Method of Fertility Regulation: Report of a Workshop", was compiled by Mr. Jeffrey Spieler, Agency for International Development. The issue is a result of a collaborative effort on the part of all of those involved in the NIH/AID/PARFR Workshop on Research and Development of Immunologic Methods of Fertility Regulation which convened at NIH on April 16-18, 1984. The issue, Volume 3, Number 4, "Transcutaneous Male Sterilization", was authored by Alfredo Goldsmith, M.D., M.P.H., David A. Edelman, Ph.D., and Gerald I. Zatuchni, M.D., M.Sc.

19. Several PARFR multicenter clinical trial reports were prepared and accepted for publication in peer review journals.

PROGRAM ACCOMPLISHMENTS

LDC Involvement

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

1. PARFR-327C -- "Time Interval MCA/FEMCEPT Study"
   Rene Guzman-Serani, M.D., Instituto de Obstetrica y Ginecologia, Valdivia, Chile

2. PARFR-327V -- "Time Interval MCA/FEMCEPT Study"
   Itic Zighelboim, M.D., Maternidad "Concepcion Palacios", Caracas, Venezuela

3. PARFR-330M -- "A Clinical Evaluation of the Subdermal Contraceptive Norethindrone Pellet (Phase II)"
   Roberto Rivera, M.D., Instituto de Investigacion Cientifica, Durango, Mexico

LDC Research Funds

As of June 30, 1985, the following funds were budgeted or expended for research in LDCs:

Country & PARFR #	Budget (Dollars)	Total Expenditures To Date
ARGENTINA		
PARFR-367	$ 9,900	$ 6,930.00
BRASIL		
PARFR-318B	14,982	14,701.49
PARFR-328B	14,122	10,792.00
TOTAL BRASIL:	$29,104	$ 25,493.49
CHILE		
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	20,880.00
PARFR-341C	45,419	10,811.00
TOTAL CHILE:	$141,775	$107,167.00
EGYPT		
PARFR-300E	11,930	11,930.00
PARFR-314E	5,400	5,400.00
TOTAL EGYPT:	$17,330	$17,330.00
HONG KONG		
PARFR-366	5,302	565.54
MEXICO		
PARFR-300M	23,056	23,056.00
PARFR-330M	28,710	28,410.00
PARFR-341M	36,135	3,326.00
TOTAL MEXICO:	$87,901	$ 54,792.00
THAILAND		
PARFR-354	9,800	9,633.61
VENEZUELA		
PARFR-305V	24,049	21,512.10
PARFR-322V	3,600	3,600.00
PARFR-327V	19,535	17,043.23
TOTAL VENEZUELA:	$47,184	$ 42,155.33
TOTAL LDC:	$348,296	$264,066.97

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 agenda for the April 12, 1985 meeting of the Scientific Advisory Committee. The agenda included 4 formal proposals, 2 informal proposals, 2 extension proposals, 2 pilot study proposals and 15 technical reports.

3. Negotiating and executing:

   8 New Subagreements: 368, 369, 370, 371, 372, 373, 374, 376

   3 Additional Funding Amendments: 309, 359, 368

   6 No-Cost Extension Amendments: 309, 337T, 343, 352, 355, 361

4. Completion of entering our entire mailing list of 4,500 into our computer.

5. Mailing of our series, Research Frontiers in Fertility Regulation. We went to an external mailing service for distribution of Volume 3, No. 3 and Volume 3, No. 4 of our RFFR series. Because our entire mailing list is now completely entered into our computer, we were able to generate our mailing labels according to zip code order enabling the mailing service to use a bulk rate for our domestic envelopes, saving considerable money. We plan to continue this practice in the future for our RFFR series because of the saved expense.

6. Upgrading our Xerox machine to Xerox's 1075 Marathon copier. It is 1 1/2 times faster than our previous copier. Copy quality is much better. The speed aided us greatly in the preparation of the SAC agenda, as well as helping with our routine work.

7. Entering all PARFR Subagreements under the present cooperative agreement into our microcomputer. In addition to technical data, this database is updated regularly to keep track of the individual current expenditures as well as other changes there may be in each subagreement.

1/1/85 - 6/30/85

DPE-0546-A-00-1003-00

Subagreement Negotiations

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	ACTION	PERIOD	FUNDING
309	"Ovulation Inhibition by Anordrin" Robert T. Chatterton, Ph.D. Northwestern University Chicago, Illinois	No-cost Extension (Amendment #4)	1/1/82-6/30/85	
		Additonal Funding (Amendment #5)	1/1/82-6/30/85	$ 27,353 ($214,928 total)
337T	"Intracervical Device Acceptability Study" Rochelle N. Shain, Ph.D. The University of Texas Health Science Center at San Antonio San Antonio, Texas	No-cost Extension (Amendment #1)	7/1/84-6/30/86	
343	"NIH/Biotek Levonorgestrel Microcapsules" Lee R. Beck, Ph.D. The University of Alabama at Birmingham Birmingham, Alabama	No-cost Extension (Amendment #1)	11/1/83-4/30/85	
352	"Baboon Testing of Duration of NET from Fused Pellets" Lee R. Beck, Ph.D. The University of Alabama at Birmingham Birmingham, Alabama	No-cost Extension (Amendment #1)	12/1/83-5/31/85	
355	"Enhancement of the Secretory Immune Response to LDH-C_4" Nancy J. Alexander, Ph.D. Medical Research Foundation of Oregon Portland, Oregon	No-cost Extension (Amendment #1)	4/15/84-6/30/85	

1/1/85 - 6/30/85

DPE-0546-A-00-1003-00

Subagreement Negotiations

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	ACTION	PERIOD	FUNDING
359	"Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins" Bonnie S. Dunbar, Ph.D. Baylor College of Medicine Houston, Texas	Additional Funding (Amendment #1)	6/1/84-5/31/85	$ 8,663 ($86,404 total)
361	"Testosterone Microcapsule Formulation Study" Ricardo H. Asch, M.D. The University of Texas Health Science Center San Antonio, Texas	No-cost Extension (Amendment #2)	7/1/84-6/30/86	
368	"NIH/Biotek Levonorgestrel Microcapsules" E.S. Nuwayser, Ph.D. Biotek, Inc. Woburn, Massachusetts	New Subagreement	2/1/85-1/31/86	$ 74,965
		Additional Funding (Amendment #1)		$ 4,141 ($79,106 total)
369	"Transuterine Application of Bicompatible Hydrogel Hypan into Fallopian Tubes" Milos Chvapil, M.D., Ph.D., D.Sc. Bio-Products, Inc. Tucson, Arizona	New Subagreement	2/1/85-7/31/85	$ 3,979
370	"Sterilization of Male Dogs by Injecting Vas Deferens with Hydrogel Hypan" Milos Chvapil, M.D., Ph.D., D.Sc. Bio-Products, Inc. Tucson, Arizona	New Subagreement	2/1/85-10/31/85	$ 22,499
371	"Vaginal Spermicidal Barrier (VSB) Postcoital Tests" James R. Dingfelder, M.D. Eastowne Ob-Gyn and Infertility Chapel Hill, North Carolina	New Subagreement	4/1/85-3/31/86	$ 15,598

1/1/85 - 6/30/85

DPE-0546-A-00-1003-00

Subagreement Negotiations

PROJECT #	TITLE/INVESTIGATOR/INSTITUTION	ACTION	PERIOD	FUNDING
372	"Progestational Agents Effects on Baboon Endometrium" Danny H. Lewis, Ph.D. Stolle Research and Development Corporation. Cincinnati, Ohio	New Subagreement	3/1/85-10/31/85	$ 75,120
373	"90-Day Levonorgestrel Microspheres" Danny H. Lewis, Ph.D. Stolle Research and Development Corporation. Cincinnati, Ohio	New Subagreement	4/1/85-3/31/86	$ 67,404
374	"Progesterone Microspheres - Clinical Doses" Danny H. Lewis, Ph.D. Stolle Research and Development Corporation. Cincinnati, Ohio	New Subagreement	5/1/85-10/31/85	$ 40,799
376	"Pre-IDE Studies - Tubal Clip" C. Irving Meeker, M.D. Maine Medical Center Portland, Maine	New Subagreement	5/1/85-4/30/86	$ 29,774

PERSONNEL

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

Staff and Title	Effort in Man-Months	Salary
John J. Sciarra, M.D., Ph.D. Director and Principal Investigator	0.6	$ 3,266.52
Gerald I. Zatuchni, M.D., M.Sc. Director of Technical Assistance	5.1	27,763.98
Alfredo Goldsmith, M.D., M.P.H. Head, Research Project Development	5.7	31,030.50
Diane Krier-Morrow, M.B.A. Director of Administration	6.0	17,050.02
Mary Nemeth Project Controller	6.0	8,500.02
Kelley Osborn Publications Coordinator	0.6	3,270.00
Ruvenia Thomas Secretary II	6.0	10,887.50
Asenath Williamson Secretary I	6.0	7,558.46
Josephine Harris Secretary I	6.0	7,762.04

Fringe Benefits	$ 22,200.56
Indirect Costs	$131,847.64

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.	Southern Illinois University
David A. Blake, Ph.D.	The Johns Hopkins University School of Medicine
William Droegemueller, M.D.	University of North Carolina
Ronald H. Gray, M.D.	The Johns Hopkins University School of Hygiene and Public Health
Gary D. Hodgen, Ph.D.	Eastern Virginia Medical School
Kamran S. Moghissi, M.D.	Wayne State University
Dean L. Moyer, M.D. (Last meeting - 4/12/85)	University of Southern California School of Medicine
C. Alvin Paulsen, M.D.	University of Washington School of Medicine
Antonio Scommegna, M.D.	Michael Reese Hospital and Medical Center
Rochelle N. Shain, Ph.D.	The University of Texas Health Science Center at San Antonio
Anne Colston Wentz, M.D.	Vanderbilt University

The Committee met once during the current reporting period, on April 12, 1985, in Chicago, Illinois.

At the meeting, the Committee reviewed 4 formal, 2 extension, 2 informal, and 2 pilot study proposals. Fifteen technical reports, including 6 final reports, were also reviewed.

Of the 4 formal proposals reviewed, 3 were approved by the Committee. Dr. Erwin Goldberg's proposal, "Immunocontraception with Synthetic Antigenic Determinants of Lactate Dehydrogenase-C_4" and Dr. C. Irving Meeker's proposal, "A Tubal Plug and Clip Method for Female Sterilization", were approved as presented. Dr. Robert T. Chatterton's proposal "Ovulation Inhibition by Anordrin: Preclinical Testing", was approved with a request that toxicology and teratology studies be rewritten in order to comply with recent FDA guidelines.

Both of the 2 extension proposals were not approved as presented. However, the Committee suggested a site visit (Reproductive Biologist) to work with the PI on a modified proposal to be funded for Dr. Bonnie S. Dunbar's proposal, "Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins." The Committee reviewed Dr. John C.M. Tsibris' proposal, "Inter- and Intra-Cycle Variation of Genital Peroxidases in Women" and voted not to approve the proposal as presented. However, once all the data is available, the Committee will consider a revised proposal dealing with the effects of the ejaculate on GP and self-sampling of cervical mucus.

Of the 2 informal proposals, the Committee recommended for Dr. Ralph M. Richart's proposal, "Clinical Evaluation of Iodine Compound for Closure of the Human Fallopian Tube", that the PI should contact the FDA (Drug Division) and

hold an information discussion with them to decide what type of studies are needed for an IND. Once the information is obtained, a revised proposal will be submitted for the July SAC meeting.

Both of the pilot study proposals were voted not to approve.

Minutes of this 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 thereof. Included in this list of consultants are members of the Scientific Advisory Committee.

Consultant	Purpose	Effort	Fee
Nancy J. Alexander, Ph.D. Reproductive Physiology	Proposal Review 2/25/85		$ 50.00
Andrzej Bartke, Ph.D. Obstetrics and Gynecology Andrology	SAC 4/11-12/85	2 days	420.00
	Project Development Site Visit 5/13-14/85	2 days	420.00
David A. Blake, Ph.D. Obstetrics and Gynecology Pharmacology	Project Development Site Visit 3/27/85	1 day	210.00
	Proposal Review 3/27/85		50.00
	SAC 4/11-12/85	2 days	420.00
Robert T. Chatterton, Ph.D.	Proposal Review 3/27/85		50.00
	Project Development Site Visit 5/6-7/85	2 days	420.00
Bernhard Cinader, Ph.D., D.Sc. Immunology	Project Development Site Visit 1/6-8/85	3 days	630.00
William Droegemueller, M.D. Obstetrics and Gynecology	SAC 4/11-12/85	2 days	420.00
David A. Edelman, Ph.D. Biostatistician	Consultant Services 10/1-12/31/85	14 days	3,290.00
	Consultant Services 1/5-3/25/85	13.5 days	3,172.50
	RFFR, Vol. 3, No. 4		646.25
Ronald H. Gray, M.D. Epidemiology	SAC 4/11-12/85	2 days	420.00
Michael J.K. Harper, Ph.D., M.Sc. Obstetrics and Gynecology	Proposal Review 2/25/85		50.00
Gary D. Hodgen, Ph.D. Reproductive Biology	Project Development Site Visit 1/16/85	1 day	210.00
	SAC 4/11-12/85	2 days	420.00
Danny H. Lewis, Ph.D.	Proposal Review 4/5-7/85	2.5 days	$ 500.00
	Project Development Site Visit 5/14/85	1 day	200.00
	IND preparation March-May, 1985	18 days	3,600.00

Consultants (cont'd)

Michael Matthews	Regulatory Affairs, 1/2/85	1 day	200.00
Kamran S. Moghissi, M.D. Reproductive Endocrinology	Proposal Review 3/14/85	1 day	50.00
	Proposal Review 3/14/85	1 day	50.00
	SAC 4/11-12/85	2 days	420.00
Dean L. Moyer, M.D. Experimental Pathology	SAC 4/11-12/85	2 days	420.00
Noel R. Rose, M.D., Ph.D. Immunology	Proposal Review 3/7/85	1 day	50.00
Antonio Scommegna, M.D. Obstetrics and Gynecology Reproductive Endocrinology	SAC 4/11-12/85	2 days	420.00
Rochelle N. Shain, Ph.D. Social Anthropology	SAC 4/11-12/85	2 days	420.00
Joyce A. Shelton, Ph.D. Biochemistry	Project Development Site Visit 5/6-8/85	3 days	240.00
Vernon C. Stevens, Ph.D. Pharmacology	Project Development Site Visit 1/6-8/85	3 days	630.00
Kenneth Tung, M.D. Pathology	Project Development Site Visit 1/6-8/85	3 days	630.00
Thomas G. Wegmann, Ph.D. Immunology	Proposal Review 3/18/85	1 day	50.00
Anne Colston Wentz, M.D. Reproductive Endocrinology	SAC 4/11-12/85	2 days	420.00
Lourens J.D. Zaneveld, D.V.M., Ph.D.	Project Development Site Visit 6/17/85	1 day	210.00
		TOTAL	$19,808.75

DETAIL OF EXPENDITURES AND COMMITMENTS UNDER AID/DPE-0546-A-00-1003-00, EFFECTIVE 7/1/81-6/30/86

TOTAL AWARD: $12,363,280

TOTAL APPROPRIATION TO DATE: $10,060,000

Category	Budget Appropriation	%	7/1/81 - 6/30/85 Expenditures	Commitments	Total
Research	$ 6,465,006	64.2	$3,365,741.40	$1,277,800.06	$4,643,541.46
Workshops and Publications	451,374	4.5	391,501.21	33,250.00	424,751.21
Consultants	110,399	1.1	96,829.89	4,010.00	100,839.89
Travel	513,584	5.1	366,123.54	12,441.95	378,565.49
Salaries and Fringe Benefits	1,089,935	10.8	984,253.99	66,578.72	1,050,832.71
Supplies, Communications and Rent	605,941	6.0	472,964.33	29,585.33	502,549.66
Equipment	26,439	.3	12,511.35	- 0 -	12,511.35
Indirect Costs	802,322	8.0	711,196.50	66,249.23	777,445.73
	$10,060,000		$6,401,122.21	$1,489,915.29*	$7,891,037.50

* This figure includes known commitments as of 6/30/85. In addition, anticipated commitments to 6/30/86 for Research and Administrative Costs are estimated at $2,622,000.

EXPENDITURES UNDER AID/DPE-0546-A-00-1003-00
DURING THE PERIOD 1/1/85-6/30/85

Category	Expended 7/1/81-12/31/84	Expended 1/1/85-6/30/85	Total Expended 7/1/81-6/30/85
Research	$2,519,912.70	$ 845,828.70	$3,365,741.40
Workshops & Publications	333,072.81	58,428.40	391,501.21
Consultants	76,909.35	19,920.54	96,829.89
Travel	296,291.51	69,832.03	366,123.54
Salaries & Fringe Benefits	845,819.39	138,434.60	984,253.99
Supplies, Communications and Rent	399,729.03	73,235.30	472,964.33
Equipment	12,511.35	- 0 -	12,511.35
Indirect Costs	579,348.86	131,847.64	711,196.50
TOTAL:	$5,063,595.00	$1,337,527.21	$6,401,122.21

TECHNICAL REPORTS

The following technical reports were reviewed at the April 12, 1985 Scientific Advisory Committee Meeting:

Project	Period Covered by Report
PARFR-330M -- "A Clinical Evaluation of the Subdermal Norethindrone Pellet Implant (Phase II)"	4/1/83-11/30/84 FINAL
PARFR-330T -- "A Clinical Evaluation of the Bioabsorbable Contraceptive Norethindrone Pellet Implant (Phase II)"	11/15/84-3/15/85 FINAL
PARFR-337F -- "Use-Effectiveness of a Levonorgestrel-Releasing Intracervical Device"	10/1/84-12/31/84
PARFR-337T -- "Intracervical Device Acceptability Study"	12/1/84-2/28/85
PARFR-348 -- "Development of Improved Methods and Materials for Injecting Microencapsulated Steroids"	11/1/83-11/30/84 FINAL
PARFR-351 -- "Development of Methods for Female and Male Contraception Based on LH-RH Antagonist"	1/1/84-12/31/84 FINAL
PARFR-353 -- "Effect of Chronic Intrauterine Release of Estradiol and Progesterone on Uterine Histology in Intact Rabbits"	Through January 11, 1985 FINAL
PARFR-354 -- "Screening of Thai Plants for Proteins (or Lectins) as Potential Vaginal Contraceptives"	4/1/84-2/28/85
PARFR-355 -- "Enhancement of the Secretory Response to LDH-C_4"	10/1/84-12/31/84
PARFR-358 -- "Development of a 30-Day Injectable Contraceptive"	5/1/84-1/31/85 FINAL
PARFR-361 -- "Testosterone Microcapsule Formulation Study"	11/1/84-2/15/85
PARFR-362 -- "Combination Injectable Steroidal Microsphere - Continuation of PARFR-332"	10/1/84-3/1/85
PARFR-363 -- "Laboratory Studies on an Antispermatogenic Agent - THP for Control of Male Fertility"	10/1/84-3/15/85

Technical Reports (continued)

Project	Period Covered by Report
PARFR-364 -- "Antifertility Effects of Microencapsulated LHRH Agonist"	9/1/84-12/31/84
PARFR-367 -- "Fertility Inhibition of *In Vivo* Immunization with Epididymal Proteins in Hamsters"	11/1/84-2/22/85

RESEARCH

PARFR SUBAGREEMENT EXPENSE SUMMARIES

This section summarizes the expenses of PARFR subagreements active during the period January 1, 1985 to June 30, 1985. Summaries are categorized according to the following AID Contraceptive Research Areas:

I. FEMALE STERILIZATION

- A. Surgical
- B. Transcervical
- C. Reversible
- D. Other

II. MALE STERILIZATION

- A. Reversible
- B. Non-Reversible
- C. Other

III. INTRAUTERINE CONTRACEPTION

IV. STEROIDAL CONTRACEPTION - FEMALE

- A. Injectable
- B. Implants
- C. Orals
- D. Other

V. STEROIDAL CONTRACEPTION - MALE

- A. Injectable
- B. Implants
- C. Orals
- D. Other

VI. NEUROPEPTIDES

- A. Female
- B. Male

VII. OTHER PHARMACEUTICAL AGENTS

- A. Female
- B. Male

VIII. BARRIER CONTRACEPTION

- A. Female
- B. Male

IX. IMMUNOCONTRACEPTION

- A. Female
- B. Male

X. MISCELLANEOUS

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

I. FEMALE STERILIZATION

B. TRANSCERVICAL

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
327C	Instituto de Obstetricia y Ginecologia de Universidad Austral de Chile Valdivia, Chile Rene Guzman-Serani, M.D.	"Time Interval MCA/FEMCEPT Study"	12/1/82-11/30/84	$ 20,880	$ 360.00	$20,880.00
369	Bio-Products, Inc. Tucson, Arizona Milos Chvapil, M.D., Ph.D., D.Sc.	"Transuterine Application of Biocompatible Hydrogel Hypan into Fallopian Tubes"	2/1/85-7/31/85	3,979	1,961.00	1,961.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

I. FEMALE STERILIZATION (continued)

C. REVERSIBLE

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
350	Tenon Hospital, University of Paris Paris, France Jacques Hamou, M.D.	"An Intra Tubal Device (ITD) for Female Sterilization"	1/1/84-6/30/85	$ 11,000	$ 2,805.00	$ 8,470.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

II. MALE STERILIZATION

A. REVERSIBLE

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
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	$ 37,476	$ 6,914.61	$29,789.10
339UI	University of Illinois at Chicago Chicago, Illinois Donald P. Waller, Ph.D.	"Toxicology of Silicone Implanted (SHUGS) in the Vas Deferens"	7/1/84-6/30/85	55,780	5,714.63	7,319.97

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

II. MALE STERILIZATION (continued)

C. OTHER

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
363	The University of Western Ontario London, Ontario, Canada John P. Wiebe, Ph.D.	"Laboratory Studies on an Antispermatogenic Agent - THP for the Control of Male Fertility"	10/1/84-3/31/86	$90,000	$25,864.75	$31,356.16
370	Bio-Products, Inc. Tucson, Arizona Milos Chvapil, M.D., Ph.D., D.Sc.	"Sterilization of Male Dogs by Injecting Vas Deferens with Hydrogel Hypan"	2/1/85-10/31/85	22,499	8,496.00	8,496.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

III. INTRAUTERINE CONTRACEPTION

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
337F	University of Helsinki Helsinki, Finland Tapani Luukkainen, M.D., Ph.D.	"Use-Effectiveness of a Levonorgestrel-Releasing Intracervical Device"	5/1/83-4/30/86	$ 97,537	$16,823.33	$60,885.53
337T	The University of Texas Health Science Center San Antonio, Texas Rochelle N. Shain, Ph.D.	"Intracervical Device Acceptability Study"	6/1/83-6/30/86	12,502	4,464.46	5,424.37
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"	11/1/83-6/30/85	98,694	33,354.83	84,864.91
353	Michael Reese Hospital and Medical Center Chicago, Illinois Antonio Scommegna, M.D.	"Effects of Chronic Intrauterine Release of Estradiol and Progesterone on Uterine Histology in Intact Rabbits"	3/15/84-12/31/84	7,895	7,895.00	7,895.00
D-52	Southern Research Institute Birmingham, Alabama	Preparation of an IND for the Fibrous E/P IUD Delivery System		5,000	- 0 -	- 0 -

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

IV. STEROIDAL CONTRACEPTION - FEMALE

A. INJECTABLE

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
309	Northwestern University Medical School Chicago, Illinois Robert T. Chatterton, Ph.D.	"Ovulation Inhibition by Anordrin"	1/1/82-6/30/85	$214,928	$33,884.88	$167,162.14
341	The University of Alabama at 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	- 0 -	36,008.46
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 -	1,971.63
341C	Centro Nacional de la Familia Santiago, Chile Horacio B. Croxatto, M.D. and Soledad Diaz, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84-12/31/85	45,419	- 0 -	10,811.00
341I	Associazione per Studio della Riproduzione Umana Roma, Italy Giuseppe Benagiano, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84-12/31/85	$ 39,655	- 0 -	2,803.35
341M	Instituto de Investigacion Cientifica, University of Durango Durango, Mexico Roberto Rivera, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84-12/31/85	36,135	- 0 -	3,326.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

IV. STEROIDAL CONTRACEPTION - FEMALE (continued)

A. INJECTABLE (continued)

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
341T	The University of Texas Health Science Center San Antonio, Texas Jose P. Balmaceda, M.D. and Ricardo H. Asch, M.D.	"Phase II Poly NET 90 Injectable Study"	1/1/84-12/31/85	$ 82,487	$ 6,285.77	$25,134.85
343	The University of Alabama at Birmingham Birmingham, Alabama Lee R. Beck, Ph.D.	"NIH/Biotek Levonorgestrel Microcapsules"	11/1/83-4/30/85	46,040	- 0 -	41,149.86
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	5,056.00	11,800.00
362	Stolle Research and Development Corp. Birmingham, Alabama Danny H. Lewis, Ph.D.	"Combination Injectable Steroidal Microsphere - Continuation of PARFR-332"	10/1/84-5/31/85	67,195	57,683.00	57,683.00
365	Southern Research Institute Birmingham, Alabama Thomas R. Tice, Ph.D.	"Optimization of Progesterone Microcapsule System"	9/1/84-12/31/84	7,998	7,998.00	7,998.00
368	Biotek, Inc. Woburn, Massachusetts E.S. Nuwayser, Ph.D.	"NIH/Biotek Levonorgestrel Microcapsules"	2/1/85-1/31/86	79,106	35,999.30	35,999.30

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

IV. STEROIDAL CONTRACEPTION - FEMALE (continued)

A. INJECTABLE (continued)

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
372	Stolle Research and Development Corporation Cincinnati, Ohio Danny H. Lewis, Ph.D.	"Progestational Agents Effects on Baboon Endometrium"	3/1/85-10/31/85	$ 75,120	$18,780.00	$18,780.00
373	Stolle Research and Development Corporation Cincinnati, Ohio Danny H. Lewis, Ph.D.	"90-Day Levonorgestrel Microspheres"	4/1/85-3/31/86	67,404	5,617.00	5,617.00
374	Stolle Research and Development Corporation Cincinnati, Ohio Danny H. Lewis, Ph.D.	"Progesterone Microspheres - Clinical Studies"	5/1/85-10/31/85	40,799	- 0 -	- 0 -
D-44	Northwestern University Chicago, Illinois	Animal Purchase and Care - 12 Cynomologous Monkeys for Anordrin Study		4,620	1,244.67	5,405.78
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-66	IIT Research Institute Chicago, Illinois	"Best Effort" Synthesis of 6 grams of Anordrin under GMP		30,000	20,000.00	20,000.00
D-67	Amersham Corporation Arlington Heights, Illinois	Custom preparation of 2mCi of Anordrin		16,000	- 0 -	- 0 -

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

IV. STEROIDAL CONTRACEPTION - FEMALE (continued)

B. IMPLANTS

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
330M	Instituto de Investigacion Cientifica Durango, Mexico Roberto Rivera, M.D.	"A Clinical Evaluation of the Subdermal Contraceptive Norethindrone Pellet (Phase II)"	4/1/83-9/30/84	$ 28,710	$ 3,838.00	$28,410.00
330T	The University of Texas Health Science Center San Antonio, Texas Ricardo H. Asch, M.D.	"A Clinical Evaluation of the Bioabsorbable Subdermal Contraceptive Norethindrone Pellet Implant (Phase II)"	3/1/83-2/28/85	48,139	16,535.41	43,888.99
352	The University of Alabama at Birmingham Birmingham, Alabama Lee R. Beck, Ph.D.	"Baboon Testing of Duration of NET from Fused Pellets"	12/1/83-5/31/85	29,420	- 0 -	19,892.68
D-65	Cornell Medical Center New York, New York	Radioimmunoassays of NET in Serum Samples for Implant Study			4,207.50	4,207.50

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

V. STEROIDAL CONTRACEPTION - MALE

A. INJECTABLE

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
361	The University of Texas Health Science Center San Antonio, Texas Ricardo H. Asch, M.D.	"Testosterone Microcapsule Formulation Study"	7/1/84-6/30/86	$122,106	$60,483.80	$60,483.80

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
7/1/84-12/31/84

VI. NEUROPEPTIDES

A. FEMALE

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
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"	2/1/84-1/31/86	$145,501	$55,566.46	$80,312.79
364	The University of Texas Health Science Center San Antonio, Texas Francisco J. Rojas, Ph.D. and Ricardo H. Asch, M.D.	"Antifertility Effects of Microencapsulated LHRH Agonist"	9/1/84-6/30/86	64,218	11,148.75	11,148.75

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

VIII. BARRIER CONTRACEPTION

A. FEMALE

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
338	Rush-Presbysterian-St. Luke's Medical Center Chicago, Illinois Lourens J.D. Zaneveld, D.V.M., Ph.D.	"Development of Sperm Enzyme Inhibitors as Vaginal Contraceptives"	7/1/83-6/30/86	$160,950	$20,883.74	$79,005.62
338UI	The University of Illinois at Chicago Chicago, Illinois Donald P. Waller, Ph.D.	"Toxicology Studies of Acrosin Inhibitors"	7/1/84-6/30/86	84,866	21,791.36	23,047.28
371	Eastowne Ob-Gyn and Infertility Chapel Hill, North Carolina James R. Dingfelder, M.D.	"Vaginal Spermicidal Barrier (VSB) Postcoital Tests"	4/1/85-3/31/86	15,598	- 0 -	- 0 -

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

IX. IMMUNOCONTRACEPTION

A. FEMALE

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
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"	3/1/82-6/30/85	$199,184	$51,935.13	$173,849.49
351	The Tulane University School of Medicine 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	65,153	19,106.18	60,702.31
355	Medical Research Foundation of Oregon Beaverton, Oregon Nancy J. Alexander, Ph.D.	"Enhancement of the Secretory Immune Response to LDH-C_4"	4/15/84-6/30/85	47,335	15,344.46	37,603.85
356a	The George Washington University Washington, D.C. Rajesh K. Naz, Ph.D.	"Development of an Immunocontraceptive Vaccine: Role of 23-Kd Antigen in Immunoinfertility and Fertility Regulation"	12/1/84-8/31/85	59,593	25,534.94	25,534.94
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	86,404	36,976.29	64,475.22
D-32	Biologic Resources Laboratory, University of Illinois at Chicago Chicago, Illinois	Animal maintenance, papio baboons for PARFR-315, and rhesus monkeys for PARFR-317			10,191.55	95,181.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

IX. IMMUNOCONTRACEPTION (continued)

B. MALE

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
351	The Tulane University School of Medicine 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	$ 65,153	$19,106.18	$ 60,702.31
366	University of Hong Kong Hong Kong, Hong Kong Steven Y.W. Chan, Ph.D.	"Immunological Contraception - Study on the Time Course of Sperm Antibodies Production in Rabbits Following Intravasal Injection of BCG (Bacillus Calmette Guerin)"	10/1/84-3/31/86	5,302	565.54	565.54
367	Instituto de Biologia y Medicina Experimental Buenos Aires, Argentina Jorge A. Blaquier, M.D.	"Fertility Inhibition of In Vivo Immunization with Epididymal Proteins in Hamsters"	11/1/84-10/31/85	9,900	6,930.00	6,930.00

RESEARCH PROJECTS BY AID CONTRACEPTIVE RESEARCH AREA
UNDER COOPERATIVE AGREEMENT AID/DPE-0546-A-00-1003-00
1/1/85-6/30/85

X. MISCELLANEOUS

PARFR #	INSTITUTION & INVESTIGATOR(S)	TITLE	DATES	BUDGET	EXPENDITURES THIS PERIOD	TOTAL EXPENDITURES
354	Mahidol University Bangkok, Thailand Montri Chulavatnatol, Ph.D.	"Screening of Thai Plants for Proteins (or Lectins) as Potential Vaginal Contraceptives"	4/1/84-3/31/85	$ 9,800	$ 5,858.17	$ 9,633.61
360	University of Illinois at Chicago Chicago, Illinois John C.M. Tsibris, Ph.D.	"Inter- and Intra-Cycle Variation of Genital Peroxidases in Women"	7/1/84-6/30/85	28,127	20,766.85	22,664.98
D-60	Reproductive Endocrinology Laboratory Chicago, Illinois	Measurement of LH and Pregnanediol Glucuronide relating to PARFR-360		7,200	4,520.00	7,200.00

FOLLOWING ARE SIX MONTH TECHNICAL REPORT SUMMARIES

OF ALL PROJECTS DURING THIS PERIOD

1/1/85 - 6/30/85

Projects are listed by PARFR number and not by

"Contraceptive Research Area."

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 309

JUL 1985

TITLE: "Ovulation Inhibition by Anordrin"

INSTITUTION: Northwestern University Medical School

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

FUNDING PERIOD: 1/1/82-6/30/85 AMOUNT FUNDED: $214,928

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

Work done during this reporting period was directed toward preclinical evaluation of anordrin.

1. Five monthly injections of anordrin resulted in 7.0±0.8 months of amenorrhea; all monkeys had evidence of an ovulatory cycle after the first post-treatment menstrual period.

2. The highest production of urinary pregnanediol occurred in all monkeys after the first injection. Two monkeys exceeded 2 μg/day during this time (mean mid-luteal phase pregnanediol in control cycles is 4 μg/day) and both had one day of vaginal bleeding shortly thereafter. Progesterone was elevated the week before menses, approximately 6 months later, in all monkeys.

3. Sporadic peaks of urinary LH occurred during anordrin treatment. As many as 6 peaks occurred during a 48 day treatment cycle. The mean concentration of LH on the day of the peaks was 27.6±7.2 (SD) ng/ml. By comparison, the mid-cycle surge of LH associated with ovulation in normal, untreated monkeys was 28.8±13.7 (SD) ng/ml.

4. Serum estradiol measured weekly throughout the study remained at early follicular-phase levels in all monkeys, 28.1±2.8 pg/ml. No values were in the preovulatory range.

5. Vaginal smears were maintained at 10:1 cornified to basal cells in treated monkeys until after the first menses when the ratio returned to lower values. Normal cycles are characterized by approximately 1:1 ratios in the follicular phase, and a rapid rise at about the time of ovulation to 10:1 or more. The 10:1 ratio is maintained until menstruation.

6. Blood coagulation factors antithrombin III and protein C were analyzed monthly from the first through the fourth month during treatment. The values remained constant throughout the study. The antithrombin III values at the beginning and end of this period were 177±32 (SD) and 173±28 (SD) as a percent of the normal human standard. The comparable values for protein C were 297±47 and 300±38.

7. Two monkeys have been given intravenous injections of ^{3}H-anordrin and one has received an intramuscular dose. Serum, urine, and feces samples have been collected for studies of pharmacokinetics. Preliminary results for volume of distribution of anordrin indicate substantial protein binding by the drug; he V_d was 863 L/kg bw. Clearance was also slow as expected, 6.5 ml/min/kg. This is a half-life of about 7 days. Absorption from an intramuscular injection site is also slow; a plateau of serum concentrations was not reached until about 5 days after the injection.

Robert Chatterton
SIGNATURE

7/12/85
DATE

37

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 315

TITLE: "Immunologic Suppression of Fertility by Synthetic Antigenic Determinants of Lactate Dehydrogenase-C_4, Extension of PARFR-232

INSTITUTION: Northwestern University

PRINCIPAL INVESTIGATOR: Erwin Goldberg, Ph.D.

FUNDING PERIOD: 3/1/82-6/30/85 AMOUNT FUNDED: $199,184

Below is a project summary for the January 1, 1985-June 30, 1985 period.

The objective of this project is to develop a contraceptive vaccine based on synthetic peptides containing antigenic determinants of sperm-specific LDH-C_4. Female baboons have been immunized with MC5-15, one such peptide, conjugated to diphtheria toxoid (DT). The nature of the immune response, suppression of fertility and its reversibility, as well as possible immunopathological effects are being examined.

Fourteen female baboons received immunizations consisting of DT MC5-15 in squalene and arlacel A plus CGP 11, 637 adjuvant. Immunizations were timed to each individual's menstrual cycle. Fourteen control animals received the adjuvant plus vehicle alone. Experimental animals all responded by making antibodies to the MC5-15 peptide that also reacted with LDH-C_4. No such antibodies were detected in control animals. The animals were then mated four times or until pregnant. Matings commenced in controls after their 4th and in experimentals after their 8th immunization. Only 3 experimental animals became pregnant, compared to 10 pregnancies in controls.

Immunization of experimental animals was discontinued after the fourth mating. Non-pregnant females were then mated up to seven additional times. Serum antibody levels were monitored and as these levels decreased, 8 out of 11 (73%) of the experimental animals conceived. Thus, the immunosuppressive effect of the injections on fertility was reversible.

Two of the DT-MC5-15 immunized females were studied to determine whether antibodies were detectable in their reproductive tract secretions following systemic immunization. In both animals, antibodies specific for LDH-C_4 were detectable by ELISA in oviductal fluids and cervical mucous. Antibody levels in oviductal fluids appeared to peak at approximately the same time as serum antibody levels. However, although both females had nearly the same serum antibody titer, the oviductal fluid titer was much higher in one than in the other. This result may indicate that monitoring the serum levels is not necessarily the best indication of the amount of antibody in the reproductive tract.

Tissue samples from kidney, liver, ovary, oviduct, uterus and lymph node were removed from these two animals for immunopathological studies. These are now being performed by Dr. K.S.K. Tung, at the University of New Mexico School of Medicine, Albuquerque, NM. Extensive chemical analysis of their serum including a screen for anti-nuclear antibodies and rheumatoid arthritis factor is also in progress. Previous medical examinations of these and the other immunized baboons do not indicate that any abnormalities resulting from the immunizations will be found.

Erwin Goldberg
SIGNATURE

July 11, 1985
DATE

38

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

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 January 1, 1985 - June 30, 1985 (FINAL) period.

This represents a final report on the study Phase II Bioabsorbable Subdermal Contraceptive Norethindrone Pellet Implant PARFR 330T. We were contracted to compare the effects of subdermal implants of 3 or 4 bioabsorbable pellets of Norethindrone in order to identify the lowest dose and duration that is contraceptively effective. A total of 19 subjects were recruited and randomly received implants of 3 (n=11) or 4 (n=8) pellets of 35mg each in the forearm without difficulty under local anesthesia. Data was collected regarding cycle length, blood loss, ovulation, steroid levels, lipoprotein profiles (HDL, LDL, VLDL, cholesterol) and blood chemistries (SMAC 24) on all subjects. Pellets were removed after 6 months from 15 of the subjects, the remaining 4 subjects continued in the study for a total period of 1 year.

None of the subjects became pregnant. Serious side effects were observed in only one subject, consisting of nausea and vomiting and a membranous dysmenorrhea with passage of an endometrial cast one month after implant. Removal of the pellets produced almost immediate dissappearance of these symptoms.

Blood samples collected during the study showed no significant changes in SMAC and lipoprotein profiles. Absence of ovulation was evidenced in most subjects as determined by serial progesterone levels.

Bleeding calendars were collected from all subjects. Cycle length and blood loss varied greatly among individuals in both groups with one subject who received 4 pellets being amenorrheic for the duration of the study.

SIGNATURE

July 10, 1985
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

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: 5/1/83-4/30/86 AMOUNT FUNDED: $97,537

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

Project was terminated as of June 30, 1985 because the modified ICD vector still resulted in a higher expulsion rate than the levonorgestrel IUD and the most recent generation of copper IUDs and PARFR does not have access to the master file on levonorgestrel. Hence, the chances for an IND were minimal.

Continuation of this project will probably be funded by ICCR since they have access to the master file.

SIGNATURE

DATE

40

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

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: 6/1/83-6/30/86 **AMOUNT FUNDED:** $12,502

Below is a project summary for the January 1, 1985 - June 30, 1985 **period.**

To date a total of 566 questionnaires have been received from Finland. These include 260 initial interviews (109-ICD, 85-LNG IUD, and 66-Nova T); 225 3-month follow-up interviews (103-ICD, 70-LNG IUD, and 52-Nova T); and 81 12-month interviews (68-ICD, 6-LNG IUD, and 7-Nova T). A total of 492 instruments have been coded; preliminary analysis has been conducted and the resultant data have been presented at the June, 1985 ICCR meeting at the Population Council.

Preliminary results (initial intake and 3-month follow-up data) indicate that both the ICD and LNG IUD devices are associated with lighter menses, more irregular cycles, more spotting, less cramping and some amenorrhea. On the other hand, the Nova T is associated with heavier menses and more cramping. These subjectively perceived menstrual changes are consistent with expectations. On most 3-month follow-up measures (can feel device, husband can feel device, change in desire for sexual intercourse, presence of especially bothersome side effects, overall satisfaction with device, overall husband satisfaction with device, confidence in device, would recommend the device to others, etc.) there are no significant differences between groups. Most users (between 89% and 95%) of all three methods are either very satisfied or satisfied with their device. However, significantly more ICD users have either expelled their device or discontinued use (9.6% of ICD users compared to no users in the two control groups). Almost half of these discontinuations were due to expulsions. (This may be associated with the higher incidence of nulliparity among the ICD users).

On the other hand, ICD users are significantly more satisfied than controls with their current device than they were with previously used IUD's. This seeming discrepancy is explained by group differences in prior contraceptive behavior patterns. Significantly more ICD users than members of the other control groups (1) stopped using a contraceptive method because of dissatisfaction (more methods were discontinued in this group as well), (2) were dissatisfied with the last method that they used, (3) experienced unacceptable side effects from oral contraceptives, (4) were dissatisfied with their prior IUD (5) experienced unacceptable side effects from their prior IUD, and (6) were dissatisfied with prior mechanical methods. While there were no basic sociodemographic group differences (other than a higher incidence of nulliparity among ICD users), the ICD group as a whole appeared to be considerably more particular about contraceptive methods in general and/or sensitive to side effects. This finding was confirmed by Tapani Luukkainen, M.D.: apparently, many women who selected the ICD were dissatisfied with other contraceptive methods and joined the study group in expection of finding a more satisfactory method.

Preliminary correlations indicate that prior contraceptive behavior patterns, in addition to other factors, are correlated with current dissatisfaction and discontinuation among all three groups. Examples of such patterns include having

Rochelle Shain

SIGNATURE

July 2, 1985

DATE

discontinued a prior IUD because of dissatisfaction, overall dissatisfaction with prior IUD use, experience of side effects with a previous IUD, the tendency to perceive side effects from contraceptive methods in general, and having felt (subject and/or partner) a prior IUD. Further analyses are necessary to determine if controlling for these variables eliminates group differences in dissatisfaction and discontinuation.

In summary, all three devices (ICD, LNG IUD and Nova T IUD) appear highly acceptable to the Finnish sample. Whereas there have been significantly more discontinuations among ICD users, this population appears unique, in that they have been more critical of contraceptive methods in general.

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

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: 7/1/83-6/30/86 AMOUNT FUNDED: $160,950

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

The objective of this project is to develop inhibitors of the sperm enzyme acrosin, specifically aryl 4-guanidinobenzoates, as a new type vaginal contraceptive with high potency and low toxicity. To date, a number of aryl 4-guanidinobenzoates have been synthesized with phenol portions that are compounds approved by the FDA for human use. These aryl 4-guanidinobenzoates are very active inhibitors of acrosin and several are about 100 times more potent as vaginal contraceptives in the rabbit than nonoxynol-9. The acute toxicity of all the synthesized inhibitors is less than that of nonoxynol-9. The aryl 4-guanidinobenzoates were also shown to inhibit the fertilizing capacity of human spermatozoa. Four inhibitors were selected for further study and were shown to possess low subacute toxicity. During the past six months, studies were performed to select one compound from these four that would be tested in women by a Phase I Clinical Trial. These four aryl 4-guanidinobenzoates were shown to possess spermicidal activity at dose levels that are lower than those that we intend to place vaginally. On mixing with whole semen at low concentrations for maximally 2 minutes, the compounds contact spermatozoa and inhibit acrosin even if the spermatozoa are washed after the two minute incubation period (as occurs during cervical mucus passage). The sponge was selected as the vaginal delivery system and experiments have shown that the sponge material does not alter the activity of the inhibitors and that the inhibitors can be released at high concentrations from the sponge. These as well as vaginal irritation experiments have led to the selection of acetominophen guanidinobenzoate (AGB) as the compound that will be used for the the Phase I Clinical Trial. The compound is presently being synthesized in large quantities, its vaginal irritation and subacute toxicity properties are being evaluated in detail, and the sponge is further being developed as delivery system for this agent. The expected outcome of this work is availability of a new vaginal contraceptive agent that is more potent and less toxic than nonoxynol-9.

L J D Zaneveld
SIGNATURE

7/16/85
DATE

12

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 338UI

TITLE: "Toxicology Studies of Acrosin Inhibitors"

INSTITUTION: The University of Illinois at Chicago

PRINCIPAL INVESTIGATOR: Donald P. Waller, Ph.D.

FUNDING PERIOD: 7/1/84-6/30/86 AMOUNT FUNDED: $84,866

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

Toxicology of Acrosin Inhibitors

The primary objective of this project is to evaluate the relative toxicity of several acrosin inhibitors (arylguanidinobenzoates) which are potential vaginal contraceptive agents. This information in combination with data on efficacy would then be used to select a single agent to be developed for Phase I clinical trials. Vaginal irritation and subchronic toxicology testing would then be performed on the selected inhibitor to support an IND for the drug. In previous reporting periods we evaluated the vaginal irritation of several acrosin inhibitors. A single compound has been selected and during this reporting period we began the development of the methods required for pharmacokinetic studies to monitor absorption and systemic exposure of the selected agent. GLP protocols for the subchronic testing have been written. When formulated compound is available it will be tested in both the vaginal irritation assay and subchronic toxicity testing. Monitoring of blood levels of animals dosed by the vaginal route will also be performed. These data will then be used to support an IND application.

SIGNATURE

12 July 1985
DATE

42

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

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: 7/1/83-6/30/85 AMOUNT FUNDED: $37,476

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

The objective of this project is to develop a reversible vas deferens occlusive device. To date, our studies have shown that a double silicone plug with the two parts held together by a nylon thread, completely obstructs sperm transport when placed in the vas deferens of primates and that normal sperm passage occurs again when the device is removed. During the past six months, an amendment was written to the FDA in request of a Phase I Clinical Trial. Additionally, a number of studies were performed to allow implantation of the device in the rat vas deferens so that the required toxicity studies can be performed. A reasonably satisfactory technique was found and 400 rats have been and are being implanted with the device. The rats will be euthanized after 6, 12 and 24 months and the site specific toxicity determined. Implantation of the device into the human vas deferens is not optimal as yet and techniques are being studied to make implantation very simple using a mechanical inserter. Contact is maintained with the FDA so that assurance can be obtained that a Phase I Clinical Trial will be approved within the next six months. The expected outcome of this work is the availability of a device that can readily and easily be implanted into the vas deferens, will obstruct sperm transport when in place and will allow the ejaculation of spermatozoa again when removed.

L J D Zaneveld
SIGNATURE

7/16/85
DATE

44

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 339UI

TITLE: "Toxicology of Silicone Implanted (SHUGS) in the Vas Deferens"

INSTITUTION: University of Illinois at Chicago

PRINCIPAL INVESTIGATOR: Donald P. Waller, Ph.D.

FUNDING PERIOD: 7/1/84-6/30/85 AMOUNT FUNDED: $55,780

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

Toxicology of Silicone Implanted in the Vas deferens

The primary objective of this project is to determine the effects of silicone implanted in the vas deferens of the rat. Silicone is the primary material used in the construction of the SHUG, a reversible plug device for the male. Previously we determined the appropriate diameter for implantation in the rat vas deferens and began developing the surgical procedure for implanting the silicone in the rat. The method for implanting the device required a great deal of work because of the nature of the silicone material and the small size of the rat vas deferens. During the end of this reporting period a method was finalized and the GLP protocols finalized. The implantation of silicone in the four hundred test animals has begun and will be completed shortly. Autopsies of groups will be performed after six months, one year and two years of exposure. Phase I clinical trials will be initiated if FDA approval can be obtained following the autopsy of the six month exposure group.

SIGNATURE

12 July 1985
DATE

45

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

AUG 1985

PARFR- 341

TITLE: "Phase I Poly NET 90 Injectable Study"

INSTITUTION: The University of Alabama in Birmingham

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

FUNDING PERIOD: 12/1/83 - 11/30/85 AMOUNT FUNDED: $79,264

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

Ten subjects will be treated with one of three doses of Poly NET 90 to determine the optimal dose for a multi-center Phase II clinical trial using the same formulation. Two subjects voluntarily withdrew from the study during their control cycle, effecting a delay in completing the treatments. Nine of the ten subjects have been treated, with three subjects each receiving 50, 75 or 100 mg norethisterone (NET) contained in PLGA microspheres. There have been no reported side effects following treatment. The last patient will receive the 100 mg dose. Blood samples are being obtained at weekly intervals to provide sufficient data for selection of the dose to be used in the Phase II trials. Posttreatment serum NET levels determined to date exceed 0.70 ng/ml, and all values are less than 4.00 ng/ml. Intergroup comparisons will be made when an adequate number of posttreatment samples have been obtained from the subjects in each group. A meeting is planned for the end of September to select the dose for use in the Phase II clinical trials.

SIGNATURE

26, 1985

DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

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 January 1, 1985-June 30, 1985 period.

Each of four women received a single injection of 75 mg NET poly (DL-lactide-co-glycolide) microspheres formulation. Blood samples were drawn during one pretreatment cycle in 2 of these women to determine estrogen and progesterone levels and assess the occurrence of ovulation. After drug administration, clinical follow up and blood sampling were continued in all until one or two normal cycles were detected. During treatment, blood samples were obtained once or twice a week in 2 women during month 1, in one during month 2, in all women during months 3 to 6 and in 2 women during month 7. An aliquot of each serum sample was lyophilized and send do Dr. Lee Beck in Alabama for NET determination. The rest of each sample was frozen for measuring estradiol and progesterone. Plasma progesterone levels compatible with ovulation were observed at various intervals after drug administration in the 4 cases.

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.

M Croxatto
SIGNATURE

June 27th 85
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

JUL 1985

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 January 1, 1985 - June 30, 1985 period.

We were contracted to test the safety, contraceptive effectiveness and effect on ovarian function of the 75 mg injectable NET poly (DL-lactidecoglycolide) microphere on up to 200 subjects.

Premature termination of Phase II was necessitated by an inadequate rise in serum NET levels after injection of the microcapsule as compared to the levels seen in Phase I.

Of the 15 original subjects injected 1 dropped out for personal reasons and 5 opted for a systemic contraceptive method, making them ineligible to continue further with the modified study. The remaining 9 subjects continued with blood sampling for serial serum NET levels in the months following injection.

All of the subjects have completed their blood sampling, termination visits have been performed and menstrual charts have been collected.

Eight of the thirteen subjects that were followed after injection, have progesterone serum levels suggestive of ovulation during the first 30 days after injection. These results are in absolute contradiction with Phase I results at our center. Furthermore, of the nine subjects that completed more than 90 days of follow-up after injection 7 had evidence of ovulation in that period.

Serum NET levels did not rise until nine weeks post injection, peaking around 12 weeks and then declining sharply over the next 3 weeks. These results are in contradiction to Phase I where NET levels rose immediately after injection and gradually declined until a second rise was evident between days 90-120. However, consistent with Phase I, ovulation was suppressed in all subjects during the weeks correlating with the higher serum NET levels.

Menstrual bleeding patterns were also analyzed in the nine subjects that continued with the follow-up. Mean values show insignificant changes in total number of bleeding days following injection. Individual variation though is very notorious with some subjects having marked oligomenorrhea and amenorrhea while others bled for 30 consecutive days.

Jose Balmaceda

SIGNATURE

7/11/85

DATE

118

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 343

TITLE: "NIH/Biotek Levonorgestrel Microcapsules"

INSTITUTION: The University of Alabama in Birmingham

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

FUNDING PERIOD: 11/1/83 - 4/30/85 AMOUNT FUNDED: $46,040

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

Two groups of five baboons each were treated with doses of Biotek microcapsules obtaining 10 (Norcaps 20) or 20 (Norcaps 100) mg levonorgestrel (LN). Blood samples obtained weekly were analyzed for estradiol (E2), progesterone (P4) and LN content by radioimmunoassay. Duplicate aliquots were provided to Mason Research Institute for parallel LN determination.

Blood sampling was discontinued at approximately 460 days post-treatment (PT). At that time, serum LN levels were $<$0.125 to 0.84 ng/ml in four of the five baboons on the low dose. The fifth baboon died at 378 days PT, following serum LN averaging 0.62 ng/ml from day 302 through day 371 PT. Serum LN ranged from 1.74 to 4.26 ng/ml in the five baboons on Norcaps 100 at 462 days PT.

Lack of menstrual cyclicity was maintained in all baboons in the Norcaps 100 group through day 565 PT, the current status of the baboons. The Norcaps 20 group has continued to have variable perineal cyclicity.

SIGNATURE

Jul 16, 1985
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

J... 1985

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: 2/1/84-1/31/86 AMOUNT FUNDED: $145,501

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

The aim of these studies is to determine the effects of chronic administration of an LH-RH inhibitory analogue in regularly cycling cynomologous monkeys. The studies were designed toward determining the adequate dose of the LH-RH antagonist that would provide effective anovulation without inducing a state of hypo- or hyperestrogenism. These studies are the continuation of those previously funded by PARFR (PARFR-302), in which we demonstrated that LH-RH antagonists are potent antigonadotropins in nonhuman primates, and that they inhibit ovulation when administered on a short-term basis to rhesus and cynomologous monkeys.

Experiment 1: screening of different doses of LH-RH antagonists that induce consistent anovulation without creating a marked hypoestrogenic state. Each animal received the drug or vehicle in subcutaneous daily injections for sixty successive days. On day 61, a progesterone challenge was performed in all animals.

As a result of this study, a dose of LH-RH antagonist was determined that induced a marked hypoestrogenic state in which the progesterone challenge was negative, and a dose in which normal vaginal bleeding followed the progesterone injection.

Groups 1 and 4 (250 mg and 1000 mg) have been completed. The results obtained, however, contradicted our previous findings in terms of ovulation suppression. Two animals in each group ovulated during the time of treatment (both dosages had been proven previously to inhibit ovulation consistently). Due to this, a new batch of the antagonist was ordered to start the protocol again. The experiment is now in progress.

SIGNATURE

DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

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: 11/1/83-6/30/85 AMOUNT FUNDED: $98,694

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

The objective of this project was to develop an IUD delivery system for the combined administration of estradiol and progesterone. The system was to consist of estradiol- or progesterone-releasing fibers wrapped around a conventional T-shaped IUD. In previous studies, SRI had developed coaxial or reservoir fibrous systems that gave constant rates of release of both estradiol and progesterone. However, when SRI began to prepare the exemption for an IND, SRI found that one of the rate-controlling membrane polymers was no longer commercially available. SRI identified five candidate materials for use as the sheath polymer, made fibers from them containing steroids, and evaluated their *in vitro* release. SRI determined that several of the polymers would give satisfactory release rates for the proposed IUD system.

Based upon these results, SRI began to prepare the IND for the fibrous estradiol/progesterone IUD. However, the data from Dr. Antonio Scommegna's study in which he had evaluated the fibrous systems in rabbits indicated that the two steroid fibers had to be intertwined with each other to obtain the desired combined effect. With this requirement and the change in the sheath polymers, SRI was unable to wrap sufficient lengths of both estradiol- and progesterone-releasing fibers around the required T-shaped IUD platform and obtain the desired release of both steroids for greater than two years. To intertwine equal lengths of both fibers around the IUD platform and still get the device into the inserter, SRI had to reduce both the length and size of the fibers. Both reductions caused SRI to decrease the core loadings of each steroid, and these lower loadings resulted in shorter durations of release. Because the duration of release was shorter than desired, PARFR, rejected it, because the device did not release for more than two years.

If, however, the fibers can be wrapped separately on the IUD platform as originally proposed or if a different platform can be used, then the currently available estradiol- and progesterone-releasing fibers can be used to prepare a steroid-releasing IUD with more than two years of release.

Richard L. Dunn/JWG
SIGNATURE

7/10/85
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 350

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

INSTITUTION: Tenon Hospital, University of Paris

PRINCIPAL INVESTIGATOR: Jacques Hamou, M.D.

FUNDING PERIOD: 1/1/84-6/30/85 AMOUNT FUNDED: $11,000

Below is a project summary for the July 1, 1984 - December 31, 1984 period.

Sixty-three patients, who requested permanent sterilization and who fulfilled the conditions of the protocol established for this study, underwent insertion of the ITD under microhysteroscopic office procedure. Last procedure for this study was performed December 13, 1984.

The device was slightly modified as from June, 1984 (patient No. 39); the distal hook being replaced by a double hook in order to reduce displacement and rejection rate.

One intrauterine pregnancy occurred; the patient underwent uneventful elective abortion.

Forty-seven follow-up microhysteroscopies were performed during this semester. Acceptability of repeated hysteroscopy was good.

The findings confirm the extreme low rate of risks and complications. The displacement rate with the modified device was reduced.

SIGNATURE DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

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 - 5/31/85 AMOUNT FUNDED: $29,420

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

Five baboons implanted with cholesterol-fused NET pellets continued to display suppressed ovarian activity through 470 days posttreatment (PT). Serum NET levels at that time were 0.43 ng/ml (2 pellets), 0.47 and 0.75 ng/ml (3 pellets), and 1.07 and 1.05 ng/ml (4 pellets). On day 475 PT, the pellet remnants were successfully removed from two of the baboons as evidenced by a resumption of perineal cyclicity.

SIGNATURE

July 26, 1985

DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

J... 1985

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 January 1, 1985-June 30, 1985 (FINAL) period.

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. This report covers the period of the last three months of the project, January 1, 1985 - March 31, 1985.

Semen samples from volunteers were collected by masturbation into clean bottles and delivered to the laboratory within 2 hours. Samples with good sperm count ($\leqslant 50 \times 10^6$/ml) and good motility ($\leqslant 50\%$) were selected for tests. Seeds and storage roots (tubers) of plants found in Thailand were collected for extraction. The fresh or frozen plant tissue (25 gm) was chopped and then homogenized is 75 ml of ice-cold phosphate-buffered saline, pH 7.4 (PBS). Any insoluble material was removed by centrifugation at 10,000 x g for 30 min at 4°C. Protein fraction was precipitated from the supernatant fluid by ammonium sulfate (80%), collected by centrifugation, redissolved in PBS and dialyzed against PBS at 4°C overnight. Human sperm agglutination test was performed by mixing an aliquot (50 µl) of semen or washed human sperm (4×10^7 cells/ml) with 50 µl of the dialyzed plant extract at room temperature. Sperm agglutination was observed under a dark-field microscope within 1 minute after mixing. The test was scored from 0 (no agglutination) to 4 (maximal massive clumping). In this period, we tested 17 species. Among these, two were found effective, namely taro *(Colocasia esculenta)* and bread fruit *(Artocarpus altilis)*. Fifteen inactive species were pomegranate, mangosteen, guava, turmeric, ginger, betal nut, galangal, leech lime, star gooseberry, pararubber, yam bean, turnip, bael fruit, santol, and carambola. Together with those tested in the previous nine-month period, a total of 50 species were tested in the full period of the project. Among these, 11 were found active and 39 were inactive. To recap, the 11 species containing human-sperm binding lectins were : Jack fruit, red kidney bean, garden pea, yard-long bean, sa-taw, broad bean, peanut, green gram, soybean, taro and bread fruit. These plants should be potentially useful for the development of new lectin-based vaginal contraceptives.

Montri Chulavatnatol

SIGNATURE

24 June 85

DATE

54

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 355

TITLE: "Enhancement of the Secretory Immune Response to LDH-C4"

INSTITUTION: Medical Research Foundation of Oregon

PRINCIPAL INVESTIGATOR: Nancy J. Alexander, Ph.D.

FUNDING PERIOD: 4/15/84-6/30/85 AMOUNT FUNDED: $47,335

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

We immunized 16 female monkeys on the seventh day after the start of menses with primary subQ injections of LDH-C_4 plus B. pertussis, in saline. The monkeys were then divided into two groups. One group was stimulated with an aerosol preparation and the other an enteric capsule both containing LDH-C_4 con A. Three days later all were given agarose vaginal plugs containing LDH-C_4 and con A. Both IgC and IgA anti-LDH-C_4 antibodies did develop. Both humoral and locally produced antibodies developed. Three months later the genital secretory immune system was restimulated with another vaginal plug. Subsequently, the females were placed in a breeding study. At no time in the study did oral lavage samples exhibit any significant antibody activity, although significant levels of anti-LDH-C_4 were found in the vaginal lavage samples. Maximal serum IgG levels coincided with maximal IgA levels. The highest levels occurred at day 28 following the primary immunization. The female macaques stimulated via the gut appeared to develop a slightly better immune response than those stimulated via the bronchioles. There was a large amount of variation in the individual responses to the immunizations. The fertility of these monkeys was not reduced; pregnancy occurred in both high and low antibody responders. We suggest that the antibody levels at the time of mating were too low to inhibit conception. The levels of antibodies seem to be generally detected for less than 42 days following immunization, after which the levels return to preimmunization levels. This reflects well on the potential reversibility of this procedure.

The results of the study indicate that there are some basic questions that need to be addressed before another contraceptive vaccine study in non-human primates is initiated.

SIGNATURE

July 11, 1985
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 356a

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

INSTITUTION: The George Washington University

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

FUNDINC PERIOD: 12/1/84-8/31/85 AMOUNT FUNDED: $59,593

Below is a project summary for the January 1, 1985-June 30, 1985 period.

As the availability of human germ cells is limited for use in the isolation of FA-1 required for large scale practical use, we tried to purify it from murine germ cells. We have purified FA-1 from either deoxycholate (DOC) - or lithium diiodosalicylate (LIS) - solubilized murine testes by immunaffinity chromatography using monoclonal antibody MA-24. The FA-1 thus isolated is homogeneous and shows a single band of 47 kilodaltons (kDa) when analyzed by slab SDS-PAGE and silver staining. Following removal of the detergent and extensive dialysis or treatment with 0.5M NaCl, PAGE analysis shows a 23 kDa band. Two-dimensional (2D) PAGE of the eluted FA-1 shows 4-5 polypeptides in the 47 kDa or 23 kDa range. The dialyzed FA-1 contains a major 23 kDa and a minor 48 kDa band when separated on both sucrose and cesium chloride gradients. High performance size-exclusion chromatography shows a major peak at 23 kDa and a minor peak at 50 kDa. Further analysis of the 23 kDa peak by reverse-phase chromatography resolves the antigen into 3 peaks, which gave similar 2D patterns as the native FA-1. Lectin affinity chromatography on a lens culinaris column demonstrates that a part of the antigen is bound to the lectin while the rest is not. The FA-1 reveals a positive reaction with periodic-Schiff reagent and contained glucose and mannose, which together constituted 18.8% of the total antigen mass. Amino acid analysis shows a high percentage of aspartic and glutamic acid, while the N-terminal analysis shows serine and aspartic acid (Naz et al, Proc. Natl. Acad. Sci., USA, manuscript, submitted).

MA-24 monoclonal antibodies inhibited fertilization and fertility in vivo in female mice and female rabbits when antibodies were administered passively. Recently we actively immunized six female rabbits with the purified, and well-characterized FA-1 and found a drastic reduction in fertility after immunization. Antiserum collected from these rabbits at various stages of immunization was negative in agglutination and immobilization techniques. All the animals responded to the antigen and raised an immune response recognizing specific bands in the Western blot enzyme immunobinding procedure. The modal ELISA titers in these antisera, as tested against the antigen coated on plates were > 1:2560. We are investigating the local immune response in the vaginal secretions.

Presently, we are investigating the effect of active immunization on fertility in female mice. Six female mice actively immunized with FA-1 showed a significant inhibition of fertility when mated with males of proven fertility. We are planning to conclude these results by summer.

Rajesh Kumar Naz
SIGNATURE

15 July 1985
DATE

56

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 359

TITLE: Active Immunization of Non-Human Primates and Rabbits with Zona Pellucida Proteins

INSTITUTION: Baylor College of Medicine

PRINCIPAL INVESTIGATOR: Bonnie S. Dunbar

FUNDING PERIOD: 7/1/84-7/1/85 AMOUNT FUNDED: $78,000

Below is a project summary for the January 1, 1985-July 15, 1985 period.

In order to evaluate the effect of immunization with purified ZP proteins on ovarian function in primates, bonnet monkeys and rhesus monkeys have been given total heat-solubilized as well as two purified deglycosylated ZP glycoproteins. All animals have developed significant antibody titers to specific ZP proteins as analyzed by enzyme-linked immunoassay and 2D-PAGE immunoblot procedures. The titers from animals receiving the heat-solubilized ZP preparation were much greater than those receiving the purified proteins. (Since these monkeys do not normally cycle during the summer months, the effects on ovarian function cannot be monitored until the fall). Some of the baboons which were immunized with the 55K protein showed alterations of ovarian function as measured by estrogen levels. The antibody titers and ovarian cycles are continually being monitored.

Bonnie S. Dunbar
SIGNATURE

7-2-85
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 360

TITLE: "Inter-and Intra-Cycle Variation of Genital Peroxidases in Women"

INSTITUTION: University of Illinois at Chicago

PRINCIPAL INVESTIGATOR: John C.M. Tsibris, Ph.D.

FUNDING PERIOD: 7/1/84-6/30/85 AMOUNT FUNDED: $28,127

Below is a project summary for the January 1, 1985 - June 30, 1985 (FINAL) period.

The levels of Guaiacol Peroxidase (GP), a Ca^{++} extracted enzyme from cervical mucus or vaginal fluids can define the fertile period in women. GP specific activity decreased rapidly 5-6 days before ovulation (the LH+1 day) and rose again 1-2 days after ovulation. GP levels were measured by a simple colorimetric assay (guaiacol + H_2O_2) and were expressed as units of activity per g wet sample; one unit of GP activity increases by one the 470 nm absorbance of the reaction mixture per min. In the luteal phase there was a strong positive correlation between GP and serum progesterone, therefore, GP was also proven useful in detecting, a) low progesterone levels, suggestive of a luteal phase defect, and b) early pregnancy. (Tsibris et al, Fertility and Sterility, August 1985 issue).

The objective of this project was to test the reproducibility of the cervical mucus GP patterns in ten volunteers, each tested for six menstrual cycles. Daily samples of cervical mucus were collected with a fertility cannula at the external os, following the insertion of a speculum, and GP activity was measured spectrophotometrically. First-morning urine was used for the determination of LH and pregnanediol.

The main conclusions drawn from our data, are as follows:

1. An average 6.4 $\pm$ 1.2 (SD) days prediction of ovulation was observed in 24 cycles of six volunteers each studied for 2-6 menstrual cycles.
2. Within each individual we tested the time period from 7 days before to 3 days after ovulation (the LH+1 day) and found (using 2-way ANOVA) that the GP "profiles" were not different ($P > 0.05$) from cycle to cycle. However, one of the six volunteers showed significant differences among her cycles; she also had lower than normal urine LH or pregnanediol in 2 of her 6 cycles.

   When the test period was expanded to days -10 to +3 from ovulation then 4 of 6 volunteers had significanlty different cycles. This suggests that there is quite a scatter of GP values at the very beginning of the cycle but not later when they approach ovulation.
3. A seventh volunteer showed consistently ($P < 0.05$) short "luteal" phases, following the GP nadir, in the 3 cycles tested which must have been anovulatory as indicated by the low pregnanediol values.

Therefore, GP "profiles" in cervical mucus can be useful diagnostic tools of some abnormalities (sporadic or permanent) of the menstrual cycle.

We conclude that serial measurements of GP specific activity in cervicovaginal secretions would constitute a useful method to predict ovulation for couples wishing to achieve or avoid pregnancy. In addition, it may supply useful information on the cause of infertility.

John C.M. Tsibris
SIGNATURE

July 12, 1985
DATE

55

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 361

TITLE: "Testosterone Microcapsule Formulation Study"

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

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

FUNDING PERIOD: 7/1/84-6/30/86 AMOUNT FUNDED: $122,106

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

The objective of this project is to determine the release rate of microcapsules loaded with testosterone (T) in non-human primate animal models.

Microcapsules (45-125µl)(85:15 poly (DL-lactide - CO glycolide)) containing a core loading of testosterone (42% T, 55mg) were used in an attempt to mimick the daily T production in this species for up to 4-6 months. Serum levels of T, DHT, FSH and LH were obtained for 1 month prior and 1 month after bilateral castration (cx); then, the microcapsules were administered as a single injection IM and the blood sampling continued for 6 months. In addition, the following tests were performed throughout the study: lipoprotein profile (HDL, LDL, VLDL), liver enzymes (SGOT, SGPT, LDH) acid phophatase, SMAC-20 and CBC. Following cx, blood levels of T, DHT, FSH and LH changed drastically. T and DHT fell from 4.4-11.2ng/ml to 0.1-0.3ng/ml ($P<.001$), and from 2-4.2ng/ml to 0.2-0.6ng/ml ($P<.001$) respectively, within a week from cx. During that same period, FSH and LH rose from 2.8-3.2µg/ml ($P<.001$) to 15-20µg/ml ($P<.001$) and from 0.8-1.4µg/ml to 2.8-3.4µg/ml ($P<.001$) respectively. By three months after the microcapsule administration, T, DHT and LH values returned to baseline precastration (precx.) values. 4 months after the microcapsule injections, serum T values had dropped below baseline precx. levels. DHT values also dropped slightly below precx. levels by 4 months. By 6 months after the microcapsule injections, serum T and DHT levels had dropped to post castration values. 4-6 months after the microcapsule injections, LH levels remained at baseline precx. values. Serum FSH values remained high at post castration levels throughout the study. Values of serum chemistries were not different before cx from those observed 3 months after the microcapsule injections. These results suggest that the availability of such a system of a long lasting biodegradable microcapsule formulation of testosterone may open new approaches of androgen administration with possible uses both as a male contraceptive as well as in the treatment of hypogonadal men.

SIGNATURE

DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 362

TITLE: "Combination Injectable Steroidal Microsphere - Continuation of PARFR-332"

INSTITUTION: Stolle Research and Development Corporation

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

FUNDING PERIOD: 10/1/84-5/31/85 AMOUNT FUNDED: $67,195

Below is a project summary for the January 1, 1985-June 30, 1985 ~~(FINAL)~~ period.

The objective of this project is the development of a contraceptive comprising microspheres of norethisterone and ethynyl estradiol. The individual steroids would be microencapsulated separately and then combined in a specific ratio in a single injection. The target duration of activity is 90 days. This type of formulation would be expected to provide greater control over uterine bleeding in women using the long-acting injectable contraceptive.

Twelve baboons in 3 groups have been treated with a combination of NET (Batch SR003-60) and EE (SR0014-7) microspheres. Endometrial biopsies are being performed at 30 day intervals during the first 90 days PT, and biweekly blood samples are being obtained during the first 120 days PT. Group A (n = 2) received 40 mg NET and 1.0 mg EE, was deturgesced by day 40 PT, and had no ovulatory P4 levels in the first 52 and 105 days PT. Group B (n = 5) was treated with 20 mg NET/0.50 mg EE. Following deturgescence in all five baboons by day 20 to 37 PT without concomitant ovulatory P4 levels, 3 baboons had P4 greater than 3 ng/ml on days 63 to 73 PT. Group C (n = 5) was injected with 10 mg NET/0.25 mg EE, and all five had at least two consecutive ovulatory serum P4 levels between days 40 to 70 PT. Serum EE levels were usually less than 100 pg/ml, and serum NET reached peaks of 3 to 4 ng/ml.

The NET microspheres tested thus far contain only 25% by weight drug. We are now preparing a new NET/EE combination formulation in which the NET microspheres are equivalent to those being tested in the Phase I clinical trial at UAB; i.e., 45-50% loaded. Baboons will be treated with this formulation within the next few weeks. We expect to prepare an IND on this product during late 1985 and clinical testing could begin in the first or second quarter of 1986.

SIGNATURE

July 3, 1985

DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 363

TITLE: "Laboratory Studies on an Antispermatogenic Agent - THP for Control of Male Fertility"

INSTITUTION: The University of Western Ontario

PRINCIPAL INVESTIGATOR: John P. Wiebe, Ph.D.

FUNDING PERIOD: 10/1/84-3/31/86 AMOUNT FUNDED: $90,000

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

The overall objective of the project is to determine the antifertility effects of intratesticular injections of 1,2,3-trihydroxypropane (THP) solution in small mammals and subhuman primates. We had already shown that in the laboratory rat a single injection results in cessation of spermatogenesis and consequent infertility for at least 6 months, without reduction in libido, secondary sex characters or differences in hormone levels and hormone receptors. During the current reporting period (January 1, 1985 to June 30, 1985) we continued to study the effects and mechanisms of action of THP treatment in rats and rabbits. It was determined that the volume of THP injected must be adequate for dispersion of the substance throughout the testis and that THP is effective also at lower concentrations. Long term experiments are in progress to determine if the antifertility effect is reversible. Treatment of pubertal (35 day old) males effectively halts spermatogenesis; serum androgen levels fall after the treatment but are back to normal at 6 weeks. In vivo experiments with ^{14}C-THP have shown that the substance is 99.8% removed from the testes by 24 hours after an injection; some of the THP is metabolized to CO_2, about 10% of the radioactivity appears in the urine and feces and only traces (<0.2%) are found in epididymis, prostate, seminal vesicles, kidney, liver, and body fat at 24 hours. In vitro studies have shown that testis homogenate converts THP to CO_2. Examination of tissues at the light and electron microscope level is in progress; to date the results indicate that the limiting membranes around the seminiferous tubules may be affected by the treatment but the myoid cells are normal and the Sertoli cell tight junctions retain the capacity to exclude lanthanum. The lumen of the epididymis decreases in size but the cells are nomal in appearance. A preliminary experiment (n=6) on rabbits shows that THP treatment results in 99.9% reduction in sperm per ejaculate, 100% infertility upon matings, without significant change in libido, serum androgen and estrogen, testicular 5-alpha-reductase activity, gonadotropin/receptor binding and secondary sex characters. A larger experiment on rabbits (n=10) is underway and will include testicular steroidogenesis, histology, and the long term effects, in addition to the above parameters. Methods of autoantibody and androgen binding protein (ABP) measurements have been tested. Preliminary tests on rabbit serum samples indicate no differences in agglutination between control and THP treated. Results from rats and ABP measurements are not yet available. Twenty Squirrel Monkeys have been purchased and are undergoing quarantine at Michigan State University. We hope to begin treatments in September and Dr. Dukelow (M.S.U.) has agreed to obtain regular semen samples and analyses on a contractual basis. If THP treatment is effective in primates without side effects, it could lead to the development of an effective human male contraceptive.

SIGNATURE

July 17/85
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 364

TITLE: "Antifertility Effects of Microencapsultated LHRH Agonist"

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

PRINCIPAL INVESTIGATOR: Francisco J. Rojas, Ph.D.

FUNDING PERIOD: 9/1/84-6/30/86 AMOUNT FUNDED: $64,218

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

At present, we have completed the analysis concerning the regularity of the menstrual cycles in each of the twenty cynomolgus monkeys involved in the study. To accomplish this, all the animals were periodically checked for regular cycles by vaginal swabbing, serial laparoscopies and serum estrogen and progesterone determinations. This phase of the project was in accordance with our initial objective to use only monkeys that underwent at least two consecutive normal, ovulatory menstrual cycles.

Having accomplished this objective, we initiated the different regimes according to our original protocol. Consequently, Group 1 received a simple s.c. injection of microcapsules releasing D-Trp-6-LH-RH (at a rate of 20μg/day, for 30 days) every 30 days. Group 2 received, in addition to the microcapsules as in Group 1, a progesterone challenge (50mg, i.m.) on day 25 of each cycle. Groups 3 and 4 represented the corresponding controls for each of the experimental groups, and therefore, they were injected with vehicle. To ensure a faster and better inhibition of pituitary-gonadol axis, we injected 100μg D-Trp-6-LH-RH/day for 7 days before the first injection of microcapsules. In subsequent months, the monkeys received only the microcapsules every 30 days.

Laparoscopies were performed at periodic intervals to determine the degree of folliculogenesis and to time the ovulation date for each month. Also, blood was drawn every week and circulatory concentrations of FSH, LH, prolactin, estradiol and progesterone were determined by radioimmunoassay.

Our data obtained in these initial months of treatment, indicate that the D-Trp-6-LH-RH microcapsules administered in a single s.c. injection every 30 days can suppress LH levels and delay ovulation for 4 consecutive months as determined by progesterone and estradiol profile, vaginal swabbing and serial laparoscopic exams.

Francisco J. Rojas 7/11/85

SIGNATURE DATE

62

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 366

TITLE: "Immunological Contraception - Study on the Time Course of Sperm Antibodies Production in Rabbits Following Intravasal Injection of BCG (Bacillus Calmette Guerin)"

INSTITUTION: University of Hong Kong

PRINCIPAL INVESTIGATOR: Steven Y.W. Chan, Ph.D.

FUNDING PERIOD: 10/1/84-3/31/86 AMOUNT FUNDED: $5,302

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

The objective of the project is to study the time course of development of serum and seminal plasma sperm--reactive antibodies in adult male rabbits following intravasal injection of BCG (Bacillus Calmette Guerin). During the period of January 1, 1985 to June 30, 1985, experimental rabbits and certain essential chemicals including the BCG were obtained. Experimental procedures including:

1. Collection of serum samples from rabbits by marginal ear vein puncture,
2. Collection of rabbit ejaculates by the use of artificial vagina,
3. Analyses of rabbit ejaculates: semen volume, pH value, colour, consistency, sperm count, percent motility and percent normal sperm forms, and
4. Surgical operation and intravasal injection

were also successfully established and all these experimental procedures are being performed regularly on both the control (intravasal injection of saline) and the experimental (intravasal injection of BCG) groups. Serum and seminal plasma samples are being frozen at -80°C for future analysis of sperm--reactive antibodies. The principal investigator has recently obtained some high-titre anti-rabbit sperm antiserum from Dr. Alan C. Menge of the Immuno-Infertility and Andrology Laboratory of the University of Michigan, Ann Arbor, Michigan, USA. This high-titre anti-rabbit sperm antiserum was a gift from Dr. Menge and will be used as a positive control antiserum in the future experiments for determination of sperm-reactive antibodies in the rabbit serum and seminal plasma samples.

SIGNATURE

DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

JUL 1 1985

PARFR- 367

TITLE: "Fertility Inhibition of In Vivo Immunization with Epididymal Proteins in Hamsters"

INSTITUTION: Instituto de Biologia y Medicina Experimental

PRINCIPAL INVESTIGATOR: Jose A. Blaquier, M.D.

FUNDING PERIOD: 11/1/84-10/31/85 AMOUNT FUNDED: $9,900

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

Evidence obtained in several laboratories indicates that androgen-dependent secretory epididymal proteins participate in the development of fertilizing capacity during epididymal maturation of spermatozoa. In addition, antibodies against these proteins can interfere with the fertility of mature sperm.

Our goal is to induce autoimmunity in hamsters with 2 epididymal proteins (EP2 and EP3) which associate with sperm during transit and study the effect of this condition on sperm function, fertility and the entry of antibodies into the lumen of the epididymis "in vivo".

During the previous reporting period (11-1 to 12-31,1984) we prepared a large batch of purified antigen, by ion exchange chromatography and gel filtration, in which proteins EP2 and EP3 represented approximately 70% of total protein. This preparation was used to immunize (150 ug antigen/animal/every 24 days) 5 male and 4 female hamsters. An equal number of animals was injected with Freund's adjuvant (250 ul) in saline (250 ul) as control. After receiving 3 injections, the animals had failed to develop titers of circulating antibodies detectable by immunodifusion.

Last January 26, 1985 we accidentally lost all our experimental and stock animals and the batch of antigen prepared for this project (a whole floor of our Institute was flooded) and had to start the experiments once again.

Once a new batch of antigen became available, immunization was begun using a different schedule: 500 ug antigen/ animal/ every 10 days. Subcutaneous injections were alternated between the back and the hind legs. Four groups of 4 animals each wre injected, one group of males and one of females with antigen and an equal number with Freund's adjuvant in saline only. Animals were bled prior to each injection by puncture of the suborbital sinus. The sera are being processed to detect the presence of circulating antibodies against the injected antigen. In addition, we are setting up an agglutination test (sheep red blood cells coated with antigen) to increase the sensitivity of the assay.

If this new approach fails to produce autoimmunity we plan to induce pasive immunization by injection of antiserum raised in rabbits against the same antigen. Two rabbits are being immunized to obtain that antiserum.

Expected results: a) demonstration of the autoantigenicity of EP2-EP3 in hamster; b) disturbance of fertility in immunized male and female hamsters; c) information on the mode of action of the immunoglobulins on sperm; d) determination of the entry of immunoglobulins into the epididymis "in vivo".

J. A. Blaquier

SIGNATURE

July 2, 1985

DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 368

TITLE: "NIH/Biotek Levonorgestrel Microcapsules"

INSTITUTION: Biotek, Inc.

PRINCIPAL INVESTIGATOR: E. S. Nuwayser, Ph.D.

FUNDING PERIOD: 2/1/85-1/31/86 AMOUNT FUNDED: $79,106

Below is a project summary for the January 1, 1985 - June 30, 1985 period.

The objective of the project is to develop a biodegradable injectable microcapsule system for the delivery of levonorgestrel over a period of 3-6 months. The following is a brief summary of the accomplishments of the project during the first five months.

D,L-lactide monomer and levonorgestrel were procured from a European supplier and Wyeth Labs in the U.S., respectively. Standard Operating Procedures (SOP) were written for the synthesis of poly-D,L-lactide polymer using Good Manufacturing Practice (GMP) procedures to meet the requirements of the U.S. Food and Drug Administration. Six batches of polymer were synthesized under GMP procedures. Three batches which met the SOP specifications were blended together to produce one kilogram of poly-D,L-lactide with a reduced specific viscosity of 1.12 dlg $\pm$ 0.02.

Three preliminary microencapsulation trial runs were made with levonorgestrel and the polymer using BIOTEK's proprietary small air suspension coating unit. This unit was used in order to conserve both drug and polymer and to define the operating conditions for the larger coating unit. The first run ended in an agglomeration of microcapsules. The second run produced two ranges of particles which were oversized and unsuitable for injection. The third run produced microcapsules in all three desired ranges (44-75; 75-106; 106-150 microns).

Levonorgestrel diffusion studies were started on all three sizes. At the time of this report the 12 day release profiles showed the typical initial burst. It is expected that the release of the drug from the microcapsules will reach steady state in the next few weeks.

In conclusion, despite the unexpected delay encountered in procuring and synthesizing the D,L polymer (instead of the L(-) polymer previously prepared for NIH), we were able to accelerate the program by performing three microencapsulation runs using BIOTEK's proprietary small air suspension coating unit. This should permit meeting the schedule submitted with the proposal. On the basis of this we expect to submit an IND in January, 1986, before the end of the first year contract in anticipation of clinical trials.

E S Nuwayser
SIGNATURE

June 28, 1985
DATE

63

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 369

TITLE: "Transuterine Application of Biocompatible Hydrogel Hypan into Fallopian Tubes"

INSTITUTION: Bio-Products, Inc.

PRINCIPAL INVESTIGATOR: Milos Chvapil, M.D., Ph.D., D.Sc.

FUNDING PERIOD: 2/1/85-7/31/85 AMOUNT FUNDED: $3,979

Below is a project summary for the January 1, 1985- June 30, 1985 period.

Nine human uteri were collected through the courtesy of the Department of Pathology at the University of Arizona and from Hermisillo, Mexico. The age of the women ranged from 32 to 81 years, the average age being 63 years. Six of the specimens had pathological changes, including three samples with either both or one tube occluded, two specimens had fibromyoatous uterus, one uterus was from a patient with viral hepatitis, one uterus was without a cervix (vaginal hysterectomy).

HYPAN N-90, 8 to 12% solutions in DMSO and containing 30% renographin as X-ray contrast material, were tested using a Fem-Cept applicator (Bio-Nexus, S.C.). Due to higher viscosity of HYPAN than that of Methylcyanoacrylate (for which Fem-Cept was developed) we modified Fem-Cept by using a larger diameter of the delivery catheter and enlarged both openings at the tip of the delivery tubing. We also changed the volume of the cartridge from 0.6 ml to 1.5 ml to see how far the hydrogel penetrates the tubes. The transcervical application of HYPAN into the tubes was observed under a fluoroscope.

In three acceptable uteri we administered 1.0 µl HYPAN. Both tubes were blocked approximately 2.5-3.5 cm from the uter-tubal junction. We found, however, almost 50% of the injected HYPAN volume left gelled in the fundus. This may be due to a too fast gelling of the polymer or the presence of highly viscous mucus in the uteri, which were not always lavaged before use.

Presently, we are preparing HYPAN solutions with lower viscosity and slower gelling time. We also received professionally modified Fem-Cepts directly from the manufacturer to meet our specifications.

Milos Chvapil

SIGNATURE

7-1-85

DATE

66

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 370

TITLE: "Sterilization of Male Dogs by Injecting Vas Deferens with Hydrogel Hypan"

INSTITUTION: Bio-Products, Inc.

PRINCIPAL INVESTIGATOR: Milos Chvapil, M.D., Ph.D., D.Sc.

FUNDING PERIOD: 2/1/85-10/31/85 AMOUNT FUNDED: $22,499

Below is a project summary for the January 1, 1985-June 30, 1985 period.

A total of 19 dogs were injected under visual inspection into both vas deferens with HYPAN N-50 acrylic hydrogel, 50 to 150 microliters was deposited inside the vas lumen via teflon 22 G intercath. The solution gelled inside the vas lumen within 120 seconds. One hundred percent effectiveness of the injection technique was achieved.

Semen was collected by manual mastarbation 68 times and analyzed for volume, sperm count and viability. Thirty analyses were made before HYPAN occlusion; 38 analyses were made at 1,3, 5 and 10 weeks after the occlusion. Twenty week analyses are still awaiting completion.

The data on semen before occlusion showed the following average values: 2.25 ml/volume of ejaculate, 50 to 500 x 10^6 spermatozoa with 90.2% average viability. After the occlusion the volume of ejaculate did not change, amounting to 2.04 ml. No viable or dead spermatozoa were found in any of the samples analyzed. The stained smears of the ejaculate showed the presence of cell debris, granulocytes and few epithelial cells.

Two dogs were terminated 5 weeks after the occlusion of vas. Gross observation showed intact vas and epididymis. When a 3 mm long incision in the longitudinal direction of the vas was made, light pressure applied proximal and distal to the incision site resulted in the HYPAN noodle popping out. Histopathology of both frozen sections or paraffin embedded specimens documented the already reported inertness of the HYPAN in the vas lumen, which was completely blocked with the hydrogel without inducing any cellular inflammatory or fibrotic reaction.

Of the 19 dogs, 15 were injected with the catheter pointing in the epididymis direction, 4 were injected in the urethra direction. A volume of 150 µl reached and filled the convoluted epididymis, approximately 7 cm long segment. We found that 50 µl is optimal to block approximately 2-3 cm long vas lumen.

We conclude that the experimental data obtained to this date show that blocking the vas deferens by direct intraluminal injection of hydrogel HYPAN is a feasible and effective method of eliminating spermatozoa from the ejaculate. The hydrogel seems to be very biocompatible, not inducing any postinjection inflammatory or fibrotic changes in the vas for up to 5 weeks.

Milos Chvapil

SIGNATURE

7-1-85

DATE

61

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 371

TITLE: "Vaginal Spermicidal Barrier (VSB) Postcoital Tests"

INSTITUTION: Eastowne OB-GYN and Infertility

PRINCIPAL INVESTIGATOR: James R. Dingfelder, M.D.

FUNDING PERIOD: 4/1/85-3/31/86 AMOUNT FUNDED: $15,598

Below is a project summary for the January 1, 1985-June 30, 1985 period.

The project has been delayed due to technical problems associated with manufacture of the vaginal spermicidal barrier (VSB) device. A recent telephone conference with the developer of the VSB disclosed that supplies of the device are expected to be available on or around August 1, 1985 and clinical trials could start shortly after that date.

James R Dingfelder MD
SIGNATURE

7/16/85
DATE

68

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

JUL 1985

PARFR- 372

TITLE: "Progestational Agents Effects on Baboon Endometrium"

INSTITUTION: Stolle Research and Development Corporation

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

FUNDING PERIOD: 3/1/85-10/31/85 AMOUNT FUNDED: $75,120

Below is a project summary for the January 1, 1985-June 30, 1985 period.

The endometrial effects of two doses each of three synthetic progestins are being determined in the baboon model. A levonorgestrel (LN) oxime at 3 and 6 mg/kg, an LN ester at 0.5 and 1.0 mg/kg, and Depo-Provera at 0.5 and 2.0 mg/kg are being evaluated in groups of five baboons each. Weekly blood samples are being obtained and will be sent to Mason Research Institute for steroid analysis. Sex skin turgescence is recorded daily to aid in evaluating treatment effects on ovarian cyclicity. Second injections are being given at 60 days posttreatment (PT) in the LN oxime group, and at 90 days PT in the other two groups. An endometrial biopsy specimen is obtained at a selected interval after each injection. To date, 15 PT endometrial samples have been obtained, and six baboons have completed the study. The biopsies are being evaluated by light, transmission and scanning electron microscopy to determine if any deleterious endometrial changes result from treatment.

SIGNATURE

7/1/85
DATE

67

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 373

TITLE: "90-Day Levonorgestrel Microspheres"

INSTITUTION: Stolle Research and Development Corporation

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

FUNDING PERIOD: 4/1/85-3/31/86 AMOUNT FUNDED: $67,404

Below is a project summary for the January 1, 1985-June 30, 1985 period.

The objective of this project is the development of a 90-day injectable microsphere system for the delivery of levonorgestrel. The availability of such a product would allow a direct comparison of levonorgestrel, norgestimate, and norethindrone to be made both in the baboon model and the Phase I human studies. All three systems would be fabricated from the same polymeric excipient and be administered in the same fashion with expected durations of action of 90 days.

The technical program was temporarily delayed due to problems in procurement of the levonorgestrel. Those problems appear to be resolved with the recent purchase of 25 g of drug from a chemical supply house. We have synthesized biodegradable copolymer for the project, and we expect to prepare microspheres for evaluation in the baboon immediately upon receipt of the drug.

It is important to recognize that we previously prepared a batch of levonorgestrel microspheres using the Stolle process and consequently we do not anticipate any unusual problems with this development.

Danny H. Lewis
SIGNATURE

July 2, 1985
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

JUL 1985

PARFR- 374

TITLE: "Progesterone Microspheres - Clinical Studies"

INSTITUTION: Stolle Research and Development Corporation

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

FUNDING PERIOD: 5/1/85-10/31/85 AMOUNT FUNDED: $40,799

Below is a project summary for the January 1, 1985-June 30, 1985 period.

The objective of this project is the development of a 90-day injectable contraceptive based on a natural steroid, progesterone for use in lactating women. The primary project task is the preparation of a batch of progesterone microspheres under GMP in the Stolle Sterile Facility for use in a Phase I clinical trial.

Results from the most recent prototype formulation produced at Southern Research Institute tested in baboons were as follows: Three additional groups of three baboons each were injected with 250 mg P4 in microsphere batches C841-052-1 (microspheres for Group E were sterilized by 2.8 - 3.5 Mrad gamma radiation; Group F by 1 Mrad) and C841-054-1 (Group G - 1 Mrad). Two baboons in Group E had peak serum P4 levels of 7.44 to 8.74 ng/ml 35-42 days PT, while serum P4 in the third baboon never reached 3 ng/ml. Sex skin cyclicity was suppressed for at least 75 days in all three baboons, and serum P4 was less than 2 ng/ml by 63-77 PT. Group F baboons had serum P4 peaks of 7.33 to 10.77 ng/ml 31-42 days PT, and sex skin cyclicity resumed 70 to 90 days PT when P4 levels fell below 2 ng/ml. Group G, which contained 1 ovariectomized and 1 hemiovariectomized baboon, reached peak serum P4 levels of 6.31 to 13.30 ng/ml 35-49 days PT, with a decrease to less than 2 ng/ml on day 77 to 98 PT.

We have reproduced this formulation in the Stolle laboratories and the samples are undergoing in vitro characterization. We plan to prepare the clinical batches during September in the Stolle facility. Samples of the clinical material will be tested in the baboon prior to their release for human studies. It is possible that the Phase I trial can be initiated during late 1985.

SIGNATURE

July 2, 1985
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 375

TITLE: "Immunopathologic Evaluations of Female Baboons Immunized with LDH-C_4 Peptide"

INSTITUTION: University of New Mexico

PRINCIPAL INVESTIGATOR: Kenneth S.K. Tung, M.D.

FUNDING PERIOD: 5/1/85-11/30/85 **AMOUNT FUNDED:** $9,560

JULY 1985

Below is a project summary for the January 1, 1985 - June 30, 1985 **period.**

Tere Yule visited the laboratory of Dr. Irv Goldberg in Chicago. With Dr. Joyce Shelton, she attended the laparotomy operation on two baboons from Dr. Goldberg's project. She collected tissue as indicated in the contract for histologic, electron microscopic, and immunofluorescent study. She also showed the local technician methods of collection of tissues for the different studies. Serum samples were drawn from the two baboons. We are currently processing all the tissues that have been sent to the University of New Mexico. The serum samples will soon be subjected to clinical chemistry analysis as indicated in the contract. Blood samples and urine samples were also obtained locally for studies outlined in the contract. Additional tissue and serum samples will be collected in the near future. The study is on schedule.

SIGNATURE

5/26/85

DATE

72

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 376

TITLE: "Pre IDE Studies - Tubal Clip"

JUL 0 - 1985

INSTITUTION: Maine Medical Center

PRINCIPAL INVESTIGATOR: C. Irving Meeker, M.D.

FUNDING PERIOD: 5/1/85-4/30/86 AMOUNT FUNDED: $29,774

Below is a project summary for the January 1, 1985-June 30, 1985 period.

The objective of this project is to implant Teflon plugs and clips in 150 female mice for the essential lifetime of these animals to assess the carcinogenicity and/or toxicity of Teflon in the reproductive tract.

150 sham operated controls will be followed at the same time. This information is required as part of our IDE application to the FDA to initiate human trials of this device. Preparations to begin the study during July are currently underway, but none of the actual procedures have as yet been carried out.

Detail on current reporting period:

A. Personnel. A certified animal care technician has been hired and began work on Monday, June 24. Her background in research will allow her to appropriately fill the position of animal techinician as well as that of research assistant on this project. As such, she will be responsible to the principal investigator for both the care of the animals and assistance with the surgery, and for maintaining all records associated with this project.

B. Device Preparation. Special small devices are required for this procedure. A new process for the manufacture of these devices has been worked out by the instrumentation facility at the University of Vermont to prepare plugs with an outside diameter of 1 mm. Devices of both 1 mm. and 1.5 mm. have been inserted in mice to determine that these size devices can be used as planned. Having determined this, device manufacture will commence immediately.

C. Animal Selection. Discussions have been held with Dr. Terrie L. Cunliffe-Beamer at the Jackson Laboratories in Bar Harbor, Maine. Dr. Cunliffe-Beamer is responsible for the small animal facility and research activities. Our initial discussion suggests that the Balb C-ByJ virus-free mice will be used for this study. Some additional references are being

(continued on next page)

SIGNATURE

July 1, 1985
DATE

PROGRAM FOR APPLIED RESEARCH ON FERTILITY REGULATION

SIX MONTH TECHNICAL REPORT SUMMARY

PARFR- 376

TITLE: "Pre IDE Studies - Tubal Clip"

INSTITUTION: Maine Medical Center

PRINCIPAL INVESTIGATOR: C. Irving Meeker, M.D.

FUNDING PERIOD: 5/1/85-4/30/86 AMOUNT FUNDED: $29,774

Below is a project summary for the January 1, 1985-June 30, 1985 period.

(continued from page one)

reviewed to determine whether any other strain may be more appropriate. It is anticipated that the animals for this study will obtained from the Jackson Laboratories, and in addition, that Maureen Marito, our new animal technician will spend several days at Jackson Laboratories learning the details of care that may assist us in managing a colony of this specific strain of mice.

It is our expectation that the mice will actually be ordered and on hand at the Maine Medical Center prior to the middle of July so that our surgical procedures may be carried out on schedule during the latter part of July and beginning of August. This time-table will allow us to meet our original projected schedule.

C. Irving Meeker
SIGNATURE

July 1, 1985
DATE

PARFR SCIENTIFIC ADVISORY COMMITTEE
MEETING XXXXVII

Friday, April 12, 1985
HYATT REGENCY O'HARE
9300 West Bryn Mawr Avenue
Rosemont, Illinois 60018
(312) 696-1234

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.
Ronald H. Gray, M.D.
Gary D. Hodgen, Ph.D.
Miriam H. Labbok, M.D., M.P.H.
Kamran S. Moghissi, M.D.
Dean L. Moyer, M.D.
C. Alvin Paulsen, M.D.
Antonio Scommegna, M.D.
Rochelle N. Shain, Ph.D.
Anne Colston Wentz, 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

Laneta Dorflinger, Ph.D.
James D. Shelton, M.D., M.P.H.

I. ANNOUNCEMENTS

A. Dr. Alvin Paulsen was introduced to the Committee.

B. The following SAC meetings were scheduled for

July 15, 1985 -- Chicago (downtown)
December 2, 1985 -- Washington, D.C. (Roslyn area)
March 28, 1986 -- Chicago

II. NEW BUSINESS:

A. EXTENSION PROPOSALS

PARFR-359 -- 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: $97,482 Length of Project: Second Year

The Committee reviewed the material provided and voted not to approve the proposal as presented. The Committee suggested a site visit (Reproductive Biologist) to work with the PI on a modified proposal to be funded.

A. EXTENSION PROPOSALS (continued)

PARFR-360 -- John C.M. Tsibris, Ph.D., University of Illinois at Chicago
"Inter- and Intra-Cycle Variation of Genital Peroxidases in Women"
Funding Requested: $61,646 Length of Project: One Year

The Committee reviewed the material provided and voted not to approve the proposal as presented. Once all the data is available, the Committee will consider a revised proposal dealing with the effects of the ejaculate on GP and self-sampling of cervical mucus.

B. FORMAL PROPOSALS

Diana Riad-Fahmy, M.Sc., Ph.D., The Tenovus Institute for Cancer Research, University of Wales, Heath Park, United Kingdom
"Microtitre Plate Enzymeimmunoassays for Salivary Progesterone and Estradiol"
Funding Requested: $37,290 Length of Project: One Year

The Committee reviewed the material provided and voted not to approve the proposal.

Erwin Goldberg, Ph.D., Northwestern University, Evanston, Illinois
"Immunocontraception with Synthetic Antigenic Determinants of Lactate Dehydrogenase-C_4"
Funding Requested: $176,421 - 1st Year Length of Project: Three Years
189,490 - 2nd Year
204,791 - 3rd Year

The Committee voted to approve the proposal.

Robert T. Chatterton, Ph.D., Northwestern University Medical School, Chicago, Illinois
"Ovulation Inhibition by Anordrin: Preclinical Testing"
Funding Requested: $ Length of Project:

The Committee approved the proposal and requested that toxicology studies should be rewritten in order to comply with recent FDA requests. Additionally, the PI should request a budget from the private sector for the teratology studies.

C. Irving Meeker, M.D., Maine Medical Center, Portland, Maine
"A Tubal Plug and Clip Method for Female Sterilization"
Funding Requested: $26,515 - 1st Year Length of Project: Two Years
36,270 - 2nd Year

The Committee approved the proposal as presented.

C. PILOT STUDY PROPOSALS

Richard Berger, M.D. and John Jessen, D.D.S., Harborview Medical Center, Seattle Washington
"Evaluation of Hydrolyzable Intraluminal Stents in Vasovasostomy"
Funding Requested: $9,987 Length of Project: One Year

The Committee reviewed the material provided and voted not to approve the proposal.

Shalender Bhasin, M.D., Harbor-UCLA Medical Center, Torrance, California
"Testosterone Microcapsules: Kinetics of Testosterone Release in Castrated Male Rats"
Funding Requested: $10,000 Length of Project: One Year

The Committee reviewed the material provided and voted not to approve the proposal.

D. INFORMAL PROPOSALS

Barbara Gross, B.Pharm., M.Sc., Ph.D., The Parramatta Hospitals/Westmead Hospital, Westmead, Australia
"Prolactin Measurement as a Means of Predicting Returning Fertility in Post Partum Breastfeeding Women"
Funding Requested: $50,640 - 1st Year Length of Project: Three Years
48,650 - 2nd Year
43,850 - 3rd Year

The Committee reviewed the material provided and voted not to request a form proposal.

Ralph M. Richart, M.D., Presbyterian Hospital in the City of New York,
"Clinical Evaluation of Iodine Compound for Closure of the Human Fallopian Tube"
Funding Requested: $9,765 + Clinical Costs Length of Project: Two Years

The Committee reviewed the material provided and recommended that the PI should contact the FDA (Drug Division) and hold an informal discussion with them to decide what type of studies are needed for an IND. Once the information is obtained, a revised proposal will be submitted for the July SAC meeting.

E. TECHNICAL REPORT REVIEW

The following technical reports were reviewed in detail:

PARFR-330M (FINAL) -- Roberto Rivera, M.D., Instituto de Investigacion Cientifica, Durango, Mexico
"A Clinical Evaluation of the Subdermal Contraceptive Norethindrone Pellet (Phase II)"

E. TECHNICAL REPORT REVIEW (continued)

PARFR-330T (FINAL) -- Ricardo H. Asch, M.D., The University of Texas Health Science Center at San Antonio
"A Clinical Evaluation of the Bioabsorbable Subdermal Contraceptive Norethindrone Pellet Implant (Phase II)"

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

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

PARFR-348 (FINAL) -- Danny H. Lewis, Ph.D., Stolle Research and Development Corporation, Cincinnati, Ohio
"Development of Improved Methods and Materials for Injecting Microencapsulated Steroids"

PARFR-358 (FINAL) -- Danny H. Lewis, Ph.D., Stolle Research and Development Corporation, Cincinnati, Ohio
"Development of a 30-Day Injectable Contraceptive"

PARFR-362 -- Danny H. Lewis, Ph.D., Stolle Research and Development Corporation, Cincinnati, Ohio
"Combination Injectable Steroidal Microsphere - Continuation of PARFR-332"

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

PARFR-364 -- Francisco J. Rojas, Ph.D., The University of Texas Health Science Center at San Antonio
"Antifertility Effects of Microencapsulated LHRH Angonist"

PARFR-353 (FINAL) -- Antonio Scommegna, M.D., Michael Reese Hospital & Medical Center, Chicago, Illinois
"Effect of Chronic Intrauterine Release of Estradiol and Progesterone on Uterine Histology in Intact Rabbits"

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"

PARFR-355 -- Nancy J. Alexander, Ph.D., Oregon Regional Primate Research Center, Beaverton, Oregon
"Enhancement of the Secretory Immune Response to LDH-C_4"

E. TECHNICAL REPORT REVIEW (continued)

PARFR-367 -- Jorge A. Blaquier, M.D., Instituto de Biologia y Medicina Experimental, Buenos Aires, Argentina
"Fertility Inhibition of *In Vivo* Immunization with Epididymal Proteins in Hamsters"

PARFR-363 -- John P. Wiebe, Ph.D., The University of Western Ontario, London, Ontario, Canada
"Laboratory Studies on an Antispermatogenic Agent - THP for Control of Male Fertility"

PARFR-361 -- Ricardo H. Asch, M.D., The University of Texas Health Science Center at San Antonio
"Testosterone Microcapsule Formulation Study"

III. MISCELLANEOUS

A. The following subagreements were executed on projects reviewed and approved at the December 12, 1984 SAC Meeting:

1. PARFR-368 -- E.S. Nuwayser, Ph.D., Biotek, Inc., Woburn, Massachusetts
"NIH/Biotek Levornorgestrel Microcapsules"
Funding Period: 2/1/85-1/31/86 Amount Funded: $74,965

2. PARFR-369 -- Milos Chvapil, M.D., Ph.D., D.Sc., Bio-Products, Inc., Tucson, Arizona
"Transuterine Application of Biocompatible Hydrogel Hypan into Fallopian Tubes"
Funding Period: 2/1/85-7/31/85 Amount Funded: $3,979

3. PARFR-370 -- Milos Chvapil, M.D., Ph.D., D.Sc., Bio-Products, Inc., Tucson, Arizona
"Sterilization of Male Dogs by Injecting Vas Deferens with Hydrogel Hypan"
Funding Period: 2/1/85-10/31/85 Amount Funded: $22,499

4. PARFR-371 -- James R. Dingfelder, M.D., Eastowne Ob/Gyn and Infertility, Chapel Hill, North Carolina
"Vaginal Spermicidal Barrier (VSB) Postcoital Tests"
Funding Period: 4/1/85-3/31/86 Amount Funded: $15,598

5. PARFR-372 -- Danny H. Lewis, Ph.D., Stolle Research & Development Corp., Cincinnati, Ohio
"Progestational Agents Effects on Baboon Endometrium"
Funding Period: 3/1/85-10/31/85 Amount Funded: $75,120

III. MISCELLANEOUS (continued)

B. Amendments to subagreements for projects reviewed and approved at the December 12, 1984 SAC Meeting:

1. Amendment #6 to PARFR-309 -- Robert T. Chatterton, Ph.D., Northwestern University Medical School, Chicago, Illinois
"Ovulation Inhibition by Anordrin"
Extended to: 6/30/86 Additional Funding: $94,056

C. Dr. Dean L. Moyer is thanked for his years of service to PARFR as a member of our Scientific Advisory Committee from March, 1982 through April, 1985.

D. SAC was asked to submit suggestions for topics for PARFR's 1986 Workshop.

E. The Fertility Monitor device developed by Zetek, Inc. to measure electrical resistance in saliva and cervical fluids as a predictor of ovulation was reviewed. The Committee suggested limited trials to be conducted at Northwestern and Wayne State Universities.

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

Diane Krier-Morrow, M.B.A.
Director of Administration, PARFR

February, 1985, Volume 3, Number 3

Program for Applied Research on Fertility Regulation
Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611
Editor: Gerald I. Zatuchni, M.D., M.Sc.
Managing Editor: Kelley Osborn
This publication is supported by AID/DPE-0546-A-00-1003-00

parfr

rffr

RESEARCH FRONTIERS IN FERTILITY REGULATION

IMMUNOLOGIC METHODS OF FERTILITY REGULATION: REPORT OF A WORKSHOP*

Reproductive immunology has been identified as a topic of interest by several national and international agencies supporting contraceptive development. The National Institute of Child Health and Human Development (NICHD) has a vested interest in both the basic and applied aspects of research on immunocontraception. Both contract and grant support mechanisms of NICHD are used to provide direct financial assistance for research in this field. The Office of Population, Agency for International Development (AID), is currently interested in identifying and defining an increased role it could play in enhancing developments in this field. Limited support for some applied aspects of immunocontraception has been provided to investigators through programs such as the Program for Applied Research on Fertility Regulation (PARFR) and the Population Council. The World Health Organization (WHO) Special Programme of Research in Human Reproduction began the Task Force on Immunological Methods of Fertility Regulation (Birth Control Vaccines) in 1973 and intends to initiate clinical trials in 1985. The Government of India accords high priority to immunocontraception.

Owing to these interests, a joint National Institutes of Health (NIH) AID PARFR Workshop on Research and Development of Immunologic Methods of Fertility Regulation was convened at NIH on April 16-18, 1984.

The purpose of the workshop was to review current research on reproductive immunology, with specific reference to research on sources of antigens that have the potential for eliciting an immune response and can interfere with reproductive processes in both the male and the female. Participants were experts in reproductive biology, endocrinology, biochemistry, immunology, and pathology (see page 11). It was the intention that the workshop result in recommendations to investigators and funding agencies interested in conducting and supporting research and development activities in this field.

One important issue considered was whether efficacious, safe, and reversible vaccines for fertility regulation can be developed. The current research in the area and related topics were reviewed and discussed (see page 10), with primary emphasis on research to develop vaccines based on 1) gonadotropins or their subunits and fragments, 2) sperm antigens, and 3) antigens derived from the zona pellucida. Prerequisites for an acceptable vaccine were discussed in detail. The workshop participants identified research needs and stressed the importance of research on both basic and applied aspects of reproductive biology and immunology. The overall complexity of developing contraceptive vaccines indicates the need for interdisciplinary research.

It was the consensus that immunologic approaches offer some unique advantages in fertility regulation. Considerable research effort, however, must be mounted to recognize their full potential. Although some approaches have advanced to the stage where clinical testing is imminent, these as well as other approaches require additional preclinical research.

The proceedings and the recommendations of the participants (indicated by italics) are summarized in the sections that follow.

*This report was published in part in Contraception 31(1):11-28, January 1985, and is presented here with permission.

The organizers of the workshop wish to acknowledge the enthusiasm and active participation of the speakers and the observers. This report was compiled by Jeff Spieler, Agency for International Development, and is the result of a collaborative effort on the part of all those involved in the workshop. Special thanks for its preparation must be extended to Nancy Alexander, Gabe Bialy, Laneta Dotts-Geier, Mike Harper, Mike McClure, Jeff Spieler, and Vern Stevens.

© Copyright PARFR 1985

ANTI-HORMONE VACCINES

Research efforts are being directed toward the development and evaluation of efficacy and safety of antifertility vaccines using the gonadotropins and their subunits and various peptide fragments. For example, prototype vaccines are being developed using the beta subunit of human chorionic gonadotropin (hCG beta), the beta subunit of ovine luteinizing hormone (oLH beta), and the carboxy-terminal peptides of hCG beta. In extensive safety tests in animals, including non-human primates, hCG beta, oLH beta and the hCG beta carboxy-terminal peptide vaccines showed no evidence of acute or chronic side effects (29).

In rhesus monkeys, immunization with oLH beta caused a shortened luteal phase with reduced progesterone levels. In this study, monkeys were observed for more than 5 years, and detailed histopathological studies were conducted at the end of that period (30). No damage to the pituitaries of the animals was observed, despite the continued existence of circulating antibodies reactive to LH. Although the mechanism of action of anti-hormone antibodies is not completely defined, it was demonstrated that animals with high levels of circulating antibodies do not become pregnant after multiple matings (36).

The observation of impaired or reduced ovarian function in animals immunized with oLH beta (30) raised a discussion of the possibility that such induced endocrine imbalances could lead to breast tumors or other malignant endocrine diseases, as has been seen in relation to an insufficient luteal phase. Obviously, subjects participating in clinical trials with oLH beta would need to be monitored for several years at least. In human trials conducted some years ago in India, Finland, Chile, and Brazil, immunization with an hCG beta subunit linked to tetanus toxoid did not produce any detectable side effects (6).

Further safety testing of hCG beta or oLH beta was not recommended by the workshop participants, since the number of animals that could be used for such studies would not likely reveal a potential, low-frequency problem resulting from immunization. However, it was agreed that sufficient safety testing should be conducted on new vaccine formulations to permit approval of clinical trials by appropriate drug regulatory authorities.

The major limitation to the anti-hormone vaccine (as well as other potential vaccines) is likely to be the genetically determined variability among individual recipients in response to immunization. It was speculated that such variation in both magnitude and duration of vaccine-induced immune response may be reduced by the use of potent adjuvants; however, the number of non-responders could still be significant. *Simple, non-invasive methods to identify such non-responders were believed to be an important area for research.* Despite this concern, it was the consensus that Phase I clinical trials to test the immunogenicity of at least two or three of the best characterized anti-hormone vaccines could be initiated as soon as approved by appropriate regulatory authorities.

Early clinical trials will answer many important questions, such as the variation in the level and duration of antibody production among women. Since several different vaccines are likely to be in clinical trials within 1 to 2 years, *it was recommended that the exchange of sera among investigators be encouraged and that a reference "standard" serum bank for comparing antibody levels attained from different vaccines be established.* The availability of standard reagents and standard methodology will be required to permit comparisons between laboratories.

Several areas of research for the development of other anti-hormone vaccines were discussed, including anti-FSH and anti-LHRH. Questions were raised as to the advantages of an anti-LHRH vaccine over a long-acting, injectable LHRH antagonist. The most advanced primate study for male immunocontraception (22) used FSH as the antigen, however early promising results were followed by an unexplained recovery of spermatogenesis in spite of high antibody titers.

While there was no immediate role envisioned for anti-steroid hormone vaccines in human fertility regulation, it was thought that research studies employing them are likely to be of immense value in animal husbandry and veterinary medicine, and will provide much needed data on basic immunology and reproductive biology. *There was a consensus that more research is needed in the area of antigen carriers, new adjuvants, and chemical modification of antigens.*

When a method is ready for clinical testing, it was thought that studies of the mechanism(s) of action should be initiated, if they are not already underway. These data will reveal how a given vaccine is affecting fertility, and may provide insight for developing new vaccines.

While the participants encouraged clinical studies of anti-hormone vaccines at an early date, they did not endorse any specific method and could not vouch for the purity or lack of toxicity of individual vaccines at this time.

ANTIGENS IN THE MALE

Development of a vaccine based on sperm represents a promising approach to contraception. Interference may be feasible at several sites, during sperm production in the testes, during sperm maturation in the epididymis, or during sperm interaction with the egg in the female

62

reproductive tract. The number of known sperm antigens that could be used as a vaccine is limited. In fact, it was postulated that only 1% of the proteins in sperm have been identified to date (3). This situation is changing rapidly, however, due to the advent of monoclonal antibody (Mab) technology. *It is important that basic studies of spermatogenesis, sperm maturation, sperm function, and fertilization be continued.* Only with such information will we know which antigens will prove most useful for vaccine development.

Lactate Dehydrogenase (LDH-C_4). Some known antigens of sperm include LDH-C_4, protamine, acrosin, hyaluronidase, plasma membrane antigens, and other differentiation products. The most completely characterized antigen is LDH-C_4, which is synthesized during spermatogenesis and becomes localized to the mid-piece and tail of spermatozoa. This sperm-specific isoenzyme has been crystallized from mouse testis. Although there are some differences in the amino acid sequences of this tetrameric protein from various species, there is considerable cross-reactivity of antibodies raised against the mouse LDH-C_4 with the comparable enzyme of other mammals including rabbits, baboons and humans.

Although LDH-C_4 is considered to be an internal antigen, a significant amount of the antigen is also on the surface of sperm. Systemic immunization of females with LDH-C_4 resulted in a reduction of fertility in mice, rabbits, and baboons (12). Infertility was correlated with high antibody titers, and in baboons the ovulatory cycles were not affected (12). When antibody titers fell, normal pregnancies occurred. Samples of oviductal fluid from systemically immunized rabbits have been shown to contain antibodies to LDH-C_4, and IgA has been detected in uterine washings of immunized mice (12). These data suggest that immunoglobulins produced following immunization with LDH-C_4 enter the female reproductive tract. Antibodies probably prevent fertilization by causing spermatozoa to become agglutinated or immobilized. Studies have recently been initiated that attempt, by immunizing with LDH-C_4, to stimulate IgA secretory systems and promote antibody secretion into cervical mucus and oviductal fluids in rhesus monkeys.

Antibodies to LDH-C_4 cause a marked reduction in fertility (approximately 80%) in female baboons. While there is some concern that the contraceptive effect is not complete, increased fertility suppression may be achieved by altering the protocol to enhance antibody production. These changes would involve the route of administration (local versus systemic), dosage and schedule, adjuvant, and antigen (primate versus murine LDH-C_4).

Until these parameters are tested, LDH-C_4 remains one of the most promising target antigens for an antifertility vaccine. Nevertheless, *participants agreed that an in vitro test to measure antibody effectiveness would circumvent problems of multiple fertility trials.*

One advantage of LDH-C_4 is that its structure is well-defined; thus, the development of a synthetic vaccine is facilitated. Since production of a vaccine will depend on the capacity to synthesize large amounts of antigen, current studies are focusing on evaluating selected peptide sequences that can be synthesized. Fertility evaluation of baboons immunized with one such peptide, consisting of amino acid residues 5 to 15 of LDH-C_4 conjugated to diphtheria toxoid, is currently underway (11). After the first mating, only one of eleven baboons conceived. There are at least five other peptide sequences that could be tested and that may be even more effective in preventing conception. Although LDH-C_4 and its fragments have some attractive properties, questions of efficacy still require resolution in order to develop a vaccine based on this antigen. However, as long as antibodies to LDH-C_4, or to any other sperm antigen, for that matter, will agglutinate spermatozoa, the potential for a contraceptive effect exists.

Other Internal Sperm Antigens. The protamines, a family of nuclear proteins, may not be as amenable to antibody attack as other sperm constituents because they are not readily accessible to the antibody. Nevertheless, these antigens, as well as other internal antigens, may be vaccine candidates at some future date. Perhaps it will be possible to induce the Sertoli cells to transport substances into the maturing spermatogenic cells prior to their release from the seminiferous epithelium, thus rendering them incapable of subsequent fertilization (3). *With a better understanding of sperm production, new immunological methods of suppressing spermatogenesis without altering steroid hormone production may be possible.*

Use of Monoclonal Antibodies. Identifying sperm specific antigens has been facilitated by monoclonal antibody (Mab) techniques. Such antibodies provide a powerful tool to deduce the role of sperm antigens in germ cell differentiation, sperm maturation, capacitation, and fertilization. Since studies in many species will provide important basic information for vaccine development, a library of anti human sperm Mabs is required. Some Mabs to specific regions of sperm have already been described. Several Mabs to the acrosome, equatorial region, post acrosomal region, and tail of human sperm have been shown to impede sperm penetration of hamster eggs. Some Mabs react only with capacitated human sperm. One Mab directed against the surface of guinea pig sperm induces an acrosome reaction. *More studies on sperm function and fertilization will allow a better definition of possible sites for intervention.* Those Mabs that are directed against phylogenetically conserved antigenic determinants will be useful in

33

permitting functional evaluation in laboratory species. For example, an anti-human sperm Mab that reacts with mouse sperm will allow studies of its antifertility action in mice. Recent advances in hybridoma technology make it feasible to utilize human Mabs for antifertility vaccines through passive immunization.

Application of Mabs as immunoaffinity ligands has permitted the isolation of new antigens. Immunization of animals with these antigens may provide important information as to whether high titers of these antibodies will prevent fertility. Mabs as reagents will greatly facilitate studies on the ontogeny of surface antigens and their organization into functional topographic domains.

Another approach to the identification of antigens has been the evaluation of serum samples from individuals considered to have immunologically mediated infertility. Western blot techniques have been used to evaluate such serum samples. Further assessment of these antigens excised from gels may provide useful data concerning naturally occurring antibodies.

In this context, participants discussed the usefulness of supporting the WHO serum bank (Task Force on Birth Control Vaccines, Special Programme of Research in Human Reproduction). Concern was expressed that some of the serum samples were from patients who had not been adequately clinically characterized. Although the WHO serum bank has been useful in allowing a comparison of tests for assessing immunologic infertility, its importance for advances in vaccine development has not yet been demonstrated.

Further basic efforts should be directed toward studies of immunosuppressive and anti-complement factors in seminal plasma. These substances should be characterized and defined, since their presence may affect the antigenicity of sperm antigens, regulate whether intercourse can provide a booster effect, and affect other immunological events at a local level.

Differences in the immune response to sperm antigens should be studied. Only through studies in genetically defined or inbred strains of animals can a better understanding of the basis for optimal immunization regimens be determined and vaccines developed capable of overcoming poor immune responsiveness.

Whether a vaccine involving sperm antigens could be used more appropriately in females than males remains open to discussion. Two feasible approaches in females involve active immunization or a passive local delivery, such as vaginal administration of the anti-sperm antibody. Possibly, a vaccine that stimulates production of antibodies to several different sperm antigens will be most efficacious, although no one has yet reported 100% fertility suppression even with crude extracts that presumably contain all sperm and/or testis antigens.

THE OVUM AND ITS INVESTMENTS

Several unique antigenic substances are associated with oocyte development and may provide suitable targets for immunologic contraception. Although there may be specific antigens associated with the oocyte itself, such antigens have not been purified. In contrast, antigens specific to the oocyte investment — the zona pellucida — have been identified. Antigens from this noncellular layer may prove to be an excellent target for immunologic inactivation. Most recent research has concentrated on studying the antigens of the zona pellucida.

It remains to be seen whether other differentiation antigens can be found that appear or are expressed in the ovum only after fertilization (perhaps associated with the fertilizing spermatozoon). The availability of such post-fertilization antigens would reduce the possibility of cross-reactions with oocytes in the ovary. A particular class of structural nuclear proteins, the lamina A and C, has been detected in the cytoplasm of the ovum before fertilization (7). Remarkably, these two polypeptides suddenly become non-immunoreactive 2 or 3 minutes after fertilization, in a wave-like manner that is dependent on the release of intracellular calcium, and then gradually reappear again a few hours later. The significance of this observation and its possible utility for immunocontraception remains to be determined.

It is clear that if antigens that are associated only with later stages of oocyte development in the preantral or antral follicles, or with fertilization, can be defined, they would have great advantages, since the risk of endocrine disturbance or, more serious, permanent damage to the germ cells and primordial follicles and associated sterility would be avoided. Although it will be ideal if a contraceptive vaccine based on ovum antigens is reversible, this cannot be guaranteed. For some individuals who have completed their family, irreversible methods of contraception are both attractive and acceptable, as is evidenced by the popularity of sterilization by surgical methods. Furthermore, irreversible methods of immunocontraception may offer non surgical approaches to sterilization that would be of value in developing and developed countries.

Zona Pellucida Antigens. It was strongly emphasized that significant progress in the isolation and characterization of zona antigens has only been made through the use of standardized separation and purification techniques. These techniques include two dimensional high resolution

gels and the most sensitive protein detection methods available, e.g. silver staining. *It was recommended that these techniques be used routinely.* Application of such techniques has facilitated the identification and isolation of several zona pellucida antigens discussed below.

The zona pellucida is a complex structure composed of three major and several minor glycoprotein families with multiple antigenic determinants. The molecular weights of these glycoproteins are difficult to determine, because of heterogeneity due to extensive glycosylation. However, the apparent molecular weights of the most abundant antigens from the major family range from 55K to 80K, and it is the 55K antigen that is being investigated most extensively.

For the present, the zona pellucida of the pig provides the best immunogen, because of its availability, and because antibodies to the zona pellucida of this species cross-react with the zona antigens of several other species, including rabbits, nonhuman primates, and humans. Current information indicates that zona antigens derived from rodent species will not be suitable as the basis for a contraceptive vaccine for humans, owing to the lack of cross-reactivity with the human zona (10, 18).

Efficacy of Immunization with Zona Antigens. Active immunization with whole zona pellucida or passive immunization procedures with antibodies to zona pellucida can have dramatic effects on fertility in a variety of species. Studies involving active immunization of several species with zona pellucida antigens have also demonstrated strong antifertility effects (10, 16, 21). In addition, passive immunization with monoclonal antibodies to purified zona pellucida antigens has been shown to be effective in preventing fertilization in mice.

The immunologic response to zona antigens is remarkably consistent among individual animals and between species, and is also long-lasting (at least 1 year). This has been demonstrated in both rabbits and squirrel monkeys (9, 17).

Potential Problems and Drawbacks. There have been indications in several species that antibodies to some of these complex immunogens do alter ovarian function and ovum development. In rabbits, ovulation ceased within 3 months after immunization with zona pellucida, and histologic examination revealed that the ovaries lacked oocytes (21). Whether this effect will occur in higher primates is not yet known. Initial immunization in primates was conducted in squirrel monkeys, which do not exhibit an overt menstrual cycle (17). Unfortunately, histologic examination of the ovaries was not conducted in that study. Future research in other species may prove enlightening.

For reversible contraception, the most desirable interference with zona function would be one preventing fertilization. Inhibition of ovulation without concomitant change in endocrine function would also be desirable. Elimination of all oocytes from the ovary would be irreversible and, therefore, may be less desirable in women. It could be of significant benefit, however, in agricultural practice and for domestic pets.

More detailed studies are needed to determine the mechanisms involved in alterations of fertility following immunization with different zona antigens. When this information is available, selection can be made of the most appropriate antigens for further development.

Aside from discerning the mode of action, there is also the larger issue of provision of an adequate supply of the chosen antigen. Large-scale collection of zonae pellucidae from slaughter houses for processing is obviously not a practical long-term solution to this problem. It seems appropriate to start using modern techniques of molecular biology to help with the preparative-scale purification of these antigens.

Quantitation of the Immune Response. Use of standardized and quantitative methods, as outlined above, will be of great benefit in achieving progress with regard to characterization of the immune response. *For quantitation, it is recommended that investigators avoid the use of assays involving the measurement of fluorescence in intact zonae. Suitable tests for quantitation would include radioimmunoassay and non-isotopic assay detection systems such as ELISA or biotin-avidin.*

Collaboration Among Investigators. *All investigators, both in the USA and abroad, currently working on zona pellucida and oocyte antigens should be identified and a workshop convened to discuss the status of ongoing research and to outline the standardization of techniques and approaches being used. When standardized methodology has been adopted, exchange of antigens and antisera can be initiated. At that time a bank of reference preparations should be established.*

New Information Obtainable from Further Research. Remarkably little is known about the biochemical and molecular events associated with the early stages of oocyte growth and follicular development. Since the zona pellucida proteins are synthesized and secreted during these early stages, they provide unique stage specific markers for development of the follicle. *The studies described above also should allow generation of specific molecular probes and antibodies that can be used to elucidate the mechanisms and control systems of oogenesis, and more precisely define the process of fertilization.*

65

ACCEPTABILITY AND EFFECTIVENESS OF CONTRACEPTIVE VACCINES

In view of the wide-scale acceptance of vaccination and parenteral administration of drugs, fertility-regulating vaccines should prove to be an acceptable, and therefore popular, approach to family planning. Furthermore, it would seem likely that immunologic methods can be selected that do not disrupt the menstrual cycle or cause the metabolic disturbance and concomitant side effects associated with some of the currently used hormonal contraceptives.

Because vaccines will provide long-acting fertility regulation and could be administered by trained paramedical and non-physician personnel, they are likely to be enthusiastically received by administrators of family planning programs. Furthermore, antifertility vaccine programs could be included within the scope of the MCH services and integrated not only with family planning but also with exclusive immunization programs (e.g., WHO Expanded Programme of Immunization), thus further improving on the availability of family planning.

There was considerable discussion among participants on how effective any birth control vaccines must be to warrant consideration. Views ranged from the opinion that even a 30% effectiveness would have demographic impact (if it were the only available or acceptable method) to the *general consensus that less than 90% effectiveness would not be acceptable.*

There are two distinct considerations, the percent of nonresponders in a population and the contraceptive effectiveness in responders. It has been demonstrated in animals that there are genetically controlled species and strain differences to immunogens. In addition, there are inter-animal differences in immune response within the same strain. *Therefore, techniques to predict or immediately identify poor responders or non-responders in a human population would be useful.*

Reversibility is an important issue. Although it is preferable to develop reversible methods, potentially irreversible methods may be acceptable, particularly since sterilization is currently the most widely used method of fertility regulation. *There was a consensus that irreversible approaches should have a greater method effectiveness than reversible ones.*

The discussion on this topic terminated with the following points:

1. A vaccine that is at least 90% effective should be sought.
2. In the absence of the desired level of effectiveness, a given approach should not be abandoned immediately. Instead, techniques for improving the response by modifying the immunogen, carrier, adjuvant, or delivery schedule should be investigated.
3. The actual or percent reversibility of any given approach will have to be determined empirically. At this time, the major consideration should be to obtain and maintain an appropriate antibody response over a desired period of time.
4. Depending on the nature of the antigen, it may be preferable to utilize a homologous immunogen-animal model system than a heterologous one in order to obtain better information on duration of action, reversibility, and safety.

SAFETY CONSIDERATIONS

Safety is an important consideration in the development of a contraceptive vaccine. Aspects of the comprehensive document (33) developed by the WHO Task Force on Birth Control Vaccines for testing the efficacy and safety of birth control vaccines were discussed. *In view of new knowledge obtained during the past several years, this document, which was formulated in 1977 and published in 1978, should be updated.* Some issues of safety and immunopathology were discussed with regard to these original guidelines. While immune complex deposition could be a worrisome complication, the effect of circulating immune complexes is an open question.

Four major potential complications were proposed for consideration.

1. Induction of organ-specific autoimmune disease (e.g., of the pituitary, ovary, or testes).
2. Induction of antibodies to hormone receptors that may follow an immune response to peptide hormones.
3. Enhancement of the risk of neoplasia to organs that carry antigens involved in a vaccine (e.g., hCG and immunization of men with sperm antigens), as well as an indirect biological effect resulting from, for example, the effect of unopposed estrogens on the uterus.
4. Genetically controlled variation in the immune response (i.e., some individuals may not develop a sufficient immune response) and long-term biological effects of immunization. It was stressed that the genetic control of the immune response must be dissociated from the biologic consequences of the immune response.

There is evidence to indicate that each of these possibilities is a valid concern. It was thought that for testing purposes in a responding species, autoimmune pathology, should it occur, would be observed histologically within 6 months after high titers of antibody are obtained.

CARRIERS, CONJUGATION METHODS, ADJUVANTS, AND VEHICLES

Methods used in development of certain vaccines were reviewed. Examples of antigen carriers were described and requirements for appropriate chemical coupling of antigens to carriers were outlined. The participants stressed the importance of the capability of preparing an immunogen with batch-to-batch reproducibility with regard to its chemical and immunologic properties. Chemical methodology suitable to industrial scale production will have to be developed.

Several experimental synthetic adjuvants with potent antibody-stimulating properties have been developed. Some of these compounds may be useful in overcoming genetically-related unresponsiveness to reproductive antigens. Vehicles or delivery systems for vaccine components are not in an advanced stage of development. Most experimental vaccines have employed oil-in-water emulsions or alum precipitates. The safety of these products is important and must be considered. *Some scientists believe that polymer-based delivery systems can be designed to release an appropriate amount of immunogen and/or adjuvants from the injection site, and research in this area of vaccine development is encouraged.* Advances in the synthesis of lymphokines and immunoregulatory products of bacteria are also of importance when considering methods of enhancing an immune response. Methods resulting from research are likely to be applicable to the development of a wide variety of antifertility vaccines.

USE OF RECOMBINANT DNA TECHNOLOGY IN DEVELOPMENT OF CONTRACEPTIVE VACCINES

An important issue discussed was the use of molecular biology and recombinant DNA technology to produce large quantities of purified antigen. Such large-scale production of pure antigen will be a critical and perhaps limiting factor in ultimately providing a contraceptive vaccine for family planning programs.

Technology is rapidly advancing in the area of genetic engineering. It is now possible to produce large proteins and even glycoproteins using this technology. Even rare messenger RNAs have been used to establish clones. It was pointed out that once a cDNA clone is available to produce a specific antigen, it might be possible to make small changes in the DNA of that clone to enhance immunogenicity of the antigen. Recombinant DNA technology can also be used to produce smaller peptide fragments of a protein, which could possibly confer immunity to the full protein.

There was discussion of the use of genetic engineering technology to produce large quantities of antibodies for passive immunization programs. For example, a DNA library can be made from a clone producing a desired monoclonal antibody and those genes inserted into a high producing cell line. Large quantities of the original specific monoclonal antibodies would then be produced.

Recombinant DNA vaccine viruses may be produced that would express specific antigens useful for a contraceptive development program. The approach would entail the molecular cloning and characterization of cDNA, encoding the antigen of interest, and incorporation of the cDNA into a common vaccine virus such as vaccinia. In this way, purified antigen would not be necessary and the immunization program would follow well-established routes. Such an approach would be favored where large numbers of doses would be necessary for large target populations.

Finally, it was thought that recombinant DNA technology will be increasingly important in the identification of new antigens specific to the ovary or testis. These new antigens might prove superior to currently known antigens as a basis for a contraceptive vaccine. By a technique such as subtractive hybridization, new differentiation-specific antigens can be identified. *This area of research, while very basic, is essential for future evolution of contraceptive vaccine technology.*

DELIVERY SYSTEMS

A concerned research effort is necessary to develop new methods for vaccine administration. Current techniques require booster injections to maintain effective antibody titers. Development of new delivery systems that could provide for timed release of the vaccine would obviate the need for booster injections. Technology of this type has already been successfully applied to the release of contraceptive drugs. For the release of steroids and LHRH analogs, constant release has been desired. In order to achieve appropriate release rates for peptides, blends of microcapsules with a range of lag periods have been used. *Therefore, it should be possible to produce a microcapsule delivery system for vaccines that will incorporate a series of suitable delays in antigen release, resulting in effective immunity with a single shot vaccine.* This is especially important in developing countries, where more follow-up difficulties may be encountered if booster shots are necessary.

CONCLUSIONS

Throughout the workshop, the need to provide both stimulation and continuing support of basic and applied research was reiterated. There was consensus that the basic-applied viewpoint of research in reproductive immunology represented a graded continuum not amenable to discrete partitioning. Both aspects warrant urgent initiatives in support of immunocontraceptive research and development. *Nevertheless, it was recommended that financial support for the field be increased by donor agencies, that programs supporting basic research via grant mechanisms issue new Requests for Applications (RFAs), and that those agencies funding applied research issue new Request for Proposals (RFPs) addressing several of the areas emphasized during this workshop.* Multidisciplinary research and collaboration between interested investigators are essential if the formidable challenge of developing immunocontraceptives is to be met successfully.

REFERENCES AND SELECTED READINGS*

1. Alexander NJ: Antibodies to human spermatozoa impede sperm penetration of cervical mucus or hamster eggs. Fertil Steril 41:433-439, 1984.

2. Anderson DJ, Alexander NJ: A new look at antifertility vaccines. Fertil Steril 40:557-571, 1983.

3. Bellve AR: Personal communication, 1984.

4. Bellve AR: The molecular biology of mammalian spermatogenesis. In Finn CA (ed): Oxford Reviews of Reproductive Biology. Oxford, Oxford University Clarendon Press, Vol. 1, pp 159-261, 1979.

5. Bellve AR, O'Brien DA: The mammalian spermatozoon: structure and temporal assembly. In Hartman JF (ed): Mechanisms and Control of Fertilization. New York, Academic Press, Inc., 1983, pp 5-137.

6. Contraception 13(2): [entire issue] 1976.

7. Donovan MJ, Mayhew PL, Bellve AR: Dynamics of the lamin proteins during fertilization and early embryogenesis in the mouse. Reflection of the calcium transient. Cell (submitted for publication, 1985).

8. Drell DW, Dunbar BS: Monoclonal antibodies to rabbit and pig zonae pellucidae distinguish species-specific and shared antigenic determinants. Biol Reprod 30:445-457, 1984.

9. Dunbar BS: Personal communication, 1984.

10. Dunbar BS, Wolgemuth DJ: Structure and function of the mammalian zona pellucida, a unique extracellular matrix. In Satir B (ed): Modern Cell Biology, Vol 3, pp 77-111. New York, Alan R. Liss, Inc., 1984.

11. Goldberg E: Personal communication, 1984.

12. Goldberg E: Current status of research on sperm antigens: Potential applications as contraceptive vaccines. In Zatuchni GI (ed): Research Frontiers in Fertility Regulation, Vol 2, No 6. Chicago, PARFR 1983, pp 1-11.

13. Isahakia M, Alexander NJ: Interspecies cross-reactivity of monoclonal antibodies directed against human sperm antigens. Biol Reprod 30:1015-1026, 1984.

14. Miller AB, Barclay THC, Choi NW, Grace MG, Wall C, Plante M, Howe M, Cinader B, Davis FG: A study of cancer, parity and age at first pregnancy. J Chron Dis 33:595-605, 1980.

*Not all references are cited in the text.

15. Naz RK, Alexander NJ, Isahakia M, Hamilton MS: Monoclonal antibody to a human germ cell membrane glycoprotein that inhibits fertilization. Science 225:342-344, 1984.

16. Sacco AG: Immunocontraception: Considerations of the zona pellucida as a target antigen. In Wynn RM (ed): Obstetrics and Gynecology Annual, Vol 10, pp 1-26. New York, Appleton-Century-Crofts, 1981.

17. Sacco AG, Subramanian MG, Yurewicz EC, DeMayo FJ, Dukelow WR: Heteroimmunization of squirrel monkeys (*Saimiri sciureus*) with a purified porcine zona antigen (PPZA): Immune response and biologic activity of antisera. Fertil Steril 39:350-358, 1983.

18. Sacco AG, Yurewicz EC, Subramanian MG, DeMayo FJ: Zona pellucida composition, specie cross-reactivity and contraceptive potential of antiserum to a purified pig zona antigen (PPZA). Biol Reprod 25:997-1008, 1981.

19. Saling PM, Raines LM, O'Rand MG: Monoclonal antibody against mouse sperm blocks a specific event in the fertilization process. J Exp Zool 227:481-486, 1983.

20. Saling PM, Waibel R, Lakoski KA: Immunological identification of sperm antigens that participate in fertilization. In Hedrick JL (ed): The Molecular and Cellular Biology of Fertilization. New York, Plenum Press (in press).

21. Skinner SM, Mills T, Kirchick HJ, Dunbar BS: Immunization with zona pellucida proteins results in abnormal ovarian follicular differentiation and inhibition of gonadotropin induced steroid secretion. Endocrinol 115:2418-2432, 1984.

22. Srinath BR, Wickings EJ, Witting C, Nieschlag E: Active immunization with follicle stimulating hormone for fertility control: a four and a half year study in male rhesus monkeys. Fertil Steril 40:110-117, 1983.

23. Stevens VC: Potential methods for immunological fertility control in the female. In Benagiano G, Diczfalusy E (eds): Endocrine Mechanisms in Fertility Regulation, pp 141-161. New York, Raven Press, 1983.

24. Stevens VC, Cinader B, Powell JE, Lee AC, Koh SW: Preparation and formulation of a human chorionic gonadotropin antifertility vaccine. I. Selection of a peptide immunogen. Am J Reprod Immunol 1:307-314, 1981.

25. Stevens VC, Cinader B, Powell JE, Lee AC, Koh SW: Preparation and formulation of a human chorionic gonadotropin antifertility vaccine. II. Selection of adjuvant and vehicle. Am J Reprod Immunol 1:315-321, 1981.

26. Stewart TA, Bellve AR, Leder P: Transcription and promotor usage of the *myc* gene in normal somatic and spermatogenic cells. Science 226:707-710, 1984.

27. Talwar GP: Structured vaccines for control of fertility and communicable diseases. In Chandara RK (ed): Critical Reviews of Tropical Medicine, pp 245-269. New York, Plenum Press, 1984.

28. Talwar GP, Gupta SK, Gaur A, Singh G, Kaur IP, Das C, Singh V, Singh O, Sadal D, Iyer KSN: Anti-hCG and anti-LHRH monoclonals and their applications. In Isojima S, Billington WD (eds): Reproductive Immunology, pp 69-80. Amsterdam, Elsevier Science Publishers, 1984.

29. Thau RB, Witkin SS, Bond MG, Sawyer JK, Yamamoto Y: Effects of long-term immunization against the β-subunit of ovine luteinizing hormone on circulating immune complex formation and on arterial changes in Rhesus monkeys. Am J Reprod Immunol 3:83, 1983.

30. Thau RB, Yamamoto Y, Sundaram K, Spinola PG: Human chorionic gonadotropin stimulates luteal function in Rhesus monkeys immunized against the β-subunit of ovine luteinizing hormone. Endocrinology 112:277, 1983.

31. Tung KSK: Antifertility vaccine: Considerations of their potential immunopathologic complications. Int J Fertil 121:197-206, 1976.

32. Wheat TE, Goldberg E: Sperm-specific lactate dehydrogenase C_4. Antigenic structure and immunosuppression of fertility. In Rattazzi MC, Scandalios JG, Whitt GS (eds): Isozymes. Curr Top Biol Med Res 7:113-130, 1983.

33. WHO Task Force on Immunological Methods for Fertility Regulation. Evaluating the safety and efficacy of placental antigen vaccines for fertility regulation. Clin Exp Immunol 33:360-375, 1978.

34. Wolgemuth DJ, Celenza J, Bundman DS, Dunbar BS: Formation of the rabbit zona pellucida and its relationship to ovarian follicular development. Develop Biol 106:1-14, 1984.

35. Yamamoto Y, Gunsalus GL, Sundaram K, Thau RB: Antibodies against the β subunit of ovine luteinizing hormone can decrease the clearance of human chorionic gonadotropin in Rhesus monkeys. Am J Reprod Immunol 5:164, 1984.

36. Yamamoto Y, Gunsalus GL, Sundaram K, Thau RB: Characterization and anti-oLHB antibodies acting as contraceptives in Rhesus monkeys. I. *In vitro* binding properties. J Reprod Immunol 4:295, 1982.

37. Yamamoto Y, Thau RB: Characterization of anti-oLHB antibodies acting as contraceptives in Rhesus monkeys. II. *In vivo* neutralizing ability for gonadotropic hormones. J Reprod Immunol 5:195, 1983.

89

AGENDA

Joint NIH/AID/PARFR Workshop on Research and Development of Immunologic Methods of Fertility Regulation.

April 16-18, 1984, Bethesda, Maryland

1. *Introduction:* Dr. G. Bialy, Mr. J. Spieler
2. *Areas for Possible Disruption of the Reproductive Process Through Immunologic Methods:* Dr. N. Alexander
3. *Current Status of Research and Development on Specific Approaches and Prospects for the Future*
   Anti-hormones: Dr. R. Thau
   Anti-sperm: Dr. E. Goldberg, Dr. P. Saling
   Anti-egg: Dr. B. Dunbar, Dr. A. Sacco
4. *The Indian Experience — Priorities, Lessons to be Learned, Current Status, and Prospects for the Future:* Dr. G. P. Talwar
5. *International Approaches to Birth Control Vaccine Development:* Group
6. *Identification of New Antigens*
   Investigation of Immunologically Conditioned Infertility: Dr. D. Anderson
   Systematic Dissection of Membrane Antigens (Sperm and Ovum): Dr. A. Bellve
7. *Vaccine Development*
   Overview of Vaccine Development: Dr. J. Robbins
   Production of Vaccine Antigens — Natural Versus Synthetic Antigens, Including Use of Recombinant DNA and Solid Phase Synthesis Techniques: Dr. B. Chin
   Carrier Proteins, Adjuvants, Conjugation Procedures, Delivery Systems/Formulation: Dr. V. Stevens
8. *Immunopathology and Other Safety Considerations:* Dr. K. Tung
9. *Strategy Formulation*
   Brainstorming: Group
   Recommendations: Group

40

PARTICIPANTS

Dr. Nancy J. Alexander
Oregon Regional Primate Research Center
Beaverton, Oregon

Dr. Deborah Anderson
Division of Immunogenetics
Dana-Farber Cancer Institute
Harvard Medical School
Boston, Massachusetts

Dr. C. Wayne Barain
Center for Biomedical Research
The Population Council
New York, New York

Dr. Anthony Bellve
Laboratory of Human Reproduction
and Reproductive Biology
Harvard Medical School
Boston, Massachusetts

Dr. Gabriel Bialy
Contraceptive Development Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Richard P. Blye
Contraceptive Development Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. William Chin
Laboratory of Molecular Endocrinology
Massachusetts General Hospital
Boston, Massachusetts

Dr. B. Cinader
Institute of Immunology
University of Toronto
Toronto, Ontario, Canada

Dr. Philip A. Corfman
Special Programme of Research in
Human Reproduction
World Health Organization
Geneva, Switzerland

Ms. Ruth Crozier
Contraceptive Evaluation Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Jurrien Dean
Laboratory of Cellular and
Developmental Biology
National Institute of Arthritis, Diabetes,
and Digestive and Kidney Disease
Bethesda, Maryland

Dr. Laneta Dorflinger
Research Division
Office of Population
Agency for International Development
Washington, D.C.

Dr. Bonnie S. Dunbar
Department of Cell Biology
Baylor College of Medicine
Houston, Texas

Dr. Henry L. Gabelnick
Contraceptive Development Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Erwin Goldberg
Department of Biochemistry, Molecular
Biology and Cell Biology
Northwestern University
Evanston, Illinois

Dr. Michael J. K. Harper
Department of Obstetrics and Gynecology
University of Texas Health Science Center
San Antonio, Texas

Dr. Marvin J. Karten
Contraceptive Development Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Hyun K. Kim
Contraceptive Development Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Mortimer B. Lipsett
Office of the Director
National Institute of Child Health
and Human Development
Bethesda, Maryland

Ms. Julia Lobotsky
Reproductive Sciences Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Michael McClure
Reproductive Sciences Branch
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Harold Nash
Biomedical Research
The Population Council
New York, New York

Dr. D. J. Patanelli
Contraceptive Development Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Malcolm Potts
Family Health International
Research Triangle Park, North Carolina

Dr. John Robbins, Chief
Laboratory of Developmental
and Molecular Immunity
National Institutes of Health
Bethesda, Maryland

Dr. Griff Ross
Department of Obstetrics and Gynecology
University of Texas Medical School
Houston, Texas

Dr. Anthony Sacco
Department of Obstetrics and Gynecology
Wayne State University
Detroit, Michigan

Dr. Patricia Saling
Department of Obstetrics and Gynecology
Duke University Medical Center
Durham, North Carolina

Dr. James Sarn
Agency Director for Health and Population
Agency for International Development
Washington, D.C.

Dr. Sheldon Segal
The Rockefeller Foundation
New York, New York

Dr. James D. Shelton
Chief, Research Division
Office of Population
Agency for International Development
Washington, D.C.

Mr. Jeffrey Spieler
Research Division
Office of Population
Agency for International Development
Washington, D.C.

Dr. Vernon Stevens
Department of Obstetrics and Gynecology
The Ohio State University Hospital
Columbus, Ohio

Dr. G. P. Talwar
National Institute of Immunology
New Delhi, India

Dr. Rosemarie B. Thau
Biomedical Research
The Population Council
New York, New York

Dr. Kenneth S.K. Tung
Department of Pathology
University of New Mexico
Albuquerque, New Mexico

Dr. Koji Yoshinaga
Reproductive Services Branch
Center for Population Research
National Institute of Child Health
and Human Development
Bethesda, Maryland

Dr. Gerald I. Zatuchni
Program for Applied Research
on Fertility Regulation
Northwestern University
Chicago, Illinois

91

April, 1985, Volume 3, Number 4

Program for Applied Research on Fertility Regulation
Northwestern University
Suite 1525
875 North Michigan Avenue
Chicago, Illinois 60611

Editor: Gerald I. Zatuchni, M.D., M.Sc.
Managing Editor: Kelley Osborn
This publication is supported by AID/DPE-0546-A-00-1003-00

parfr

rffr

RESEARCH FRONTIERS IN FERTILITY REGULATION

TRANSCUTANEOUS MALE STERILIZATION

Alfredo Goldsmith, M.D., M.P.H.
Associate Professor
Department of Obstetrics and Gynecology,
Northwestern University Medical School, Chicago, Illinois
David A. Edelman, Ph.D.
Medical Research Consultants, Inc., Chapel Hill, North Carolina
Gerald I. Zatuchni, M.D., M.Sc.
Professor
Department of Obstetrics and Gynecology,
Northwestern University Medical School, Chicago, Illinois

Vasectomy is one of the safest, simplest, and most effective methods of fertility regulation. Compared to female sterilization, it is safer and cheaper and has a similar rate of effectiveness. In spite of these advantages, in most countries the number of contraceptive female sterilizations performed each year continues to exceed the number of male sterilizations performed (30). In Latin America, the Caribbean, the Middle East, and Africa, fewer than one-half million couples rely on vasectomy for contraception, compared to about 5 million couples in the United States and about 12 million couples in both India and China (30). The reasons cited (30) for the low prevalence of vasectomy in some countries include:

1. Lack of vasectomy services
2. Emphasis on providing female and not male sterilization services
3. Negative attitudes of physicians toward vasectomy.

Even if there were a greater emphasis on the provision of male sterilization services there might not be a greatly increased demand for these services, especially in cultures where men have a fear of surgery in the scrotal area and where vasectomy is equated with castration. However, many experts argue that a simple nonsurgical method of male sterilization would have the potential to overcome the fears associated with the standard methods of vasectomy requiring a scrotal incision.

Advantages of Transcutaneous Procedures. For over 20 years, research has been conducted to develop a transcutaneous method of male sterilization in which the scrotum is punctured by a needle or needle-like instrument. By means of this instrument, chemical agents or electro-coagulation can be used to block the vas lumen. The ideal has been to develop a procedure that is inexpensive, can be administered by trained paramedical personnel, and can be performed with simple instrumentation. The potential advantages of transcutaneous vas occlusion compared to standard surgical vasectomies include the following (12):

1. The risk of postsurgical hemorrhage is eliminated. At present, about 1.6% of surgical vasectomy patients develop postoperative hematomas (31).
2. The risk of postoperative infections should be greatly diminished. Presently, about 1.5% of men undergoing surgical vasectomy procedures develop infections (31).
3. The procedure should be more acceptable to men who have a fear of genital operations.
4. Surgery and surgical equipment are not required.
5. The procedure can be taught to paramedical personnel. Once learned, the procedure can be done rapidly at low cost.

Despite the many advantages of transcutaneous male sterilization, only a minimal amount of research has been conducted to develop this method for widespread use in sterilization programs.

In spite of the apparent simplicity and advantages of a transcutaneous sterilization procedure, outside of the People's Republic of China, very little work has been done to evaluate the safety and effectiveness of these

©Copyright PARFR 1985

procedures in men. Most of the work has been limited to sporadic efforts to investigate the effects of various sclerosing agents using animal models. Some efforts have been made to improve the technical feasibility of the procedures through the development of improved equipment.

The purpose of this report is to review and summarize these research efforts and to provide an update on current research. In the following sections, current research and the results from animal and human studies are reviewed for each of the listed approaches to transcutaneous sterilization.

METHODS OF TRANSCUTANEOUS STERILIZATION

The following methods of transcutaneous sterilization have been evaluated in animals and/or man:

1. Intratesticular injection of chemical agents to affect spermatogenesis.
2. Intraepididymal injection of chemical agents to affect sperm transport.
3. Obstruction of the vas lumen by the intravasal injection of chemical (sclerosing) agents or by electrocoagulation of the vas lumen.

Intratesticular Methods. For over 25 years, research has been underway to develop a male contraceptive pill that would interfere with spermatogenesis, either by direct action on the pituitary by suppressing the production of gonadotropins or by direct interference with spermatogenesis in the testes. Numerous steroidal and non-steroidal agents have been evaluated, including luteinizing hormone releasing hormone (LHRH) agonists and antagonists, progestogens, and androgens, either in combination or alone, and other drugs such as gossypol, a phenolic compound isolated from the cotton plant (15, 22, 29). These drugs are usually administered orally or by systemic injection, and their use has been associated with undesirable side effects, including loss of libido and various effects on accessory sex glands. Although many of these compounds can produce oligospermia, they do not consistently produce azoospermia. Also, the effects of most of these compounds are temporary, and repeated administrations are required to maintain oligospermia/azoospermia. As an alternative to the interference of drugs, the direct injection of compounds into the testes has been investigated (32, 33).

Wiebe and Barr evaluated the effects of the direct injection of aqueous 1, 2, 3-trihydroxypropane (THP; glycerol), a normal component of living cells, into the testes of Sprague-Dawley rats (32, 33). Spermatogenesis was inhibited by a direct and local action of THP on the seminiferous tubles. The THP injections had no apparent effects on mature sperm stored in the epididymides. The first mating of treated rats resulted in normal offspring. After the fourth postinjection week no matings resulted in pregnancy. The THP injections produced long-term infertility (up to 21 weeks) without producing any significant effects on Leydig cell steroidogenesis, on testosterone and LH and FSH serum levels, or on secondary sexual characteristics and mating behavior. No undesirable side effects were noted. The investigators found that by 14 days after injection of THP, there was a 50% reduction in the weight of the testes. Although this is not considered an undesirable side effect in animals, in man a reduction in testicular size might limit the acceptability of the procedure. Additional studies are currently being undertaken to determine the mechanism of action of THP on the testes.

The only other intratesticular method that has been investigated is the use of ultrasonic energy. In one study of the effects of different ultrasound intensities on the testes of mature rabbits, ultrasound at 1.5 W per sq cm for 15 minutes produced degeneration of the seminiferous tubules (23). Whether the use of ultrasound can be developed into a practical method of nonsurgical sterilization will require further evaluation. One obvious limitation of the method, especially in developing countries, is the cost of the equipment and the difficulty in obtaining routine maintenance.

Intraepididymal Injection. Although the injection of sclerosing agents directly into the epididymis is technically simpler than injection into the vas lumen, the intraepididymal approach to nonsurgical sterilization is known to have been evaluated in only three studies. The advantages of intraepididymal over intravasal injections are that the cauda epididymis is easily palpated and intraluminal placement of the needle in the epididymis is not necessary.

Bowman and coworkers evaluated the effects of injections of anhydrous calcium chloride dissolved in sterile saline directly into the cauda epididymis of mature rams on ejaculate volume, sperm concentration, and mounting time (4). Five rams received injections of calcium chloride and five received injections of saline. By 2 weeks after the injections, the calcium chloride-treated rams had a significant reduction in ejaculate volume and sperm concentration. None of the animals became azoospermic, but semen analysis was characterized by the absence of sperm motility and by head-tail fragmentation. Mounting times were not affected. The sterilizing effects of the calcium chloride most probably were due to its necrosing effects on the epididymis. No histopathology studies were performed.

Lewis and Garcia evaluated the effects of three sclerosing agents (formaldehyde, methylcyanoacrylate [MCA], and quinacrine hydrochloride) in mature *Macaca fascicularis*

93

monkeys (18). These agents were injected directly into the cauda epididymis either transcutaneously or after exposure of the epididymis through a scrotal incision. All animals were sedated with ketamine hydrochloride intramuscular injection (0.1 ml/kg of 100 mg/ml solution). The results of the study are summarized in Table 1 and show that none of the agents evaluated was effective. Histologic evaluations of testicular biopsies performed after 6 months showed normal testicles in all monkeys but one. That MCA-treated monkey had testicular atophy. Other complications included abscess formation at the site of injection in two MCA-treated animals and two quinacrine-treated animals. In the formaldehyde-treated group, a moderate to marked inflammatory cell infiltrate and fibrosis were noted in most testicles evaluated. Four animals (1 MCA-treated; 3 quinacrine-treated) died after the intraepididymal injections. Three deaths occurred on the day of the procedure and one (in a quinacrine-treated animal) occurred 1 week after the procedure. Autopsies did not reveal the cause of death in any of the animals. In the quinacrine-treated group, death may have resulted from the quinacrine or from the combined toxicity of quinacrine and ketamine. Based on the unsatisfactory results obtained with all of the agents evaluated, no additional studies are being undertaken with intraepididymal injections of sclerosing agents.

Experimental Group	*No. Initially Azoospermic*	*Recanalization*	*No. Azoospermic After 6 Months*
Formaldehyde (4%) in glycerate (n=5)	4	2	2
MCA (0.5 cc) (n=5)	5	2	3
Quinacrine hydrochloride (100 mg) in water (n=5)	4	3	1

Source: Lewis and Garcia (18).

Table 1. Results of a study of intraepididymal injections of various agents.

Davis evaluated the intraepididymal injection of formaldehyde in alcohol and MCA in adult mongrel dogs (8). In the first two animals evaluated (one treated with formaldehyde in alcohol and one with MCA) severe toxicity and death occurred within 1 week of the injection. The cause of death of these animals was not stated, but may have resulted from the relatively large doses of the drugs administered. Gross examination indicated bilateral acute necrosis of the epididymides and testes in both animals. In other animals, in which lower doses of these chemicals were injected directly into the epididymis, extensive necrosis of the epididymides and testes was observed.

The appeal of the intraepididymal approach to transcutaneous sterilization is that it is easier to inject a chemical into the epididymis than into the vas lumen. To date, the limited evaluations of the intraepididymal injection of chemical agents has shown this to be an unsatisfactory approach to male sterilization. Whether improved results can be obtained with other chemical agents remains to be evaluated.

Intravasal Methods. The concept of transcutaneous vas occlusion with either chemical agents or electrocoagulation is not a recent one. Over 20 years ago Lee, in a series of experimental studies in dogs, evaluated the effects of electrocoagulation of the vas through a transcutaneously inserted electrode, or the transcutaneous injection of different concentrations of phenol, glycerophenol, or a combination or quinine and urethane (17). He also investigated the effects of the transcutaneous injection of liquid Biowax, a wax used in cosmetic surgery (17). Shortly after injection, the liquid Biowax solidifies, causing obstruction of the vas. Lee noted that if a radiopaque material were mixed with the Biowax, its placement could be checked using x-rays. Although none of the transcutaneous methods tested by Lee resulted in vas occlusion in all animals injected, the studies did demonstrate that the transcutaneous approach was a feasible method of vas occlusion.

Chemical Agents. Numerous chemical agents have been injected into the vasa of rats, dogs, and rabbits to evaluate their effects in producing vas occlusion. The agents evaluated are listed in Table 2. Most of the studies have been experimental, in that they have evaluated relatively few animals and have been conducted to screen chemicals that might be worthy of either more extensive trials in animals or preliminary trials in man.

Of the many chemical agents that have been evaluated in animals, only two are known to have been tested in man: 3.6% formaldehyde in 90% ethanol (5, 11) and 4% formaldehyde in 90% ethanol (6, 7) and a carbolic acid, n-butyl alpha cyanoacrylate mixture (21). The mode of action of all of the sclerosing agents tested is thought to be similar; they produce local necrosis and fibrosis and vasal closure through scarring.

One of the principal objectives in choosing a chemical agent for use in human sterilization procedures is to select one that has minimal toxic effects and will produce a minimal amount of damage if injected into structures other than the vas. Ideally, damage caused by the chemical should be limited to the basal epithelium of the vas, without damage to the muscularis. Although it is not the intent of this review to summarize the effects of each of the chemical agents used for vasal occlusion in various animals, it is noteworthy that in most of these procedures the epididymides and testes were not affected by the intravasal injections. Also, toxicity appeared to be minimal. None of the investigators reported any animal deaths or severe adverse reactions that could be attributed to the intravasal injections.

Chemical Agent & Reference	Animals Evaluated
Sodium chloride (5, 12)	Rat
Ethanol (5, 12, 13)	Rat, dog
Formaldehyde (5, 12)	Rat, dog
Formaldehyde with ethanol (5, 12)	Rat, dog
Silver nitrate (5, 12)	Rat, dog
Acetic acid (5, 12)	Rat
Sodium tetradecyl sulfate (5, 12)	Rat
Potassium permanganate (5, 12)	Rat
Sodium morrhuate (5, 12)	Rat
Camphor (28)	Rat
Potash (28)	Rat
Quinacrine dihydrochloride (28)	Rat
Quinacrine, urethane (17)	Dog
Phenol (17)	Dog
Glycerophenol (17)	Dog
Biowax (17)	Dog
Phenol, cyanoacrylate (25)	Rabbit
Methyl cyanoacrylate (8, 25)	Rabbit, dog
Tincture of iodine, potassium iodide, sinograffin, carboxymthyl cellulose (25)	Rabbit, dog
Phenol, glycerine, sinograffin, gum tragacanth (25)	Rabbit
Silver acetate alginate (8)	Dog
Calcium chloride, gum tragacanth, glycerine (21)	ns
Sodium, cod liver oil acid, gum tragacanth, glycerine (21)	ns
Carbolic acid, gum tragacanth, glycerine (21)	ns
Carbolic acid, n-butyl alpha cyanoacrylate (21)	ns
Ethyl alpha-cyanoacrylate (21)	ns
n-Butyl alpha cyanoacrylate (21)	ns

ns = not stated

Table 2. Chemical agents evaluated to produce occlusion of the vas in animals.

Based on their studies of the intravasal injection of various sclerosing agents in rats and dogs (whose vasa are similar to those of man), Coffey and Freeman elected to evaluate a combination of 3.6% formaldehyde in 90% ethanol in human trials (5, 11). One advantage to the use of this combination is that both chemicals are easily metabolized and leave no residuals to produce adverse effects. The method used by these investigators was simple and did not require the use of any elaborate surgical equipment. One disadvantage of the procedure is that the investigators found it necessary to inject 1 ml of 1% lidocaine alongside each vas before injection of 0.25 ml of the formaldehyde and ethanol mixture. After each vas was located and stabilized between the thumb and forefinger, the mixture was injected through a 25 gauge needle. By the 24th post-procedure week, 7 of the 8 men injected were azoospermic. The other man had a sperm count of 67 million sperm per ml at the 14th post-procedure week. Once azoospermia was established, none of the men followed-up for the 14-40 weeks was found to again have sperm in his semen.

Additional trials of the intravasal injection of formaldehyde in ethanol have been conducted by Davis (6, 7). The procedure used by Davis was essentially the same as the one used by Coffey and Freeman. Davis injected 0.5 ml 4% formaldehyde in 90% ethanol into each vas. In the first 27 procedures, an Allis clamp was used to stabilize the vas. In the next group of 27 men, a specially designed clamp was used to stabilize the vas. A 25-gauge needle was passed through holes in the jaws of the clamp to inject the formaldehyde in ethanol mixture into the vas. The results of the studies by Davis are summarized in Table 3.

Series	No. Subjects	Percent
First		
Azoospermic within 16 weeks	13	48.2
Failures	11	40.7
Decease in sperm count, further follow-up required	3	11.1
Second		
Azoospermic within 10 weeks*	17	65.4
Failures	4	15.4
Decrease in sperm count, further follow-up required	5	19.2
Failed injection (retractile testes)	1**	—

* Includes two men who had repeat injections
**Not included in calculation of percentages
Source: Davis (6, 7)

Table 3. Results of studies of the transcutaneous injection of 4% formaldehyde in 90% ethanol.

In the second series of subjects there was a significant increase in the incidence of azoospermia. Whether this was due to the use of the specially designed clamp or was due to the increased experience of the operator cannot be determined from the study data. For six men, the duration of follow-up was insufficient to determine success of the procedure. Davis noted that once a man became azoospermic, he remained that way (7). Some of the men, for whom the procedures had been classified as a failure, had a transient drop in their sperm counts that later returned to near baseline levels. This drop was most likely due to an initial inflammatory reaction in the vas following the injection, causing temporary obstruction of the vas.

The combined data on the intravasal injection of formaldehyde in ethanol into men show that the time to achieve azoospermia is highly variable and may take up to several months. The differences in the failure rates reported in the series by Freeman (11) and Davis (6, 7) may be due to the different lengths of follow-up rather than to factors related to the way in which the procedures were performed.

Using dogs, Davis compared the effects of direct injections of MCA or silver acetate alginate into the vas lumen with effects of perivasal injections of these agents (7). In most cases, the injections produced vasal occlusion, whereas perivasal injections did not. These data indicate that for transcutaneous injections of

95

sclerosing agents to be effective in occluding the vas, they must be made directly into the vas lumen. Although this is a technically more difficult procedure, the requirement should not limit its widespread use.

In sharp contrast to the few studies on transcutaneous sterilization procedures with sclerosing agents in the United States, reports from the Peoples' Republic of China indicate that since 1972, this procedure has been used in over 500,000 men, with satisfactory results (2, 20, 21). The sclerosing agent used is carbolic acid and n-butyl alpha cyanoacrylate. In spite of the very extensive experience with intravasal injections of chemical agents, very little information is available on the procedure, either in Chinese or Western medical journals. Precise data are not available on the effectiveness of the procedure or on the incidence and types of complications occurring either at the time of or after the procedure. In the most recent report from the Peoples' Republic of China, long-term follow-up data were given for two series of men (21). The first series included 919 men who had been followed up for up to 10 years. The second series included 640 men who had been followed for up to 8 years. The only stated difference between the two series was that in the second series 0.02 ml compared to 0.01 ml of the sclerosing agent was used. Of the 1,345 men who were followed up and examined, small nodules could be palpated at the site of intravasal injection. In 99.4% of the vasa examined, the diameter of the nodules was estimated to be less than 0.5 cm. Only one man reported that the nodules were painful. Follow-up data relating to the effectiveness of the procedures are summarized in Table 4. For both series, azoospermia was achieved in 95.9% of the men. In the second series, the pregnancy rate among spouses was reduced from 11.5% to 2.6%. Of the 109 pregnancies recorded in both series, 56% occurred to the spouses of men who had been shown to be azoospermic. Since no information was given on the time between vas injection and the time the pregnancies occurred, it cannot be determined if the pregnancies occurred before azoospermia had been confirmed.

The transcutaneous intravasal sterilization procedure developed by the Chinese is simple and requires the use of minimal surgical equipment (19, 20, 21). The following briefly describes the principal aspects of this procedure, which has been widely and successfully used in China since 1972.

The vas is stabilized by use of a vas deferens fixing clamp. This is a straight hemostat with flattened tips that permits the vas to be grasped without injuring the scrotum. With the patient under local anesthesia, the operator clamps the vas and grasps it between the thumb and index finger. A sharp needle is inserted into the vas perpendicularly. The needle is withdrawn and a blunt needle is placed through the puncture hole. The operator can usually feel when the needle has entered the vas lumen. Two simple tests can be performed to determine whether the needle is in the vas lumen:

1. A syringe containing 4 ml of air is attached to the needle. With the vas firmly compressed by an assistant at the site of puncture and at the distal end, 2 ml of air is injected into the vas. The syringe plunger is released. If the needle has been placed in the vas lumen, the plunger should return to its original position within a few seconds.
2. A small amount of saline is injected through the blunt needle. If the needle is in the vas lumen, the injection should proceed easily and should be made without undue pressure. Examination of the scrotal skin and subcutaneous tissues should indicate no local edema.

If the second test is used, all of the saline is aspirated from the vas before injection of the sclerosing agent. The vas sclerosing agent (0.045 ml of carbolic acid, n-butyl alpha cyanoacrylate) is then injected through the blunt needle into the vas. The drug polymerizes after about 20 seconds. The needle is then withdrawn. Only about 0.02 ml of the drug is actually injected into the vas. The procedure is then repeated for the other vas and both puncture sites are covered with sterile gauze. The procedure takes about 10 minutes to perform.

Electrocoagulation. The use of transcutaneous sterilization with electrocoagulation was first reported by Lee in 1964 (17). In that study, bilateral closure of the vas was obtained in four dogs and unilateral closure on two others. In one dog, testicular atrophy was noted. This atrophy was attributed to extensive burns to spermatic vessels other than the vasa. In the Peoples' Republic of

First Series: 919 men followed up to 10 years

Semen analysis performed: 456 men
Azoospermic (%): 95.6

Couples evaluated: 829
Pregnancies (%): 11.5
Semen analysis for the 95 men whose spouses became pregnant:
Azoospermic — 54.7%
Sperm in semen — 42.1%
No semen analysis — 3.2%

Second Series: 640 men followed for up to 8 years

Semen analysis performed: 404 men
Azoospermic (%): 96.3

Couples evaluated: 577
Pregnancies (%): 2.6
Semen analysis for the 14 men whose spouses became pregnant:
Azoospermic — 64.3%
Sperm in semen — 21.4%
No semen analysis — 14.3%

Source: Li S, Zhu J (21).

Table 4. Summary of long-term follow-up data from The Peoples' Republic of China on the effectiveness of the transcutaneous injection of a sclerosing agent.

96

China the use of transcutaneous electrocoagulation of the vas has been investigated in animals, but no information on the procedures was given (21).

In 1966, Schmidt first reported on a vasectomy procedure he had used in 144 men. A bilateral incision was made in the scrotum, the vasa were divided, and the vas ends were electrocoagulated with unipolar electrodes (27). Schmidt found that the incidence of sperm granulomas could be minimized if the vas ends were electrocoagulated rather than ligated. In a later series of 1,000 vasectomies in which electrocoagulation of the cut vas ends was used, Schmidt reported no failures, and fewer than 1% of the men had clinically significant sperm granulomas or other complications (26). In the procedure used by Schmidt, the vasa were electrocoagulated in such a way that the lesion was confined to the vasal epithelium, lamina propria, and part of the muscle wall.

To further advance the electrocoagulation procedure developed by Schmidt, bipolar electrodes have been developed. The use of these electrodes can eliminate the danger of damage to vessels other than the vas resulting from stray currents, and at the same time confine the lesion to the vasal epithelium and lamina propria. Preservation of most of the vasal muscles is thought to be important, because the muscle is the source of the fibrous tissue that ultimately results in occlusion of the vas.

Adair developed a transcutaneous electrocoagulation procedure that used bipolar electrodes (1). In an initial series performed by the investigator, there were no failures and no clinically significant complications attributable to the procedures. Subsequently, an independent, multiclinic evaluation of the procedure developed by Adair that included 33 men was curtailed due to the high failure rate of the procedure to attain azoospermia (24). In the second series, the only complication was a scrotal hematoma. It has been suggested that the failure rate of the procedure was high because placement of the tip of the bipolar needle into the vas lumen was extremely difficult, due to the relative diameters of the bipolar needle and vas lumen (10). The bipolar needle has a diameter of 1.6 mm; the average diameter of the human vas lumen is 0.55 mm, but it may be distended to 1.2 mm (16).

Black, at the Marie Stopes Clinic in the United Kingdom, is currently investigating a transcutaneous electrocoagulation procedure (3). The procedure gradually evolved through several steps designed to provide a simpler, quicker, and less traumatic method of sterilization. Initially, the vasa were electrocoagulated after they were pulled out through a small scrotal incision. Electrocoagulation was then performed through a small scrotal incision, but with the vas in the scrotum. The final step in the development process was to perform the electrocoagulations transcutaneously. Black has performed about 80 of these procedures using prototype bipolar electrodes (3). Although the success rate of his transcutaneous procedure does not yet approach that of the standard vasectomy procedure used at the Marie Stopes Clinic, Black is of the opinion that improvement of the electrodes and some changes in the technique of performing the electrocoagulation will result in an effective procedure.

In the United States, Denniston will evaluate whether electrocoagulation of the vas by insertion of the electrode into the vas lumen under direct vision, and without division of the vas, will result in high rates of vasal closure (9). The effectiveness of this procedure will be compared to the standard electrocoagulation procedure. If electrocoagulation of the vas under direct vision, and without division of the vas, results in high rates of vasal closure, additional trials will be undertaken in which electrocoagulation will be performed transcutaneously.

COMMENT

Over the past 20 years, a transcutaneous method of male sterilization has been sought in a haphazard manner and the limited developmental efforts have been sporadic. In spite of the apparent simplicity of the procedure and its potential for widespread use and acceptance, no systematic efforts have been undertaken in the United States to develop a transcutaneous procedure, except for the research supported by the Program for Applied Research on Fertility Regulation (PARFR) over the past 7 years.

Compared to standard surgical vasectomy procedures, there are many advantages to the transcutaneous procedures that are applicable to both developed and developing countries, including a possible reduction in the incidence of certain complications commonly associated with vasectomy, such as scrotal hematomas and infections.

In the United States, fewer than 100 men have had transcutaneous sterilization procedures either by injection of formaldehyde in alcohol or electrocoagulation. In sharp contrast to this, reports from the Peoples' Republic of China refer to over 500,000 transcutaneous sterilization procedures using a cyanoacrylate mixture. Unfortunately, the reports from China provide few data on the effectiveness of the procedure or on its short-term or long-term complications. Also, information on the development of the procedures and any associated toxicology is not available in the literature.

In view of the many advantages of the transcutaneous sterilization procedure, the importance of an organized research program is obvious if such a procedure is to become widely available in the United States and elsewhere. Electrocoagulation of the vas and occlusion of the vas with sclerosing agents are the two best candidates for further development. Regardless of the methods of transcutaneous sterilization used, criteria should be developed for determining acceptable failure rates of the procedures. Failure can be defined in terms of either the pregnancy rates of the partners of the sterilized men or the proportion of men who achieve azoospermia within a specified time period. Consideration should also be given to defining an acceptable proportion of men who do not achieve azoospermia but who become severely oligospermic.

The scanty literature on transcutaneous male sterilization procedures indicates that the research efforts have been directed to developing a procedure that will result in azoospermia in a very high proportion of men after a single application of the agent. This may not be feasible. Therefore, research efforts might concentrate on a two application procedure. Such a procedure could be highly effective with minimal rates of complications or side effects. If, for example, a single application procedure produces bilateral vasal occlusion and azoospermia in only 90% of the men, then a two-application procedure can be expected to produce azoospermia in 99%.

In women, nonsurgical methods of sterilization have been evaluated extensively (14). Methods currently under investigation in the United States include one or two applications of MCA and three applications of quinacrine. However, in the Peoples' Republic of China, nonsurgical methods of sterilization have been developed and used successfully since the late 1960s (34, 35). All of these methods are more difficult to perform than transcutaneous male sterilization and they have a higher risk of potentially serious complications, such as uterine perforation and intraperitoneal placement of the chemical agents.

Also, assessing the effectiveness of the procedure in the female is more difficult. If radiopaque MCA is used, flatplate x-rays can be taken to determine if MCA was present in the fallopian tubes. Alternatively, some time after the procedure, hysterosalpingograms may be performed to determine if there is bilateral tubal closure.

Neither of these two procedures is 100% accurate and neither is appropriate for areas where there are scarce medical resources. In countries that have the available medical resources, flatplate x-rays and hysterosalpingograms significantly add to the cost of the procedure. Evaluation of sterility in the male is a relatively easy matter. Semen analysis may be performed with simple laboratory equipment by laboratory technicians who have minimal training. If the two or three application nonsurgical sterilization procedure will be acceptable to women, there is no reason why a two application procedure should not also be acceptable to men.

Future research efforts on nonsurgical methods of male sterilization need to focus on the use of sclerosing or occluding agents or electrocoagulation of the vas when delivered as either a one or two application procedure.

REFERENCES

1. Adair EL: Transcutaneous closure of the vas deferens: a new procedure. Unpublished, 1980.

2. Anonymous: New method of male sterilization. Chin Med J 93:205, 1980.

3. Black T: Population Services. Personal communication, 1984.

4. Bowman TA, Senger PL, Koger LM, Gaskins CT, Hillers JK: Blockage of sperm transport using intraepididymal calcium chloride injections in rams. J Anim Sci 46:1063, 1978.

5. Coffey DS, Freeman C: Vas injection: a new nonsurgical procedure to induce sterility in human males. In Sciarra JJ, Markland C, Speidel JJ (eds): Control of Male Fertility. Hagerstown, Harper & Row Publishers, 1975.

6. Davis JE, Richart RM: A new method for obstructing the vas deferens by direct injection of chemical agents: a non-operative technique of male sterilization. Unpublished, 1980.

7. Davis JE: New methods of vas occlusion. In Zatuchni GI, Labbok MH, Sciarra JJ (eds): Research Frontiers in Fertility Regulation. Hagerstown, Harper & Row Publishers, 1980.

8. Davis JE: Study of the vas occlusion in animals using chemical agents. Unpublished, 1982.

9. Denniston G: Population Dynamics. Personal communication, 1984.

10. Free M: Program for the Introduction and Adaptation of Contraceptive Technology (PIACT). Personal communication, 1984.

11. Freeman C: Preliminary human trial of a new male sterilization procedure: vas sclerosing. Fertil Steril 26:162, 1975.

12. Freeman C, Coffey DS: Sterility in male animals induced by injection of chemical agents into the vas deferens. Fertil Steril 24:884, 1973.

13. Freeman C, Coffey DS: Male infertility induced by ethanol injection into the vas deferens. Int J Fertil 18:129, 1973.

14. Goldsmith A, Edelman DA: Nonsurgical methods of female sterilization. In Sciarra JJ (ed): Obstetrics and Gynecology V. 6. Philadelphia, Harper & Row Publishers (in press).

15. Heber D, Swerdloff RS: Brain peptides and fertility control in the male. In Zatuchni GI, Labbok MH, Sciarra JJ (eds): Research Frontiers in Fertility Regulation. Hagerstown, Harper & Row Publishers, 1980.

16. Hulka JF, Davis JE: Vasectomy and reversible vas occlusion. Fertil Steril 23:683, 1972.

17. Lee HY: Studies on vasectomy. I. Experimental studies on nonoperative blockages of vas deferens and permanent introduction of nonreactive foreign body in the vas. New Med J 7:117, 1964.

18. Lewis RW, Garcia RR: The results of epididymal ablation by sclerosing agents in the nonhuman primate. Fertil Steril 41:465, 1984.

19. Li S: Clinical application of the vas deferens puncture. Chin Med J 93:69, 1980.

20. Li S: Non-operative sterility research with intravasal injecting drug: a clinical report. Reprod Contracept 1:24, 1981.

21. Li S, Zhu J: Non-operative sterility research with intravasal injection drug (clinical report). Unpublished, 1984.

22. Lobl TJ, Bardin CW, Chang CC: Pharmacologic agents producing infertility by direct action on the male reproductive tract. In Zatuchni GI, Labbok MH, Sciarra JJ (eds): Research Frontiers in Fertility Regulation. Hagerstown, Harper & Row Publishers, 1980.

23. Mahmoud KZ, Abdou MS, Hemeida NA, Safouri LS, Mahmoud S, Girgis SM, Fahim MS: Effect of ultrasound on mature rabbit testes. (Abstract). Arch Androl 9:87, 1982.

24. Program for Applied Research on Fertility Regulation (PARFR): A multisite evaluation in developed and developing countries of a technique and equipment for transcutaneous closure of the vas deferens by electrocoagulation. Unpublished, 1981.

25. Richart RM, Li XZ: A rapid and effective percutaneous intra vas injection for male sterilization. Unpublished, 1984.

26. Schmidt SS: Prevention of failure in vasectomy. J Urol 109:296, 1973.

27. Schmidt SS: Technics and complications of elective vasectomy. Fertil Steril 17:467, 1966.

28. Setty BS, Dasgupta PR, Kar AB: Chemical occlusion of vas in rats. Contraception 6:329, 1972.

29. Smith DK, Rodriguez-Rigau LJ, Steinberger E: Hormonal methods for male contraception. In Zatuchni GI, Labbok MH, Sciarra JJ (eds): Research Frontiers in Fertility Regulation. Hagerstown, Harper & Row Publishers, 1980.

30. Vasectomy — safe and simple. Population Reports Series D, No. 4, November-December 1983, Population Information Program, The Johns Hopkins University.

31. Vasectomy. What are the problems? Population Reports Series D, No. 2, January, 1975. The George Washington University Medical Center.

32. Weibe JP, Barr KJ: Suppression of spermatogenesis without inhibition of steroidogenesis by a trihydroxypropane (glycerol) solution. (Abstract). Biol Reprod 28 (suppl 1):256, 1981.

33. Weibe JP, Barr KJ: The control of male fertility by 1,2,3-trihydroxypropane (THP; glycerol): rapid arrest of spermatogenesis without altering libido, accessory organs, gonadal steroidogenesis, and serum testosterone, LH and FSH. Contraception 29:291, 1984.

34. Wu Y-H, Qian P-L, Tien S-P: Nonsurgical procedures for manual instillation of a phenol-atabrine paste (PAP) for female tubal occlusion. In Zatuchni GI, Shelton JD, Goldsmith A, Sciarra JJ (eds): Female Transcervical Sterilization. Philadelphia, Harper & Row Publishers, 1983.

35. Zheng H-G, Chen X-H: A study of phenol-mucilage-induced tubal occlusion for sterilization. Analysis of the results in 4,784 cases. In Zatuchni GI, Shelton JD, Goldsmith A, Sciarra JJ (eds): Female Transcervical Sterilization. Philadelphia. Harper & Row Publishers, 1983.

This publication was supported by the United States Agency for International Development (USAID). The contents do not necessarily reflect USAID policy.