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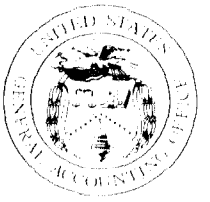
Report to the Chairman, Subcommittee on Regulation, Business Opportunities and Energy, Committee on Small Business, House of Representatives

December 1989

HUMAN EMBRYO LABORATORIES

Standards Favored to Ensure Quality



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GAO

United States
General Accounting Office
Washington, D.C. 20548

Human Resources Division

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December 19, 1989

The Honorable Ron Wyden
Chairman, Subcommittee on Regulation,
Business Opportunities and Energy
Committee on Small Business
House of Representatives

Dear Mr. Chairman:

In response to your request of December 6, 1988, this report provides information on the personnel employed and the equipment, procedures, and quality control used in human embryo laboratories. Such laboratories form an important component of medical programs that use advanced reproductive technologies to treat patients with infertility problems. The successful application of these technologies has raised the hopes of many infertile couples.

Two methods used to overcome infertility are in vitro fertilization (IVF) and gamete intrafallopian transfer (GIFT). These techniques combine eggs and sperm outside the body, in a laboratory. The procedures consist of removing one or more eggs from the ovary, preparing and processing them in the laboratory, and transferring them into the patient's body with the aim that pregnancy will occur. The proliferation of programs offering IVF and GIFT, coupled with the low probability of success of some programs, has raised questions regarding the supply of qualified personnel and the extent of quality control in human embryo laboratories.

The activities in the human embryo laboratory are considered important to successful IVF/GIFT outcomes. Although the American Fertility Society (AFS)[1] has issued minimum standards for IVF/GIFT programs, they do not specify the methods or protocols for performing the complex laboratory procedures required for these technologies.[2] Instead, each new program must attempt to duplicate another's laboratory protocols or develop its own.

To determine the practices and procedures currently in use, we surveyed laboratory managers at IVF/GIFT programs nationwide. This report

[1]AFS is a professional organization representing about 11,000 specialists in the field of reproductive medicine.

[2]AFS is currently revising its 1984 minimum standards for IVF programs. The proposed standards include specific training and experience requirements for personnel.

highlights selected factors covered by our questionnaire relating to treatment outcomes, personnel, and practices, including quality controls and techniques, currently in use. The results of items included in the questionnaire are presented in appendix I.

Results in Brief

Personnel qualifications, certain quality control measures, and techniques employed by the laboratories we surveyed varied. Most of the practitioners of these technologies who responded to our survey generally agreed that some oversight of human embryo laboratories would improve the quality of care provided to individuals. A majority of the respondents favored more uniform personnel qualifications and quality control requirements. Many IVF/GIFT program directors, however, opposed mandating standardized techniques, noting that similar results may be obtained when different methods, materials, and techniques are used. These findings support the statement by a former AFS official that

"... there is still no set recipe or 'cook book' approach shown to be widely effective in generating reproducible pregnancy rates when employed by the numerous investigators across the country."

Background

The first human IVF program in the United States was established in 1980. Since then, the number of infertility centers offering IVF and GIFT therapy has increased dramatically. Although the exact count is not available, approximately 200 programs are estimated to be in operation in the United States.

The IVF treatment cycle consists of: (1) retrieval of eggs from the ovary following the use of stimulation drugs to produce multiple eggs; (2) insemination, fertilization, and early embryonic development in the laboratory; and (3) the transfer of the pre-embryo[3] to the uterus of the patient, where implantation occurs to establish a pregnancy. GIFT differs from IVF in that the retrieved eggs and sperm are transferred directly to the patient's fallopian tubes so that fertilization and pre-embryo development may occur in the natural environment.

Current practitioners believe that a significant factor influencing the ability to become pregnant by IVF therapy is the number of pre-embryos transferred. The highest success rates have been reported when four to

[3]For the purposes of this study, "pre-embryo" is defined as a fertilized egg in the earliest stages of development.

seven pre-embryos are transferred at one time. With the use of multiple embryos in IVF treatments, about 26 percent of the transfers resulted in pregnancies and about 18 percent in deliveries, 1987 data[4] show. This method, however, introduces the possibility of multiple births, which raise the incidence of infant morbidity.

In some cases, the treatment cycle is repeated several times before a live birth may be achieved. But repetition of fertility drug stimulation (to generate a large number of eggs for insemination) and surgery for obtaining eggs can present a serious health risk[5] and may diminish the quality of the pre-embryos produced. One alternative is to retrieve several eggs at a single operation, fertilize them, and store them in a freezer for use in successive transfer cycles. Should the patient become pregnant in the first cycle, the frozen eggs or pre-embryos may be kept until a subsequent pregnancy is desired, donated to another patient, or discarded.

Objective, Scope, and Methodology

In response to your request, we conducted a survey to obtain information on the personnel employed, and the equipment, procedures, and quality controls used in human embryo laboratories. With the assistance of AFS and its specialty group, the Society for Assisted Reproductive Technology (SART),[6] we developed a questionnaire. We pretested it with laboratory directors at four sites representing a range of program sizes and affiliations. In 1988, these programs performed from 61 to 559 retrieval procedures. One program site was a university-based hospital, two were private community hospitals, and one was a free-standing infertility center. In May 1989, we sent the questionnaire to 254 IVF/GIFT program directors.[7] Of 198 respondents, 160 reported having active IVF

[4]Medical Research International and the Society of Assisted Reproductive Technology, The American Fertility Society, "In Vitro Fertilization/Embryo Transfer in the United States: 1987 Results from the National IVF-ET Registry," Fertility and Sterility, vol. 51, no. 1, Jan. 1989, p. 16.

[5]An alternative, nonsurgical method of egg retrieval uses ultrasound as a guide. With proper measures taken to prevent infection, this method may reduce the risk and cost associated with surgery.

[6]Membership in SART requires compliance with AFS minimal standards for IVF/GIFT programs and demonstrated success in producing pregnancies. Programs must perform at least 40 treatment cycles per year resulting in three live deliveries. This is considered the minimum number of cycles per year that would allow a human embryo laboratory to maintain the necessary expertise.

[7]IVF/GIFT programs were identified by either AFS or Serono Laboratories, Inc. Serono is the sole U.S. source of the pharmaceutical product Pergonal, used to stimulate multiple egg development in in vitro fertilization.

or GIFT programs.[8] All conduct IVF cycles and 141 conduct GIFT cycles. Most human embryo laboratories are hospital-based—67 at university hospitals, 73 at nonuniversity hospitals, and 20 at private clinics.

Many factors influence the outcome of IVF/GIFT treatment, including the drugs administered to the woman, laboratory conditions, and surgical procedures. We collected data on only the laboratory component of infertility clinics, not the patient's age, underlying infertility disorder, type of fertility drugs used, method of retrieval, or other influences outside of the laboratory. Due to this lack of clinical data, we did not attempt to identify which laboratory factors are of primary importance to success. To assess the relative contribution of laboratory and clinical influences to outcomes would require extensive further research.

In addition to standardized questions regarding personnel, protocols and outcomes, we asked respondents for their views on the establishment of standards. Specifically, we solicited their opinions on the need for and appropriateness of developing operating standards in human embryo laboratories. These open-ended responses were coded and organized into several categories.

To encourage a high rate of responsiveness and accuracy, we pledged confidentiality that precludes the reporting of responses from individual programs. We did not verify the data submitted.

Programs' Success Rate Low for 1988

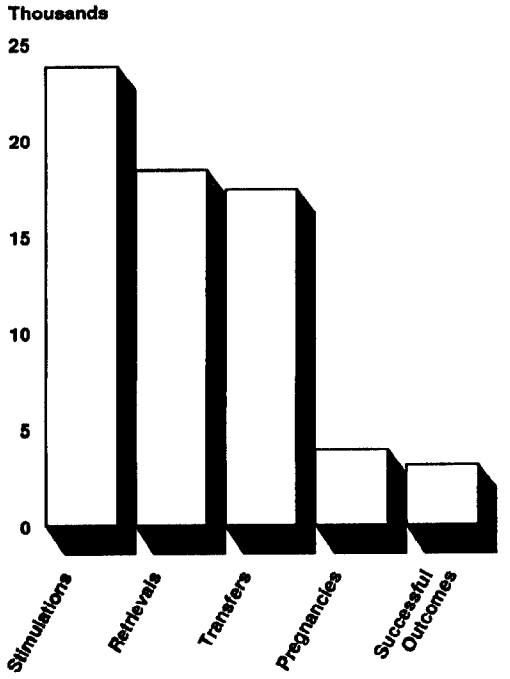
During 1988, IVF/GIFT programs started 23,815 treatment cycles and conducted 18,439 egg retrievals. (Half of the programs reported performing at least 73 egg retrieval procedures). The 17,400 transfers performed in 1988 resulted in 3,942 clinical pregnancies[9] and approximately 3,088 successful outcomes (the sum of deliveries and ongoing pregnancies, in the second or third trimester, at the time of our survey). (See fig. 1.)

The programs achieved an overall median clinical pregnancy rate of 20 percent for all 1988 procedures. We calculated this rate by dividing the number of women achieving a clinical pregnancy in 1988 by the number of retrieval procedures in 1988.

[8]The remaining 38 were programs that were temporarily or permanently closed, different addressees using the same laboratory, or responses received too late to be included in our tabulations.

[9]Clinical pregnancies are confirmed when ultrasound demonstrates a gestational sac in the uterus and the pregnancy hormone, hCG, is found in the woman's blood.

Figure 1: Treatment Cycles and Outcomes for 154 IVF/GIFT Programs (1988)

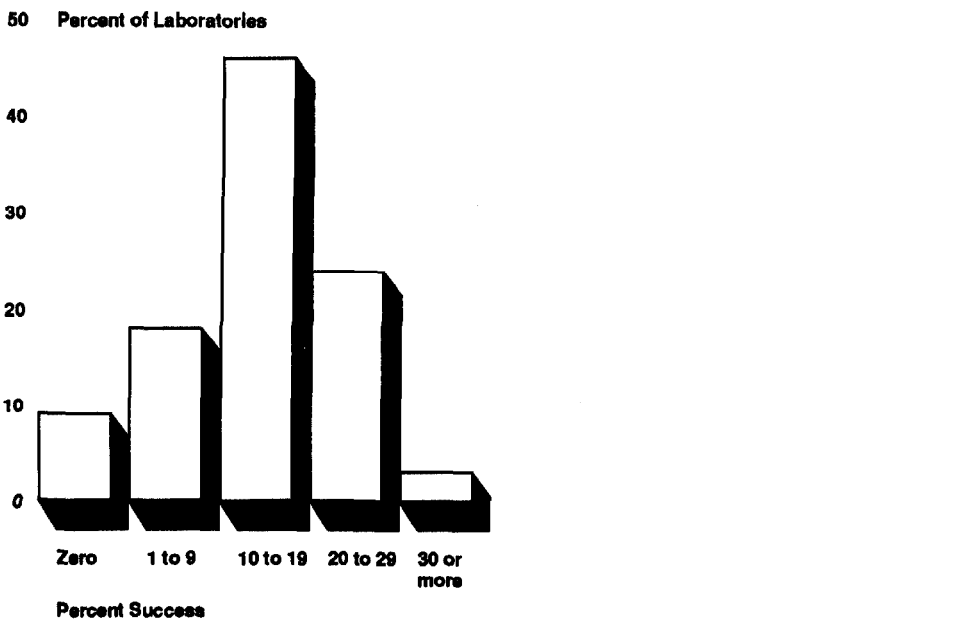


Note: Successful outcomes represent deliveries plus ongoing (second or third trimester) pregnancies resulting from 1988 transfers.

The median success rate for all 1988 treatments by responding programs was 14 percent, and for individual programs, ranged from zero to 38 percent (see fig. 2). The 14-percent rate is consistent with success rates reported by other studies in recent years. Data for 1987 from 96 programs showed an overall success rate of 13 percent.[10] We calculated the success rate by dividing the number of successful outcomes resulting from 1988 transfers by the number of retrieval procedures in 1988. Other outcome measures are sometimes used but SART officials contend that this ratio is the only statistic that patients find relevant in their decision-making.

[10]Medical Research International and the Society of Assisted Reproductive Technology, The American Fertility Society, "In Vitro Fertilization/Embryo Transfer in the United States: 1987 Results from the National IVF-ET Registry," Fertility and Sterility, vol. 51, no. 1, Jan. 1989.

Figure 2: Frequency Distribution of Success Rates for 154 IVF/GIFT Programs (1988)



Note: Success rate is defined as the number of deliveries plus ongoing pregnancies resulting from 1988 transfers divided by the number of retrievals in 1988.

Because IVF/GIFT requires the manipulation of eggs, sperm, and/or preembryos for a variable length of time, laboratory personnel and practices can affect outcomes. Failure to maintain stringent laboratory conditions may explain, in part, many programs' low rates of success. However, such factors alone do not adequately explain the variability in success rates. In testimony before your subcommittee on March 9, 1989, the president of SART pointed out that,

"Everyone doing IVF has had the experience that sometimes, for no apparent reason, no patients get pregnant while the next week or the next month, using completely identical procedures, culture media, and equipment, a highly satisfactory pregnancy rate is achieved."

The potential for success has been correlated with a number of physician-specific or patient-specific variables, as we noted on page 4. (In fact, SART members have cautioned that some programs, to appear more successful than others, may select particular types of patients.) Without more information than we obtained by our survey, these other

variables make it difficult to evaluate the appropriateness of the different protocols or determine the effect of specific laboratory factors on treatment outcomes.

Differences Found in Personnel, Environmental Controls, and Techniques

Through our questionnaire, we examined in some detail the various factors that contribute to the proper functioning of a human embryo laboratory. Among these are personnel, environmental conditions, and techniques. Both IVF and GIFT require complex laboratory arrangements. In IVF procedures, the function of the laboratory is to provide an optimal environment for the retrieval and preparation of eggs and sperm; insemination and fertilization of eggs; and pre-embryo development, transfer, and/or freezing. During GIFT, the laboratory identifies and isolates mature eggs for transfer back to the patient along with the appropriate number of viable sperm.

The laboratories we surveyed differed in some practices that are followed to maintain proper environmental conditions as well as the specific techniques employed in the handling of eggs, sperm, and pre-embryos. Many practitioners believe that careful measures for maintaining a controlled environment and for IVF/GIFT techniques can improve the quality of the pre-embryo and thereby raise the pregnancy rate.

Laboratories Headed by Ph.D.'s Predominate

Staff knowledge and experience are critical to proficiency in managing a human embryo laboratory. Responding laboratories range in size from 1 to 12 staffpersons. The typical laboratory team consists of a director, a supervisor, and one technical person. The personnel who handle the eggs, sperm and pre-embryos vary in both education and experience, our survey showed. For instance, the laboratory director

- at 43 percent of laboratories had a Ph.D. degree in some field of biology; at 34 percent was a clinically trained physician; and at 12 percent had both Ph.D. and M.D. degrees.
- at about half of the laboratories had at least 5 years of work experience in a human embryo lab.
- at about 70 percent of the laboratories had experience working in an animal embryo laboratory for at least 3 years.
- at about 70 percent of the laboratories has been employed with his or her present lab for at least 2 years.

Environmental Controls Vary

While eggs, sperm, and pre-embryos are outside the human body, the laboratory must provide a controlled, nontoxic environment. Such conditions are largely dependent on the use of equipment to control the temperature and atmosphere, and on the quality of the culture media,[11] the liquid nutrient used for supporting egg and sperm growth. For instance, when culture dishes are removed from the incubator for microscopic observation, changes in temperature and gas atmosphere that may affect egg/pre-embryo quality can occur very rapidly.

Survey respondents indicated some variability in the use of laboratory equipment and quality control measures to maintain optimal culturing conditions. In general, IVF/GIFT laboratories are designed to limit the exposure of eggs to variations in atmosphere and temperature. Nearly 80 percent of the laboratories responding to our survey are located in rooms adjacent to the egg retrieval room, thereby reducing the time it takes to transport the eggs. About 90 percent indicated that they use warming trays for culture containers to maintain a constant temperature. Other precautions taken during observation periods, but used by far fewer laboratories, include use of a water or dry bath to prewarm fluids, heated stages on the microscope, or warmer-than-normal room temperature. To maintain a sterile environment, about 50 percent of the laboratories reported using a laminar flow hood when examining eggs and pre-embryos, and for other procedures. By surrounding the items within it with filtered air, the laminar flow hood reduces the chance of microbial contamination.

Most human embryo laboratories follow routine quality control procedures such as periodic monitoring and inspection of equipment to achieve a controlled environment. However, the frequency with which these quality assurance measures are undertaken varies considerably. For instance, over 80 percent of respondents check daily on incubator temperature and atmosphere; most others check weekly. At least 16 percent of laboratories with such equipment reported that they never check incubators for proper humidity or evidence of microbial contamination, the sterility of the laminar flow hood, or the accuracy of their centrifuge.

Another way to minimize exposure to an uncontrolled biochemical environment is to ensure the quality of the culture media, an element regarded as critical to the viability of the eggs and pre-embryos.

[11]Culture media usually is prepared by supplementing a stock solution, composed of a chemical nutrient mixture and highly purified water, with a protein source.

Through quality control checks, laboratories can detect contaminants in the culture media that could interfere with fertilization and pre-embryo development. To test the suitability of the media for human IVF, tissue is grown in the same culture media and under the same conditions to be used for human eggs and pre-embryos.

The most common method our respondents use to identify good versus poor quality media for human IVF is mouse embryo growth, although a number of other methods were also reported. This method places fertilized mouse embryos collected at the 2-cell stage into the culture media to demonstrate development in the laboratory. Eighty-one percent of respondents test each batch of medium prior its use. The shelf life for a batch of culture media is limited to less than 2 weeks at half of the laboratories.

IVF/GIFT Techniques Differ

Techniques for handling eggs and sperm vary depending on egg maturity, semen quality, and other factors. Immediately after eggs are retrieved from a woman's ovaries, they are examined under a microscope and graded as to maturity to determine the best timing for insemination. Respondents indicated a variety of criteria used for evaluating egg maturity. Shortly after the egg retrieval procedure, semen is collected and processed. Depending on the quality of the semen, several techniques are used to prepare sperm for insemination.

Our survey revealed differences in many facets of IVF procedures, e.g.:

- the length of time eggs are incubated before insemination,
- the concentration of sperm added to each egg,
- whether a second insemination is performed when fertilization is not evident after the initial insemination,
- the length of time allowed for pre-embryo culturing before transfer, and
- the number of pre-embryos transferred to the patient.

The number of pre-embryos produced during an IVF treatment cycle often exceeds the number immediately replaced in the patient. In 83 percent of responding laboratories, excess pre-embryos were frozen and stored for transfer to the patient during a subsequent natural, unstimulated cycle. The techniques for freezing pre-embryos differ among laboratories in both the substance used to protect the pre-embryo from damage by freezing and the developmental stage at which the pre-embryo is frozen. In 63 percent of the responding programs, limits have been set on how long a laboratory will keep frozen pre-embryos. The

times vary from less than 2 years (11 percent), 2 to 5 years (41 percent), and more than 5 years (11 percent).

Where the freezing option is unavailable or not preferred, respondents indicated that, at least sometimes, they dispose of excess pre-embryos in the following ways:[12]

- Discard them (41 percent of laboratories),
- Use them for diagnostic purposes (28 percent of laboratories),
- Donate them to another woman (25 percent of laboratories), or
- Donate them for research[13] (8 percent of laboratories).

Standards Favored for Human Embryo Laboratories

SART considers IVF and GIFT therapies to be appropriate only for couples for whom all conventional infertility treatments have been tried unsuccessfully. By undertaking IVF or GIFT as an option of last resort, patients desperate to have children become vulnerable to unscrupulous practitioners and substandard care. The existence of fraudulent practices in this field has been brought to public attention by your Subcommittee, the Federal Trade Commission, and the news media.

In testimony before your Subcommittee on March 9, 1989, SART members stated that the potential for abuses in IVF/GIFT programs can be minimized and the chances for successful outcomes maximized by the establishment and enforcement of standards. Our survey respondents' comments indicate their nearly unanimous support for standards for human embryo laboratories, to improve the quality of laboratory performance and protect patients from untrained or unethical practitioners.

Comments from respondents identified support for

- establishment of uniform requirements for quality control methods;
- standard requirements in the area of staff qualifications;
- viewing IVF/GIFT procedures as "operator-sensitive," requiring flexibility in techniques;

[12]Percentages total more than 100 due to multiple responses to this question on our survey.

[13]In a recent study of issues in research relating to reproductive and developmental biology, a committee of scientists and IVF clinicians recommended the development of guidelines for human embryo research that are based on both scientific knowledge and societal values. See: Institute of Medicine and the National Research Council's Board on Agriculture, Committee on the Basic Science Foundations of Medically Assisted Conception, Medically Assisted Conception: An Agenda for Research (1989).

- licensing of laboratories, with periodic facilities inspection as part of an accreditation system;
- self-regulation through accreditation by the professional societies, not the federal government; and
- more scrutiny of clinical aspects of IVF/GIFT programs.

We will send copies of this report to our survey respondents and make copies available to interested persons on request. Please contact me at (202) 275-5451 if you or your staff have any questions. The major contributors to the report are listed in appendix II.

Sincerely yours,

Mark V. Nadel
Associate Director for National
and Public Health Issues

Contents

Abbreviations

AFS	American Fertility Society
GIFT	gamete intrafallopian transfer
IVF	in vitro fertilization
SART	Society for Assisted Reproductive Technology

Human Embryo Laboratory Survey Results

Overview

This appendix presents a summary of questionnaire responses received from the 160 human embryo laboratories that reported having IVF or GIFT programs. N_L represents the number of laboratories that responded to each question; N_S represents the total number of laboratory staff reported. Also, note the following:

- For some responses, the percentages total more than 100 because respondents were asked to select as many choices as applicable.
- To present the results of items 27, 34, 52b, 53, and 54, which were open-ended on our questionnaire, we organized the responses into categories.

1. Number of labs that perform IVF and GIFT:

IVF	160
GIFT	141

2a. Aggregate statistics reported for all types of procedures (IVF, GIFT, IVF/GIFT combinations, and alternative procedures) performed January 1-December 31, 1988 (N_L= 154):

	Total	Median
Stimulation cycles begun	23,815	97
Oocyte retrieval procedures	18,439	73
Oocytes retrieved	125,211	495
Oocyte/pre-embryo[a] transfer procedures	17,400	66
Oocytes/pre-embryos transferred	54,896	212
Clinical pregnancies[b]	3,942	14
Live births (deliveries) to date resulting from 1988 transfers	1,830	7
Ongoing (second or third trimester) pregnancies resulting from 1988 transfers	1,258	4

[a]For the purposes of this survey, pre-embryo is defined as a fertilized oocyte in the pronuclear or cleavage stage of development.

[b]Clinical pregnancy is confirmed by rising beta hCG and gestational sac, documented by ultrasound.

2b. Frequency distribution of 1988 success rates[3] for all types of procedures (N_L = 154):

Success rate (percent)	Percent of labs
0	9
1-9	18
10-19	46
20-29	24
30 or more	3

	Success rate (percent)
Median	14
Range (low to high)	0–38

3. Year in which human embryo labs began operation (N_L = 160):

	Percent of labs
1980-1982	6
1983-1985	44
1986-spring 1989	50

4. Human embryo lab setting (N_L = 160):

	Percent of labs
Affiliated with a university hospital	42
Affiliated with a nonuniversity hospital	46
Not affiliated with a hospital	12

[3]Calculated as live births plus ongoing pregnancies resulting from 1988 transfers divided by the number of retrieval procedures in 1988. By including births from frozen-thawed pre-embryos in the numerator, our calculation overstates somewhat the chance for success.

5. Location of the human embryo lab in relation to the oocyte recovery facility (N_L = 160):

	Percent of labs
In adjacent rooms	79
Elsewhere in the same building	16
In another building	3
Multiple locations	2

Staff

6. Person directly responsible for the daily operation of the human embryo lab (N_L = 158):

	Percent of labs
Lab director(s)	83
Other staff person	17

7a. Percent of human embryo labs by size of staff (N_L = 160):

No. of persons	Director level	Supervisory level	Technical level
0	3	41	18
1	84	44	40
2	10	10	23
3 or more	3	5	19

7b. Percent of lab staff by length of employment with present lab:

	Director level N_S = 181	Supervisory level N_S = 130	Technical level N_S = 251
Less than 6 months	1	2	15
6 to less than 12 months	8	7	14
1 to 2 years	16	25	32
More than 2 years	71	65	38
No answer given	4	1	1

7c. Percent of lab staff by length of workweek:

	Director level N_S = 181	Supervisory level N_S = 95	Technical level N_S = 131
Less than 20 hours	7	5	7
20 to less than 35 hours	4	8	8
35 hours or more	85	86	86
No answer given	4	1	0

7d. Percent of labs having one or more persons with their highest degree as:

	Director level N_L = 155	Supervisory level N_L = 95	Technical level N_L = 131
M.D. and Ph.D.	12	4	1
M.D.	34	14	5
Ph.D. in biological science	43	15	3
Ph.D. in another field	1	2	1
M.S. in biological science	4	16	12
M.S./M.A. in another field	0	3	5
B.S. in biology or medical technology	4	38	61
B.S./B.A. in another field	0	4	4
Associate or other degree	2	2	8
No answer given	0	2	0

7e. Percent of labs having one or more persons with their most experience working in a human embryo lab for:

	Director level N_L = 155	Supervisory level N_L = 95	Technical level N_L = 131
Less than 1 year	3	0	18
1 to less than 2 years	6	10	25
2 to less than 3 years	10	21	24
3 to 5 years	28	35	20
More than 5 years	52	32	12
No answer given	1	2	1

7f. Percent of labs having one or more persons with their most experience working in an animal embryo lab for:

	Director level N_L = 155	Supervisory level N_L = 95	Technical level N_L = 131
0 or less than 1 year	20	21	39
1 to less than 2 years	5	6	13
2 to less than 3 years	2	17	17
3 to 5 years	19	23	15
More than 5 years	53	31	15
No answer given	1	2	1

Equipment

8. Number of incubators used by each lab (N_L = 157):

	Incubators
Median	3
Range (low to high)	1–12

9. Use of portable incubators for oocyte identification and/or transportation (N_L = 160):

	Percent of labs
Oocyte identification only	7
Oocyte transporting only	6
Both identification and transportation	26
Neither identification nor transportation	61

10a. Gas mixture maintained in incubators during culturing (N_L= 160):

	Percent of labs
5% oxygen, 5% carbon dioxide, 90% nitrogen	22
5% carbon dioxide, 95% room air	76
Other	2

10b. Temperature level maintained in incubators during culturing (N_L = 160):

	Degrees Centigrade
Median	37
Range (low to high)	36–38

10c. Humidity level present in incubators during culturing (N_L = 142):

	Percent humidity
Median	98
Range (low to high)	30 to 100

11a. Methods used to detect incubator carbon dioxide failure (N_L = 153):

	Percent of labs
External readings	97
Incubator alarm	84
Remote alarm	41
Fyrite analyzer	39
Other	15

11b. Methods used to detect incubator temperature failure (N_L = 160):

	Percent of labs
External readings	98
Internal thermometer	95
Incubator alarm	39
Recording thermometer	21
Other	6

12. Methods, in addition to incubation, used to maintain a constant temperature environment for human gametes/pre-embryos (N_L = 158):

	Percent of labs
Warming trays for culture containers	91
Water bath to prewarm fluid substances	67
Heated stages on microscope	49
Warmer than normal room temperature	41
Isolette	15
Heating blocks, dry bath, or other	27

13. Procedures usually performed under a laminar flow hood:

		Percent of labs		
	N_L	Not performed under hood	Hood turned on	Hood turned off
Culture media preparation	150	4	92	4
Oocyte identification	142	27	51	22
Sperm preparation	150	19	69	12
Pre-embryo examination	146	29	48	23
Catheter loading	144	26	51	23

14. Method, if any, usually used to test supplies for toxicity (N_L = 160):

	Percent of labs				
	Do not test	One-cell mouse pre-embryos	Two-cell mouse pre-embryos	Human sperm	Multiple methods used
Oocyte needles	44	9	31	1	15
Culture vessels	24	12	37	5	22
Test tubes, beakers, or other vessels	28	11	36	6	19
Pipets	34	11	35	3	17
Transfer catheters	37	11	35	2	15

15. Method, if any, usually used to sterilize supplies (N_L= 159):

	Percent of labs			
	Do not sterilize	Gas/steam autoclave	Dry heat	Radiation or other
Oocyte needles	26	59	14	1
Culture vessels	84	8	1	7
Test tubes, beakers, or other vessels	50	27	20	3
Pipets	20	48	30	2
Transfer catheters	50	40	8	2

16. Type of water usually used for the final rinse when cleaning supplies (N_L = 159):

Water	Percent of labs
Distilled	7
Deionized	3
Combination distilled and deionized	16
Ultrapure (HPLC or Milli-Q)	74

17a. Frequency with which each type of equipment is checked for proper operation:

		Percent of labs						
	N_L	Once or more a day	Once or more a week	Once or more a month	Once or more every 6 months	Once or more a year	Less frequent than once a year	Never
Incubator—gas	155	81	16	3	0	0	0	0
Incubator—temperature	160	87	11	2	0	0	0	0
Incubator—humidity	140	42	29	5	3	1	1	19
Incubator—microbial contamination	154	8	26	21	16	7	4	18
Laminar hood—sterility	148	7	10	12	27	20	5	19
Laminar hood—air flow	152	5	2	5	45	34	4	5
Centrifuge	154	2	4	12	25	29	12	16
Osmometer	151	17	48	23	5	3	3	1
pH meter	151	20	47	21	5	4	2	1
Refrigerator	157	41	23	10	9	7	4	6
Freezer	150	39	24	10	8	8	3	8
Automatic pipet	97	4	4	13	28	11	16	24
Scale (weight)	156	4	15	19	22	30	8	2

17b. Persons who conduct equipment checks:

		Percent of labs		
	N_L	Human embryo lab staff	Service company staff	Hospital staff or other
Incubator—gas	156	100	20	3
Incubator—temperature	160	100	15	4
Incubator—humidity	115	98	11	1
Incubator—microbial contamination	127	90	2	14
Laminar hood—sterility	124	53	46	9
Laminar hood—air flow	146	26	71	12
Centrifuge	136	60	29	27
Osmometer	151	91	13	8
pH meter	151	96	12	3
Refrigerator	151	93	10	7
Freezer	141	92	9	7
Automatic pipet	77	88	18	1
Scale (weight)	149	66	42	6

Culture Media Preparation

18. Preparation of culture media (N_L = 160):

	Percent of labs
Self-prepared only	76
Commercially prepared only	16
Both	8

19a. Type of water usually used in preparing culture media (N_L = 137):

Water	Percent of labs
Ultrapure (HPLC or Milli-Q)	84
Combination distilled and deionized	10
Other	6

19b. Type of nutrient stock usually used in preparing culture media (N_L = 134):

	Percent of labs
Ham's F-10	80
Human tubal fluid	12
Other	8

19c. Type of protein supplement usually used in preparing different types of culture media:

		Percent of labs				
Type of media	N_L	Patient serum	Fetal cord serum	Bovine serum albumin	Other	More than one indicated
Insemination	147	58	14	10	6	12
Sperm washing	144	53	12	12	9	14
Growth	149	62	13	7	5	13
Transfer	151	67	11	4	3	15

20. Types of adverse conditions for which protein supplements, when derived from multiple human sources, usually are tested (N_L = 70):

	Percent of labs
HIV	96
Hepatitis	94
Herpes	24
Sperm antibodies	11
Other	31

21a. pH level of culture medium at time of use (N_L = 155):

	pH
Median	7.35–7.44
Range (low to high)	(7.15–7.50) to (7.25–7.80)

21b. Osmolarity of culture medium at time of use (N_L = 158):

	mOsm/kg
Median	200–285
Range (low to high)	(270–290) to (279–310)

22a. Whether each batch of culture medium is tested before it is used (N_L = 158):

	Percent of labs
Yes	81
No	19

22b. Method usually used to test the quality of the culture media (N_L = 160):

	Percent of labs
Two-cell mouse pre-embryos only	43
One-cell mouse pre-embryos only	16
Survival of human sperm only	14
Other or more than one of the above	24
Not specified—tested by supplier	3

22c. Whether records are kept of quality checks performed on culture media (N_L = 158):

	Percent of labs
Yes	92
No	8

23. Maximum amount of time each batch of medium is usually kept before it is discarded (N_L = 160):

	Percent of labs
Less than 1 week	6
1 to less than 2 weeks	43
2 to less than 4 weeks	46
1 month or more	5

Techniques

24. Types of adverse conditions for which donors are usually tested, when donor sperm or eggs are used (N_L = 136):

	Percent of labs
HIV	84
Hepatitis	82
Herpes	57
Other venereal diseases	53
Not specified—tested by supplier	18

Sperm Preparation

25a. Techniques usually used when preparing normal sperm (N_L = 160):

	Percent of labs
Washing and swim-up	96
Percoll	14
Other	9

25b. Techniques usually used when preparing male-factor sperm (N_L = 157):

	Percent of labs
Washing and swim-up	81
Percoll	39
Test yolk buffer	26
Follicular fluid	12
Other	17

26a. Number of times sperm is usually centrifuged (N_L = 159):

	Times
Median	2
Range (low to high)	1–3

26b. Length of time sperm is usually centrifuged per wash (N_L = 159):

	Minutes
Median	10
Range (low to high)	1–20

26c. Force at which sperm is usually centrifuged (N_L = 138):

	Gs
Median	300
Range (low to high)	100–800

IVF: Insemination

27. Criteria used to evaluate the stage of oocyte maturity (N_L = 152):

	Percent of labs
Corona-cumulus configuration	94
First polar body visible	51
Observation of germinal vesicle	32
Appearance of cytoplasm	15
Appearance of granulosa cells	13
Other	11

28a. Length of time an oocyte that is mature at the time of aspiration usually is incubated before insemination (N_L = 158):

	Hours
Median	6
Range (low to high)	1–8

28b. Length of time an oocyte that is immature at the time of aspiration usually is incubated before insemination (N_L = 152):

	Hours
Median	21
Range (low to high)	1.5–36

29. Concentration of sperm, in cases with normal semen, usually used for insemination (N_L = 159):

	Percent of labs
Less than 50,000/ml.	7
50,000 to less than 100,000/ml.	55
100,000 to less than 200,000/ml.	30
200,000/ml. or more	8

30. Methods used, at least sometimes, to increase the likelihood of fertilization, when inseminating with semen that has few motile sperm (N_L = 151):

	Percent of labs
Add highly concentrated motile fraction to multiple oocytes	94
Add highly concentrated motile fraction to a single oocyte	76
Add oocyte directly to microdrop containing sperm	34
Micromanipulation	13
Other	3

31. Length of time after insemination that an oocyte usually is first checked for fertilization (N_L = 160):

	Percent of labs
12 to less than 24 hours	98
24 hours or more	2

32a. Frequency with which a second insemination is performed when the first insemination does not result in fertilization (N_L = 160):

	Percent of labs
Rarely, if ever	16
Sometimes	17
About half the time	6
Most of the time	21
Always or almost always	40

32b. Length of time after the first insemination that a second insemination is usually performed (N_L = 135):

	Percent of labs
Less than 6 hours	5
6 to less than 12 hours	0
12 to less than 24 hours	85
24 hours or more	10

IVF: Pre-Embryo Transfer

33. Length of time pre-embryos are usually cultured before they are transferred (N_L = 159):

	Percent of labs
Less than 24 hours	5
24 to 48 hours	72
49 to 60 hours	23

34. Criteria used for assessing pre-embryo quality (N_L = 154):

	Percent of labs
Blastomere shape, size, evenness	90
Presence of fragments and blebs	81
Number of cells, cleavage rate	64
Presence of only two pronuclei	18
Zona intactness	18
Other	50

35. Extent to which pre-embryo quality is considered when determining transferability (N_L = 159):

	Percent of labs
To little/no extent	11
To some extent	24
To a moderate extent	27
To a great extent	22
To a very great extent	16

36a. Average number of pre-embryos transferred per attempt (N_L = 156):

	Pre-embryos
Median	3
Range (low to high)	1–6

36b. Maximum number of pre-embryos transferred per attempt (N_L = 156):

	Pre-embryos
Median	5
Range (low to high)	2–14

37. Disposition of excess pre-embryos, at least sometimes, when not all pre-embryos are immediately transferred (N_L = 145):

	Percent of labs
Frozen for future transfer to the same woman	83
Discarded without being frozen or donated	41
Cultured for diagnostic purposes	28
Donated for transfer to another woman	25
Donated for research	8

GIFT: Gamete Transfer

38. Criteria used for assessing oocyte quality and maturity for transferability (N_L = 136):

	Percent of labs
Corona-cumulus configuration	95
First polar body visible	45
Appearance of cytoplasm	25
Observation of germinal vesicle	19
Appearance of granulosa cells	11
Other	19

39. Concentration of sperm, in cases with normal semen, usually used for insemination (N_L = 142):

	Percent of labs
Less than 50,000/ml.	4
50,000 to less than 100,000/ml.	36
100,000 to less than 200,000/ml.	40
200,000/ml. or more	20

40a. Average number of oocytes placed in a single tube (N_L = 142):

	Oocytes
Median	2
Range (low to high)	1–5

40b. Maximum number of oocytes placed in a single tube (N_L = 141):

	Oocytes
Median	4
Range (low to high)	2–7

41. Disposition of excess oocytes, at least sometimes, when not all oocytes are immediately transferred (N_L = 137):

	Percent of labs
Fertilized in vitro and/or frozen for future transfer to the same woman	90
Discarded without being frozen or donated	49
Donated for transfer to another woman	23
Cultured for diagnostic purposes	22
Donated for research	12

Cryopreservation

42. Whether lab does cryopreservation (N_L = 160):

	Percent of labs
Yes	74
No	26

43. Method of freezing used for cryopreservation (N_L = 116):

	Percent of labs
Programmable freezer	94
Direct plunge	4
Vitrification	2

IVF: Freezing Pre-Embryos

44. Whether lab freezes pre-embryos (N_L = 118):

	Percent of labs
Yes	74
No	26

45. Type of cryoprotectant usually used when freezing pre-embryos at different stages of development:

		Percent of labs		
	N_L	DMSO	Propylene glycol	Glycerol or other
Pronuclear	42	0	98	2
Cleavage	72	28	83	4
Blastocyst	4	25	0	75

46. Proportion of frozen pre-embryos that have been transferred (N_L = 117):

	Percent of labs
None	13
1 to 25 percent	45
26 to 50 percent	26
51 to 75 percent	13
76 to 100 percent	3

47. Maximum length of time frozen pre-embryos are kept (N_L = 115):

	Percent of labs
Less than 2 years	11
2 through 5 years	41
More than 5 years	11
No limit	37

GIFT: Freezing Oocytes

48. Whether lab freezes oocytes (N_L = 119):

	Percent of labs
Yes	10
No	90

49. Type of cryoprotectant usually used when freezing oocytes (N_L = 11):

	Percent of labs
Propylene glycol	64
DMSO	36

50. Proportion of frozen oocytes that have been transferred (N_L = 12):

	Percent of labs
None	58
1 - 25 percent	33
26 - 50 percent	9

51. Maximum length of time frozen oocytes are kept (N_L = 12):

	Percent of labs
1 year or less	25
More than 1 year	33
No limit	42

Standards

52a. Whether respondent supports the establishment of operating standards for human embryo labs (N_L = 151):

	Percent of labs
Yes	91
No	9

52b. Reasons given for supporting the establishment of standards for human embryo labs (N_L = 100):

	Percent of labs
Quality assurance is lacking	74
Patients need protection	36
To weed out inferior programs	10
To provide ethical guidance	8

53. Specific areas for which operating standards could be developed (N_L = 130):

	Percent of labs
Develop quality control standards for media testing and culturing conditions	69
Establish requirements for lab personnel, such as training, certification, and continuing education	69
Specify the correct operation and maintenance of laboratory equipment	39
Establish a nationally consistent format and process for recording and reporting outcome data	27
Set up uniform IVF/GIFT techniques	17

54. Additional comments (N_L = 71):

	Percent of labs
Various IVF/GIFT techniques can produce successful outcomes. Need to maintain flexibility, allow for innovation.	41
An accreditation system is needed to ensure that programs meet minimal acceptable standards. Laboratories should be licensed.	39
Government regulation should be avoided. AFS should oversee the profession.	25
Standards should encompass clinical aspects of IVF/GIFT, especially ovarian stimulation protocols.	14
Other	14

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