

**THE UNIVERSITY OF WESTERN ONTARIO  
BIOLOGICAL AGENTS REGISTRY FORM**  
Approved Biohazards Subcommittee: October 14, 2010  
Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1<sup>st</sup> edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or [biosafety@uwo.ca](mailto:biosafety@uwo.ca). If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)

PRINCIPAL INVESTIGATOR	<u>Bonnie J. Deroo</u>
DEPARTMENT	<u>Biochemistry</u>
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PHONE NUMBER	<u>519-685-8500 X55988</u>
EMERGENCY PHONE NUMBER(S)	<u>519-614-8371 (mobile)</u>
EMAIL	<u>bderoo2@uwo.ca</u>

Location of experimental work to be carried out: Building(s) 4<sup>th</sup> floor VRL, Deroo bay \_\_ Room(s) A4-114

\*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR, NSERC, UWO  
GRANT TITLE(S): Grant numbers: R4169A01 (startup), R4169A03 (NSERC), R4169A05 (CIHR)

List all personnel working under Principal Investigators supervision in this location:

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Erin Parker	<a href="mailto:eparka3@uwo.ca">eparka3@uwo.ca</a>	September 2005
Adrian V. Buensuceso	<a href="mailto:adrianvincent@gmail.com">adrianvincent@gmail.com</a>	September 2008
Alexandra Zalewski	<a href="mailto:azalews3@uwo.ca">azalews3@uwo.ca</a>	September 2009
Caitlin Cudmore	<a href="mailto:ccudmore@uwo.ca">ccudmore@uwo.ca</a>	September 2009

**Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.**

### ***E. coli***

We transform these bacteria with plasmids, then grow up to IL cultures so that ample plasmid quantities may be isolated for use in transfection studies in some of our cell lines (see below). These bacteria are lysed (and therefore destroyed) during the isolation of the plasmids. Any bacteria remaining in growth flasks are killed by bleach.

### **Plasmids**

We carry out little plasmid "genetic modification" in the lab, but we have done the following:

**pCDNA3:** We have used this as a "backbone" vector into which we clone the coding regions for genes of interest. We have so far created the following expression plasmid using this vector: mNid2-pcDNA3.

**pGL3-basic:** We have used this as a "backbone" vector into which we clone the promoter regions for genes of interest. We have so far created the following reporter plasmids using this vector: mComp-pGL3, mSpon1-pGL3, and hSpon1-pGL3.

Plasmids are not disposed of except after use in experiments with cultured cells; thus, their eventual disposal location is in biohazardous waste containers. Plasmids are stored at -20°C.

### **Primary mouse ovarian granulosa cells**

We routinely isolate granulosa cells from mouse ovaries, and either freeze the cells immediately at -80°C or culture them for up to two days in standard tissue culture medium without serum, after which the cells die. If cultured, we frequently treat the cells with FSH or 17beta-estradiol for up to 48h. In either case, the RNA or protein is isolated from the isolated cells. No live cells remain after these experiments.

### **Human granulosa cells from patients**

We isolate RNA from patient granulosa cells obtained from the University Hospital IVF clinic. Granulosa cells are initially isolated from a mixture that also contains red blood cells and ovarian fluid. Any containers or plastics that come in contact with these patient samples are extensively bleached during and after the isolation procedure. Isolated granulosa cells are stored at -80°C.

### **Cell lines**

We routinely work with three main cell lines: PANC-1, MCF-7, GFSHR-17, and KGN cells. We also sporadically use HEK293 cells. PANC-1 cells are used to study the location of the protein, Thioredoxin Interacting Protein by confocal microscopy. MCF-7 cells are used for promoter-reporter (luciferase) studies using plasmids as described above. GFSHR-17 and KGN cells are not transfected, but are treated with various reagents or substances, and then gene expression or cell morphology is studied in response to these agents. HEK293 cells are used to over-express various proteins using transfection of expression plasmids. We then use these cell extracts as controls for protein-related experiments, such as Western blots. All cell lines are stored in a 150°C ultra-low freezer. After they are cultured and/or transfected, any remaining cells are disposed in biohazardous waste containers.

**Luteinizing hormone (LH) and Follicle stimulating hormone (FSH):** These peptides are stored at -80°C. We use these peptides to treat either primary mouse granulosa cells or the GFSHR-17 and KGN cell lines, after which we isolate RNA or protein for downstream analysis. The medium containing these reagents is treated with bleach before disposal.

**Pregnant mare's serum gonadotropin and human chorionic gonadotropin:** We store these compounds at -80°C and use them for injection into mice. Mice that receive these compounds are later dissected and the ovaries removed for further study. The remainder of the animal is disposed of in proper storage containers.

**17beta-estradiol:** Estradiol is stored in ethanol at -20°C. We use this compound to treat cell lines such as MCF-7, GFSHR-17 and KGN, as well as primary mouse granulosa cells.

**Please include a one page research summary or teaching protocol.**

#### **Grant R4169A03**

When the cells in our body use oxygen, they naturally produce very reactive molecules known as "free radicals" which can damage the body. This damage may lead to cancer. However, cells protect themselves from free radical damage using other molecules known as "free radical scavengers". These scavengers convert the dangerous free radicals molecules into less reactive molecules that no longer harm the cell. One free radical scavenger that is common in human cells is a very small protein known as Thioredoxin. However, Thioredoxin does not act alone, but must make contact with other proteins to scavenge free radicals. One of these other proteins is called Thioredoxin Interacting Protein (Txnip). Thioredoxin and Txnip interact in the cell and form what is called a dimer (meaning two proteins interact). However, it is very likely that other proteins also interact with Thioredoxin and Txnip, and that Thioredoxin and Txnip form part of a larger protein complex made up of many proteins. So far, very few proteins are known that are part of this complex. Therefore, the purpose of my research program is to find the proteins, which are part of the Thioredoxin/Txnip complex. We expect to find several proteins that are part of this complex and that make contact with Thioredoxin and/or Txnip. Knowing what makes up this complex will tell us more information about where and how Thioredoxin scavenges free radicals in the cell. Understanding how Thioredoxin carries out its role as a scavenger is important because laboratory experiments indicate that scavengers such as Thioredoxin may prevent cancer. If we understand Thioredoxin better, it may lead to the development of anti-cancer medications or strategies to prevent cancer in Canadians.

#### **Grant R4169A05**

The most common cause of infertility in women is impaired ovulation, and although few genetic causes of infertility are known, a genetic basis for infertility is suspected. Genes associated with the production of a healthy egg are likely candidates for female infertility because many carefully-timed signals from both the brain and the ovary are required for the production of a healthy egg. Estrogen is not only produced by a woman's ovary, but the ovary itself requires estrogen for proper function. Estrogen is required by the ovary for the production and release of a healthy fertilizable egg, and in mice, lack of estrogen results in reduced fertility. Our goal is to understand the role of estrogen within the ovary, and the mechanisms by which estrogen regulates ovarian function. To pursue this goal, we study mice that are unable to respond to estrogen because they lack a protein, Estrogen Receptor Beta, which is normally present in the ovary. These mice are less fertile because they do not ovulate: eggs cannot be released, but are "stuck" within the ovary. We have found that one reason for this is that the extracellular matrix, the substance that holds cells together within the ovary, is not properly formed. By studying the matrix of these infertile mice, we can better understand why estrogen is required for ovulation. Understanding the role of estrogen in ovulation may provide genetic clues to the basis of some cases of infertility in women.

**1.0 Microorganisms**

1.1 Does your work involve the use of biological agents?  YES  NO  
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA?  YES  NO

If YES, please give the name of the species. \_\_\_\_\_

What is the origin of the microorganism(s)? \_\_\_\_\_

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in litres)	Source/Supplier	PHAC or CFIA Containment Level
<i>E. coli</i> <i>dH5 alpha</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input checked="" type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>Luteinizing hormone (LH) /human chorionic gonadotropin</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>Follicle stimulating hormone (FSH) /pregnant mare's serum gonadotropin</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>17beta-estradiol</i>	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			<input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

\*Please attach a Material Safety Data Sheet or equivalent from the supplier.

**2.0 Cell Culture**

2.1 Does your work involve the use of cell cultures?  YES  NO

If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="checkbox"/> Yes <input type="checkbox"/> No		Not applicable
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<i>AUS: 2008-047</i>	<i>granulose cells of mouse ovary</i>
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No		

*As per e-mail*

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	HEK293, MCF-7, KGN, PANC-1	2 (all)	Drs. A. Babwah, J. Torchia, RIKEN Clone Bank, Dr. Chris Pin
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	GFSHR-17	2 (all)	The Weizmann Institute
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No			

\*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required  1  2  2+  3

**3.0 Use of Human Source Materials**

3.1 Does your work involve the use of human source materials?  YES  NO  
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)	University Hospital IVF clinic: human ovarian cells	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> Unknown	N/A	<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

**4.0 Genetically Modified Organisms and Cell lines**

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0?  YES  NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done?  YES, complete table below  NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
<i>E. coli</i> <i>dHS alpha</i>	<i>pcDNA3, pGL3-basic</i>	Dr. J. Mymryk	<i>mNid2, hSpon1, mSpon1, mComp</i>	none

\* Please attach a Material Data Sheet or equivalent if available.

\*\* Please attach a plasmid map.

See E-mail

per E-mail JS

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below  NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

\* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV  YES, please specify \_\_\_\_\_  NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens  YES, specify \_\_\_\_\_  NO
- ◆ SV 40 Large T antigen  YES  NO
- ◆ E1A oncogene  YES  NO
- ◆ Known oncogenes  YES, please specify \_\_\_\_\_  NO
- ◆ Other human or animal pathogen and or their toxins  YES, please specify \_\_\_\_\_  NO

4.5 Will virus be replication defective?  YES  NO N/A

4.6 Will virus be infectious to humans or animals? N/A  YES  NO N/A

4.7 Will this be expected to increase the containment level required?  YES  NO N/A

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent?  YES  NO  
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)  
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: \_\_\_\_\_  
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host?  YES  NO

5.3 How will the biological agent be administered? \_\_\_\_\_

5.4 Please give the Health Care Facility where the clinical trial will be conducted: \_\_\_\_\_

5.5 Has human ethics approval been obtained?  YES, number: \_\_\_\_\_  NO  PENDING

6.0 Animal Experiments

6.1 Will live animals be used?  YES  NO If no, please proceed to section 7.0

6.2 Name of animal species to be used \_\_\_\_\_ mouse \_\_\_\_\_

6.3 AUS protocol # \_\_\_\_\_ 2008-047 \_\_\_\_\_

6.4 Will any of the agents listed in section 4.0 be used in live animals  YES, specify: \_\_\_\_\_  NO

6.5 Will the agent(s) be shed by the animal:  YES  NO, please justify: N/A

\_\_\_\_\_

**7.0 Use of Animal species with Zoonotic Hazards**

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)?  YES  No If no, please proceed to section 8.0

7.2 Will live animals be used?  YES  No

7.3 If yes, please specify the animal(s) used:

- ◆ Pound source dogs  YES  NO
- ◆ Pound source cats  YES  NO
- ◆ Cattle, sheep or goats  YES, please specify species \_\_\_\_\_  NO
- ◆ Non-human primates  YES, please specify species \_\_\_\_\_  NO
- ◆ Wild caught animals  YES, please specify species & colony # \_\_\_\_\_  NO
- ◆ Birds  YES, please specify species \_\_\_\_\_  NO
- ◆ Others (wild or domestic)  YES, please specify \_\_\_\_\_  NO

7.4 If no live animals are used, please specify the source of the specimens:  
\_\_\_\_\_

**8.0 Biological Toxins**

8.1 Will toxins of biological origin be used?  YES  NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) \_\_\_\_\_  
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD<sub>50</sub> (specify species) of the toxin \_\_\_\_\_

8.4 How much of the toxin is handled at one time\*? \_\_\_\_\_

8.5 How much of the toxin is stored\*? \_\_\_\_\_

8.6 Will any biological toxins be used in live animals?  YES, Please provide details: \_\_\_\_\_  NO

\*For information on biosecurity requirements, please see:

[http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\\_Requirements.pdf](http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf)

**9.0 Insects**

9.1 Do you use insects?  YES  NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. \_\_\_\_\_

9.3 What is the origin of the insect? \_\_\_\_\_

9.4 What is the life stage of the insect? \_\_\_\_\_

9.5 What is your intention?  Initiate and maintain colony, give location: \_\_\_\_\_  
 "One-time" use, give location: \_\_\_\_\_

9.6 Please describe the risk (if any) of escape and how this will be mitigated:  
\_\_\_\_\_  
\_\_\_\_\_



**13.0 Containment Levels**

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required.

01  02  02+  03

13.2 Has the facility been certified by OHS for this level of containment?

- YES, permit # if on-campus \_\_\_\_\_
- NO, please certify
- NOT REQUIRED for Level 1 containment

*Certified Level 2  
NOV. 26, 2010  
Maile Ryden*

**14.0 Procedures to be Followed**

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures that are unique to this agent.

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:

Wash with water and/or soap for skin exposure for all agents listed in Section 1.2. Needlestick injury due to self-injection with LH, FSH, or 17beta-estradiol: fill out Accident Report for both LHSC/LHRI and UWO and monitor wound and general health of injured worker regularly after incident.

14.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE *[Signature]*

Date: 23 November 2010

**15.0 Approvals**

1) UWO Biohazards Subcommittee: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

2) Safety Officer for the University of Western Ontario  
SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

3) Safety Officer for Institution where experiments will take place (if not UWO):  
SIGNATURE: *[Signature]*  
Date: Nov. 26, 2010

Approval Number: \_\_\_\_\_ Expiry Date (3 years from Approval): \_\_\_\_\_

Special Conditions of Approval:

**Subject:** Re: Biological Agents Registry Form: Deroo  
**From:** Bonnie Deroo <bderoo2@uwo.ca>  
**Date:** Wed, 12 Jan 2011 12:52:31 -0500  
**To:** Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer!

re: E.coli DH5alpha. Thanks for fixing that for me.

For the box in Table 4.2 that is missing the information regarding "change that results from transformation or transfection" please replace "none" with: "changes in gene expression, cell proliferation, and steroidogenesis."

If any other information is required, please let me know!

--Bonnie

--  
Bonnie Deroo, PhD  
Department of Biochemistry  
The University of Western Ontario, Canada  
(mailto: [bderoo2@uwo.ca](mailto:bderoo2@uwo.ca))  
Telephone +1 519 685 8600 X55988



E-mail

On Tue, Jan 4, 2011 at 6:03 PM, Jennifer Stanley <[jstanle2@uwo.ca](mailto:jstanle2@uwo.ca)> wrote:

Hi Dr. Deroo

There are a few questions from the Committee:

Please note that E.coli DH5alpha listed in section 1.2 should not be listed as a human pathogen, animal pathogen or zoonotic agent. I can make the adjustment(s) on the form.

The changes that will result from transfection or transformation are not listed in table 4.2. Please explain the change(s).

Regards  
Jennifer

**Subject:** Re: Biological Agents Registry Form: Deroo  
**From:** Bonnie Deroo <bderoo2@uwo.ca>  
**Date:** Mon, 29 Nov 2010 10:41:31 -0500  
**To:** Jennifer Stanley <jstanle2@uwo.ca>

Yes, that's right, Jennifer: you have the correct protocol number.  
I will check on the E. coli strain and get back to you ASAP.

--Bonnie

On Fri, Nov 26, 2010 at 4:41 PM, Jennifer Stanley <[jstanle2@uwo.ca](mailto:jstanle2@uwo.ca)> wrote:  
Hi Dr. Deroo -

I understand that your AUS protocol number is 2008-047.

Regards,  
Jennifer

On 11/26/2010 3:40 PM, Jennifer Stanley wrote:  
Hi Dr. Deroo

Thank you for your submission. I received the signed version today. I have a couple of questions:

1. For Table 2.2, I assume that you get the "granulosa cells of mice ovary" from live mice? If so, what is your AUS protocol number?
2. For Tables 1.2 and 4.2, what strain(s) of E.coli do you use? (DH5 alpha, etc...)

Have a great weekend.

Regards,  
Jennifer

--

Bonnie Deroo, PhD  
Department of Biochemistry  
The University of Western Ontario, Canada  
(mailto: [bderoo2@uwo.ca](mailto:bderoo2@uwo.ca))  
Telephone +1 519 685 8600 X55988

**Subject:** Re: Biological Agents Registry Form: Deroo  
**From:** Jennifer Stanley <jstanle2@uwo.ca>  
**Date:** Tue, 30 Nov 2010 09:55:34 -0500  
**To:** Bonnie Deroo <bderoo2@uwo.ca>

Thanks Dr. Deroo  
FYI - E. coli DH5alpha is normally considered Level 1.  
Thanks for getting back to me so quickly,  
Jennifer

On 11/29/2010 2:59 PM, Bonnie Deroo wrote:

Hi Jennifer!

It's DH5alpha that we work with.

Have a great night,  
Bonnie

On Fri, Nov 26, 2010 at 3:40 PM, Jennifer Stanley <jstanle2@uwo.ca> wrote:  
Hi Dr. Deroo

Thank you for your submission. I received the signed version today. I have a couple of questions:

1. For Table 2.2, I assume that you get the "granulosa cells of mice ovary" from live mice? If so, what is your AUS protocol number?
2. For Tables 1.2 and 4.2, what strain(s) of E.coli do you use? (dH5 alpha, etc...)

Have a great weekend.

Regards,  
Jennifer

--

Bonnie Deroo, PhD  
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The University of Western Ontario, Canada  
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Telephone +1 519 685 8600 X55988

# SAFETY DATA SHEET



Order Number

Customer Number

## 1. Identification of the substance/preparation and of the company/undertaking

Product name : Luteinizing Hormone, Human Pituitary, Iodination Grade

Chemical formula : N/A

Synonym : hLH

Material Safety Data Sheets

Emergency telephone number : Call Chemtrec®  
(800)424-9300 (within U.S.A.)  
(703)527-3887 (outside U.S.A.)

## 2. Composition / information on ingredients

Substance/Preparation : Substance

Chemical name*	CAS No.	EC Number	Symbol	R-Phrases
1) Luteinizing Hormone, Human Pituitary, Iodination Grade	39341-83-8	Not available.	T	R60

## 3. Hazards identification

Physical/chemical hazards : Not applicable.

Human health hazards : CAUTION!  
POSSIBLE BIRTH DEFECT HAZARD.  
MAY CAUSE BIRTH DEFECTS BASED ON ANIMAL DATA.  
MAY CAUSE DAMAGE TO THE FOLLOWING ORGANS: REPRODUCTIVE SYSTEM, OVARY, TESTES.

## 4. First-aid measures

### First-Aid measures

- Inhalation** : If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
- Ingestion** : Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.
- Skin Contact** : In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Cold water may be used. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.
- Eye Contact** : Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention.
- Aggravating conditions** : Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

## 5. Fire-fighting measures

Flammability of the Product : May be combustible at high temperature.

### Extinguishing Media

- Suitable** : SMALL FIRE: Use DRY chemical powder.  
LARGE FIRE: Use water spray, fog or foam. Do not use water jet.
- Hazardous thermal (de)composition products** : These products are nitrogen oxides (NO, NO2...).

Special fire-fighting procedures : Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear.

Protection of fire-fighters : Be sure to use an approved/certified respirator or equivalent.

## 6. Accidental release measures

- Personal precautions** : Splash goggles. Full suit. Boots. Gloves. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.
- Small Spill and Leak** : Use appropriate tools to put the spilled solid in a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.
- Large Spill and Leak** : Use a shovel to put the material into a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system

## 7. Handling and storage

- Handling** : Keep locked up. Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe dust. Wear suitable protective clothing. If you feel unwell, seek medical attention and show the label when possible.
- Storage** : Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above -20°C (-4°F).
- Packaging materials**
- Recommended use** : Use original container.

## 8. Exposure controls/personal protection

- Engineering measures** : Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.
- Hygiene measures** : Wash hands after handling compounds and before eating, smoking, using lavatory, and at the end of day.

<u>Ingredient Name</u>	<u>Occupational Exposure Limits</u>
1) Luteinizing Hormone, Human Pituitary, Iodination Grade	Not available.

### Personal protective equipment

- Respiratory system** : Wear appropriate respirator when ventilation is inadequate.
- Skin and body** : Lab coat.
- Eyes** : Safety glasses.
- Protective Clothing (Pictograms)** :



## 9. Physical and chemical properties

- Physical state** : Solid.
- Color** : Not available.
- Molecular Weight** : 28500 g/mole
- Solubility** : Easily soluble in cold water.
- Flash point** : Not available.
- Explosive properties** : Risks of explosion of the product in presence of mechanical impact: Not available.  
Risks of explosion of the product in presence of static discharge: Not available.

## 10. Stability and reactivity

- Stability** : The product is stable.
- Conditions to avoid** : Not available.
- Hazardous Decomposition Products** : These products are nitrogen oxides (NO, NO2...).

## 11. Toxicological information

- RTECS #** : **OK6367000**
- Local effects**
- Skin irritation** : Not available.
- Acute toxicity** : LD50: Not available.  
LC50: Not available.
- Chronic toxicity** : Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Other Toxic Effects on Humans : Not available.  
No specific information is available in our database regarding the other toxic effects of this material for humans.  
May cause congenital malformations in the foetus.  
Not available.

#### Specific effects

Carcinogenic effects : Not available.  
Mutagenic effects : Not available.  
Reproduction toxicity : Classified Reproductive system/toxin/female, Reproductive system/toxin/male [SUSPECTED].  
Teratogenic effects : Classified POSSIBLE for human.

## 12. Ecological information

Ecotoxicity : Not available.  
Toxicity of the Products of Biodegradation : The product itself and its products of degradation are not toxic.

## 13. Disposal considerations

Methods of disposal; Waste of residues; Contaminated packaging : Waste must be disposed of in accordance with federal, state and local environmental control regulations.

## 14. Transport information

#### International transport regulations

##### Land - Road/Railway

ADR/RID Class : Not controlled under ADR (Europe).

##### Sea

IMDG Class : Not controlled under IMDG.

##### Air

IATA-DGR Class : Not controlled under IATA.

Special Provisions for Transport : Not applicable.

## 15. Regulatory information

#### EU Regulations

Hazard symbol(s) :



Classification : Toxic

Risk Phrases : R60- May impair fertility.

Safety Phrases : S22- Do not breathe dust.  
S36/37/39- Wear suitable protective clothing, gloves and eye/face protection.  
S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Contains : - Luteinizing Hormone, Human Pituitary, Iodination Grade

#### U.S. Federal Regulations

TSCA: No products were found.  
SARA 302/304/311/312 extremely hazardous substances: No products were found.  
SARA 302/304 emergency planning and notification: No products were found.  
SARA 302/304/311/312 hazardous chemicals: No products were found.  
SARA 311/312 MSDS distribution - chemical inventory - hazard identification: No products were found.  
SARA 313 toxic chemical notification and release reporting: No products were found.  
Clean Water Act (CWA) 307: No products were found.  
Clean Water Act (CWA) 311: No products were found.  
Clean air act (CAA) 112 accidental release prevention: No products were found.  
Clean air act (CAA) 112 regulated flammable substances: No products were found.  
Clean air act (CAA) 112 regulated toxic substances: No products were found.

HCS Classification : CLASS: Target organ effects.

State Regulations :

#### WHMIS (Canada)

: CLASS D-2A: Material causing other toxic effects (VERY TOXIC).  
No products were found.

## 16. Other information

Hazardous Material  
Information System  
(U.S.A.)

Health	0
Fire Hazard	1
Reactivity	0
Personal Protection	a

National Fire  
Protection  
Association (U.S.A.)



### Notice to Reader

*To the best of our knowledge, the information contained herein is accurate. However, neither the above named supplier nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.*

Date of issue

3/26/2003.

Catalog # 869003

Page: 4/4

## SIGMA-ALDRICH

## MATERIAL SAFETY DATA SHEET

Date Printed: 07/27/2007

Date Updated: 02/01/2006

Version 1.6

## Section 1 - Product and Company Information

Product Name CHORIONIC GONADOTROPIN FROM HUMAN &  
Product Number C0434  
Brand SIGMA

Company Sigma-Aldrich Canada, Ltd  
Address 2149 Winston Park Drive  
Oakville ON L6H 6J8 CA

Technical Phone: 9058299500  
Fax: 9058299292  
Emergency Phone: 800-424-9300

## Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
CHORIONIC GONADOTROPIN FROM HUMAN PREGNANCY URINE	9002-61-3	No

Chemical Family Human source material.  
Synonyms Ambinon \* Antuitrin S \* APL \* APL (hormone) \*  
Apoidina \* Chorigon \* Choriogonadotropin \*  
Choriogonin \* Chorionic gonadotrophin \* Chorionic  
gonadotropic hormone \* Chorionic gonadotropin \*  
Chorulon \* Coriantin \* Follutein \* Gonabion \*  
Gonadex \* HCG \* Human chorionic gonadotropin \*  
Korotrin \* Physex \* Praedyn \* Pregnyl \*  
Primogonyl \* Randonos \* Synaphorin

RTECS Number: MD6953000

## Section 3 - Hazards Identification

## EMERGENCY OVERVIEW

Biohazard.

Handle as if capable of transmitting infectious agents.

## HMIS RATING

HEALTH: 0

FLAMMABILITY: 0

REACTIVITY: 0

SPECIAL HAZARD(S): Human Source

## NFPA RATING

HEALTH: 0

FLAMMABILITY: 0

REACTIVITY: 0

SPECIAL HAZARD(S): Human Source

For additional information on toxicity, please refer to Section 11.

## Section 4 - First Aid Measures

## ORAL EXPOSURE

If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately.

#### INHALATION EXPOSURE

If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

#### DERMAL EXPOSURE

In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician.

#### EYE EXPOSURE

In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

---

### Section 5 - Fire Fighting Measures

---

#### FLASH POINT

N/A

#### AUTOIGNITION TEMP

N/A

#### FLAMMABILITY

N/A

#### EXTINGUISHING MEDIA

Suitable: Use extinguishing media appropriate to surrounding fire conditions.

#### FIREFIGHTING

Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.  
Specific Hazard(s): Emits toxic fumes under fire conditions.

---

### Section 6 - Accidental Release Measures

---

#### PROCEDURE(S) OF PERSONAL PRECAUTION(S)

Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves.

#### METHODS FOR CLEANING UP

Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Wash spill site with 10% bleach and ventilate area after material pickup is complete.

---

### Section 7 - Handling and Storage

---

#### HANDLING

User Exposure: Avoid inhalation. Avoid contact with eyes, skin, and clothing. Avoid prolonged or repeated exposure.

#### STORAGE

Suitable: Keep tightly closed.  
Store at -20°C

---

### Section 8 - Exposure Controls / PPE

---

#### ENGINEERING CONTROLS

Safety shower and eye bath. Mechanical exhaust required.

#### PERSONAL PROTECTIVE EQUIPMENT

Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a dust mask type N95 (US) or type P1 (EN 143) respirator.

Hand: Compatible chemical-resistant gloves.

Eye: Chemical safety goggles.

#### GENERAL HYGIENE MEASURES

Wash thoroughly after handling.

---

#### Section 9 - Physical/Chemical Properties

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Appearance	Physical State: Solid	
Property	Value	At Temperature or Pressure
Molecular Weight	N/A	
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
SG/Density	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	N/A	

N/A = not available

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#### Section 10 - Stability and Reactivity

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##### STABILITY

Stable: Stable.

Materials to Avoid: Strong oxidizing agents.

##### HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Carbon monoxide, carbon dioxide, and nitrogen oxides.

##### HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

---

## Section 11 - Toxicological Information

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### ROUTE OF EXPOSURE

Skin Contact: May cause skin irritation.  
Skin Absorption: May be harmful if absorbed through the skin.  
Eye Contact: May cause eye irritation.  
Inhalation: Material may be irritating to mucous membranes and upper respiratory tract. May be harmful if inhaled.  
Ingestion: May be harmful if swallowed.

### SIGNS AND SYMPTOMS OF EXPOSURE

Potentially biohazardous material.

### CHRONIC EXPOSURE - TERATOGEN

Species: Rat  
Dose: 875 MG/KG  
Route of Application: Subcutaneous  
Exposure Time: (15-17D PREG)  
Result: Effects on Embryo or Fetus: Extra embryonic structures (e.g., placenta, umbilical cord).

Species: Hamster  
Dose: 60 MG/KG  
Route of Application: Intraperitoneal  
Exposure Time: (4-6D PREG)  
Result: Effects on Embryo or Fetus: Fetal death.

### CHRONIC EXPOSURE - REPRODUCTIVE HAZARD

Result: Overexposure may cause reproductive disorder(s) based on tests with laboratory animals.

Species: Woman  
Dose: 36 MG/KG  
Route of Application: Unreported  
Exposure Time: (6D PRE)  
Result: Effects on Fertility: Other measures of fertility

Species: Rat  
Dose: 150 MG/KG  
Route of Application: Intraperitoneal  
Exposure Time: (1-3D PREG)  
Result: Effects on Fertility: Other measures of fertility  
Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).

Species: Rat  
Dose: 40 MG/KG  
Route of Application: Intraperitoneal  
Exposure Time: (10D MALE)  
Result: Paternal Effects: Spermatogenesis (including genetic material, sperm morphology, motility, and count).

Species: Rat  
Dose: 1250 UG/KG  
Route of Application: Subcutaneous  
Exposure Time: (1D PRE)  
Result: Maternal Effects: Ovaries, fallopian tubes. Maternal Effects: Uterus, cervix, vagina.

Species: Rat  
Dose: 5250 UG/KG  
Route of Application: Subcutaneous  
Exposure Time: (7-13D PREG)  
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species: Rat  
Dose: 16 MG/KG  
Route of Application: Subcutaneous  
Exposure Time: (4D MALE)  
Result: Paternal Effects: Other effects on male.

Species: Rat  
Dose: 8890 UG/KG  
Route of Application: Intravenous  
Exposure Time: (1D PRE)  
Result: Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).

Species: Rat  
Dose: 83 UG/KG  
Route of Application: Intramuscular  
Exposure Time: (9D PREG)  
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species: Rat  
Dose: 125 MG/KG  
Route of Application: Parenteral  
Exposure Time: (4D PRE)  
Result: Maternal Effects: Ovaries, fallopian tubes. Maternal Effects: Uterus, cervix, vagina.

Species: Rat  
Dose: 420 MG/KG  
Route of Application: Parenteral  
Exposure Time: (7D MALE)  
Result: Paternal Effects: Testes, epididymis, sperm duct.

Species: Rat  
Dose: 200 MG/KG  
Route of Application: Parenteral  
Exposure Time: (1D MALE)  
Result: Paternal Effects: Other effects on male.

Species: Rat  
Dose: 1650 MG/KG  
Route of Application: Unreported  
Exposure Time: (1-22D PREG)  
Result: Effects on Newborn: Growth statistics (e.g., reduced weight gain).

Species: Rat  
Dose: 50 MG/KG  
Route of Application: Unreported  
Exposure Time: (4D PREG)  
Result: Maternal Effects: Parturition.

Species: Rat  
Dose: 21212 NG/KG

Route of Application: Unreported  
Exposure Time: (7-13D PREG)  
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species: Rat  
Dose: 12 MG/KG  
Route of Application: Unreported  
Exposure Time: (1D MALE)  
Result: Paternal Effects: Other effects on male.

Species: Rat  
Dose: 280 IU/KG  
Route of Application: Unreported  
Exposure Time: (2W MALE)  
Result: Paternal Effects: Testes, epididymis, sperm duct.

Species: Mouse  
Dose: 24 MG/KG  
Route of Application: Intraperitoneal  
Exposure Time: (6D PRE)  
Result: Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).

Species: Mouse  
Dose: 400 IU/KG  
Route of Application: Intraperitoneal  
Exposure Time: (1D PRE)  
Result: Maternal Effects: Oogenesis.

Species: Mouse  
Dose: 560 MG/KG  
Route of Application: Subcutaneous  
Exposure Time: (7-13D PREG)  
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species: Mouse  
Dose: 6 MG/KG  
Route of Application: Intramuscular  
Exposure Time: (3D PRE)  
Result: Maternal Effects: Uterus, cervix, vagina.

Species: Monkey  
Dose: 10 MG/KG  
Route of Application: Subcutaneous  
Exposure Time: (5D PRE)  
Result: Maternal Effects: Menstrual cycle changes or disorders.

Species: Monkey  
Dose: 224 MG/KG  
Route of Application: Intramuscular  
Exposure Time: (18-36D PREG)  
Result: Maternal Effects: Menstrual cycle changes or disorders.

Species: Monkey  
Dose: 30 MG/KG  
Route of Application: Unreported  
Exposure Time: (15D PRE)  
Result: Maternal Effects: Parturition.

Species: Pig  
Dose: 833 UG/KG  
Route of Application: Intravenous  
Exposure Time: (1D PRE)  
Result: Effects on Fertility: Other measures of fertility

Species: Pig  
Dose: 833 UG/KG  
Route of Application: Intramuscular  
Exposure Time: (1D PRE)  
Result: Effects on Fertility: Other measures of fertility

Species: Hamster  
Dose: 240 MG/KG  
Route of Application: Intraperitoneal  
Exposure Time: (1-3D PREG)  
Result: Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).

Species: Hamster  
Dose: 280 MG/KG  
Route of Application: Subcutaneous  
Exposure Time: (7-13D PREG)  
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

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#### Section 12 - Ecological Information

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No data available.

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#### Section 13 - Disposal Considerations

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##### APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION

Contact a licensed professional waste disposal service to dispose of this material. Disposal should be made in accordance with existing disposal practices employed for infectious waste at your institution. Observe all federal, state, and local environmental regulations.

---

#### Section 14 - Transport Information

---

##### DOT

Proper Shipping Name: None  
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

##### IATA

Non-Hazardous for Air Transport: Non-hazardous for air transport.

---

#### Section 15 - Regulatory Information

---

##### EU ADDITIONAL CLASSIFICATION

Symbol of Danger: B  
Indication of Danger: Biohazard.

##### US CLASSIFICATION AND LABEL TEXT

Indication of Danger: Biohazard.  
US Statements: Handle as if capable of transmitting infectious agents.

UNITED STATES REGULATORY INFORMATION  
SARA LISTED: No

CANADA REGULATORY INFORMATION

WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.

DSL: No

NDSL: No

---

Section 16 - Other Information

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DISCLAIMER

For R&D use only. Not for drug, household or other uses.

WARRANTY

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale. Copyright 2007 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only.

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Search follitropin msds



MSDS 250,000+

MSDS : **Follicle-stimulating hormone**  
 CAS : 9002-68-0  
 SYNONYMS : \* Anthrogon  
 \* Follitropin  
 \* FSH  
 \* FSH-P  
 \* Gonad stimulating factor  
 \* Hebin  
 \* Luteoantine  
 \* Menotrophin  
 \* Menotropins  
 \* Prolan B  
 \* Thylakentrin  
 \* Urinary hebin

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[www.supprelin.ca](http://www.supprelin.ca)

Catalog of Chemical Suppliers, Buyers, Custom Synthesis Companies And Equipment Manufacturers [ Follicle-stimulating hormone 9002-68-0 ]

Suppliers:

Not Available

Buyers:

Not Available

[How to Get Pregnant Fast](#) | Stopped these common mistakes and got Pregnant almost immediately! [www.MaximumFr](http://www.MaximumFr)

[Hcg Injections](#) Official Site - Cosmetic Info. Answering All Your Questions! [BestPlasticSurgeon.com/Injectables](http://BestPlasticSurgeon.com/Injectables)

[International Donor Egg](#) Top rated US Fertility doctors Learn About Our Cost Saving Program [www.twostopdonoregg.co](http://www.twostopdonoregg.co)

\*\*\* CHEMICAL IDENTIFICATION \*\*\*

RTECS NUMBER : LP6330000  
 CHEMICAL NAME : Follicle-stimulating hormone  
 CAS REGISTRY NUMBER : 9002-68-0  
 LAST UPDATED : 198910  
 DATA ITEMS CITED : 5  
 COMPOUND DESCRIPTOR : Reproductive Effector Hormone

SYNONYMS/TRADE NAMES :

- \* Anthrogon
- \* Follitropin
- \* FSH
- \* FSH-P
- \* Gonad stimulating factor
- \* Hebin
- \* Luteoantine

\* Menotrophin  
\* Menotropins  
\* Prolan B  
\* Thylakentrin  
\* Urinary hebin

\*\*\* HEALTH HAZARD DATA \*\*\*

\*\* REPRODUCTIVE DATA \*\*

TYPE OF TEST : TDLo - Lowest published toxic dose  
ROUTE OF EXPOSURE : Subcutaneous  
SPECIES OBSERVED : Rodent - rat  
DOSE : 2 mg/kg  
SEX/DURATION : female 2 day(s) pre-mating  
TOXIC EFFECTS :

Reproductive - Maternal Effects - ovaries, fallopian tubes

REFERENCE :  
SCIEAS Science. (American Assoc. for the Advancement of Science, 1333 H  
St., NW, Washington, DC 20005) V.1- 1895- Volume(issue)/page/year:  
204,854,1979

TYPE OF TEST : TDLo - Lowest published toxic dose  
ROUTE OF EXPOSURE : Subcutaneous  
SPECIES OBSERVED : Rodent - rat  
DOSE : 25 gm/kg  
SEX/DURATION : female 1-10 day(s) after conception  
TOXIC EFFECTS :

Reproductive - Fertility - other measures of fertility

REFERENCE :  
JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams &  
Wilkins Co., 428 E. Preston St., Baltimore, MD 21202) V.1- 1909/10-  
Volume(issue)/page/year: 49,146,1933

TYPE OF TEST : TDLo - Lowest published toxic dose  
ROUTE OF EXPOSURE : Intracerebral  
SPECIES OBSERVED : Rodent - rat  
DOSE : 625 ug/kg  
SEX/DURATION : female 4-9 day(s) after conception  
TOXIC EFFECTS :

Reproductive - Maternal Effects - ovaries, fallopian tubes

REFERENCE :  
ENDOAO Endocrinology (Baltimore). (Williams & Wilkins Co., 428 E. Preston  
St., Baltimore, MD 21203) V.1- 1917- Volume(issue)/page/year:  
83,1273,1968

TYPE OF TEST : TDLo - Lowest published toxic dose  
ROUTE OF EXPOSURE : Parenteral  
SPECIES OBSERVED : Rodent - rat  
DOSE : 24 gm/kg  
SEX/DURATION : male 6 day(s) pre-mating  
TOXIC EFFECTS :

Reproductive - Paternal Effects - testes, epididymis, sperm duct

REFERENCE :  
ANREAK Anatomical Record. (Alan R. Liss, Inc., 41 E. 11th St., New York, NY  
10003) V.1- 1906/08- Volume(issue)/page/year: 65,261,1936

TYPE OF TEST : TDLo - Lowest published toxic dose  
ROUTE OF EXPOSURE : Intratesticular  
SPECIES OBSERVED : Rodent - rat  
DOSE : 8 ug/kg  
SEX/DURATION : male 1 day(s) pre-mating  
TOXIC EFFECTS :

Reproductive - Paternal Effects - testes, epididymis, sperm duct

REFERENCE :  
PSEBAA Proceedings of the Society for Experimental Biology and Medicine.  
(Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1903/04-  
Volume(issue)/page/year: 120,671,1965

\*\*\* END OF RECORD \*\*\*

**ALL Chemical Property And Toxicity Analysis PAGES IN THIS GROUP**

NAME	CAS
<a href="#">Formamide, N-(4-(5-nitro-2-thienyl)-2-thiazolyl)-</a>	58139-46-1
<a href="#">Formamide, N-(5-oxo-4,5-dihydro-1,2-dithiolo(4,3-b)pyrrol-6-yl)-</a>	21787-66-6
<a href="#">Formamide, N-(2-oxo-2-(4-(2-phenylethyl)phenyl)ethyl)-</a>	126150-86-5
<a href="#">Formamide, N-((tert-pentylamino)(3-pyridylamino)methylene)-</a>	67026-78-2
<a href="#">Follicle-regulatory protein (pig)</a>	67026-78-2
<a href="#">Formamide, N-(1-(9-(phenylmethyl)-9H-carbazol-3-yl)ethyl)-</a>	52969-60-5
<a href="#">Formamide, N-(1-(phenylmethyl)cyclopentyl)-</a>	20937-45-5
<a href="#">Formamide, 2-piperidino-1-piperidinocarbonyl-</a>	6050-25-5
<a href="#">Formamide, N-n-propyl-</a>	6281-94-3
<a href="#">Formamide, N-(1-(9-propylcarbazol-3-yl)ethyl)-</a>	52916-25-3
<a href="#">Formamide, N-(3-(3-(1-(1-pyrrolidinyl)ethyl)phenoxy)propyl)-</a>	78043-80-8
<a href="#">Formamide, N-(2,3,6,7-tetrahydro-1H,5H-benzo(ij)quinolizin-9-yl)-</a>	101418-51-3
<a href="#">Formamide, N-(4-(thiocarbamoyl)-5-imidazolyl)-</a>	19494-94-1
<a href="#">Formamide, N-(2,2,2-trichloro-1-(morpholinyl)ethyl)-</a>	60029-23-4
<a href="#">Formamide, N-((4-((3-(trifluoromethyl)phenyl)amino)-3-pyridinyl)sulfonyl)-</a>	56175-87-2
<a href="#">Follicle-stimulating hormone</a>	9002-68-0
<a href="#">Formamidine, N'-(5-acenaphthenyl)-N,N-dimethyl-</a>	101398-41-8
<a href="#">Formamidine, N-(p-acetamidophenyl)-N',N'-dimethyl-,hydrochloride</a>	2417-13-2
<a href="#">Formamidine, N'-(9-acridinyl)-N,N-dimethyl-</a>	101398-42-9
<a href="#">Formamidine, N'-(9-acridinyl)-N,N-dimethyl-, compdwith methyl iodine</a>	101398-43-0
<a href="#">Formamidine, N'-(1,3-benzodioxol-5-yl)-N,N-dimethyl-</a>	62331-09-3
<a href="#">Formamidine, N'-(2,1,3-benzothiadiazol-4-yl)-N,N-dimethyl-</a>	101398-44-1
<a href="#">Formamidine, N'-(1,3-benzothiadiazol-4-yl)-N,N-dimethyl-, compd.with methyl iodine</a>	101398-45-2
<a href="#">Formamidine, N'-benzyl-N,N-dimethyl-</a>	27159-75-7
<a href="#">Formamidine, N'-(2-benzyloxyethyl)-N,N-dimethyl-</a>	101398-46-3
<a href="#">Formamidine, N,N'-bis(3-methoxyphenyl)-</a>	3200-36-0
<a href="#">Follicle Stimulating Hormone, SJ-0021SUBSTANCE DEFINITION :A Recombinant human Follicle Stimulating HormoneREFERENCE :YACHDS Yakuri to Chiryo. Pharmacology and Therapeutics. (Raifu SaiensuShuppan K.K., 2-5-13, Yaesu, Chuo-ku, Tokyo 104, Japan) V.1- 1972-</a>	3200-36-0
<a href="#">Formamidine, 1-(6-bromo-2-methyl-4-quinolyl)-N-phenyl-, dihydrochloride</a>	78812-07-4
<a href="#">Formamidine, N'-(3-bromo-9-oxo-2-fluorenyl)-N,N-dimethyl-</a>	101398-47-4
<a href="#">Formamidine, N'-(4-tert-butyl-1-cyclohexyl)-N,N-dimethyl-</a>	101398-48-5
<a href="#">Formamidine, N'-carvacryl-N,N-dimethyl-</a>	101398-49-6
<a href="#">Formamidine, 1-chloro-N'-(3,4-dichlorophenyl)-N,N-dimethyl-</a>	6022-33-9
<a href="#">Formamidine, N'-(5-chloro-2,4-dimethoxyphenyl)-N,N-dimethyl-</a>	62331-11-7
<a href="#">Formamidine, N'-(3-chloro-2-methylphenyl)-N,N-dimethyl-</a>	13181-62-9
<a href="#">Formamidine, N'-(3-(2-chloro-10-phenothiazinyl)propyl)-N,N-dimethyl-, hydrochloride</a>	101398-50-9
<a href="#">Formamidine, N'-(alpha-(4-chlorophenyl)benzyl)-N,N-dimethyl-</a>	101398-51-0
<a href="#">Formamidine, N'-(p-chlorophenyl)-N,N-dimethyl-</a>	2103-46-0
<a href="#">Follicular fluid, porcine</a>	2103-46-0
<a href="#">Formamidine, N-((p-chlorophenyl)thio)-N-methyl-N'-(2,4-xylyl)-</a>	55311-62-1
<a href="#">Formamidine, N'-(5-chloropyridin-2-yl)-N,N-dimethyl-</a>	36172-58-4
<a href="#">Formamidine, N-(4-chloro-o-tolyl)-</a>	57151-04-9
<a href="#">Formamidine, N'-(4-chloro-o-tolyl)-N,N-dimethyl-</a>	6164-98-3

<u>Formamidine, N'-(4-chloro-o-tolyl)-N,N-dimethyl-,hydrochloride</u>	19750-95-9
<u>Formamidine, N-(4-chloro-o-tolyl)-N'-methyl-</u>	21787-80-4
<u>Formamidine,N'-(4-cyano-3-cyanomethyl-1-phenylpyrazol-5-yl)-N,N-dimethyl-</u>	101398-52-1
<u>Formamidine, 1-cyano-N,N'-diphenyl-</u>	6343-76-6
<u>Formamidine, N-((dibenzylamino)thio)-N'-(2,4-xylyl)-</u>	73839-60-8
<u>Formamidine, N,N'-dicarbamoyl-</u>	6289-14-1
<u>FO-439</u>	6289-14-1
<u>Follicular fluid, porcine, retentate</u> <u>SUBSTANCE DEFINITION :The porcine follicular fluid preparation has a</u> <u>molecular weight greater than10,000 and is charcoal treated</u> <u>REFERENCE :CCPTAY Contraception, (Geron-X,</u> <u>Inc., POB 1108, Los Altos, CA 94022) V.1-1970-</u>	6289-14-1

Chemcas Corporation  
 10000  
 10000  
 10000

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 04/13/2007  
Date Updated: 02/01/2006  
Version 1.4

Section 1 - Product and Company Information

Product Name GONADOTROPIN FROM PREGNANT MARES' SERUM  
Product Number G4877  
Brand SIGMA  
Company Sigma-Aldrich Canada, Ltd  
Address 2149 Winston Park Drive  
Oakville ON L6H 6J8 CA  
Technical Phone: 9058299500  
Fax: 9058299292  
Emergency Phone: 800-424-9300

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
GONADOTROPIN FROM PREGNANT MARE SERUM	9002-70-4	No

Synonyms Anteron \* Antex-490 \* Antostab \* Eleagol \* Equine cyonin \* Equine gonadotrophin \* Equine gonadotropin \* Gestyl \* Gonadotraphon FSH \* Gonadyl \* Gorman \* Lobulantina \* PMS \* PMSG \* Predalon-S \* Priatin \* Primantron \* Seragon \* Seragonin \* Serogan \* Serotropin \* Serum gonadotrophin \* Serum gonadotropic hormone \* Serum gonadotropin  
RTECS Number: TU4517000

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Toxic.  
Toxic if swallowed.  
Target organ(s): Reproductive system.

HMIS RATING

HEALTH: 2\*  
FLAMMABILITY: 0  
REACTIVITY: 0

NFPA RATING

HEALTH: 2  
FLAMMABILITY: 0  
REACTIVITY: 0

\*additional chronic hazards present.

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE

If swallowed, wash out mouth with water provided person is conscious. Call a physician immediately.

#### INHALATION EXPOSURE

If inhaled, remove to fresh air. If not breathing give artificial respiration. If breathing is difficult, give oxygen.

#### DERMAL EXPOSURE

In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. Call a physician.

#### EYE EXPOSURE

In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

---

### Section 5 - Fire Fighting Measures

---

#### FLASH POINT

N/A

#### AUTOIGNITION TEMP

N/A

#### FLAMMABILITY

N/A

#### EXTINGUISHING MEDIA

Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

#### FIREFIGHTING

Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.  
Specific Hazard(s): Emits toxic fumes under fire conditions.

---

### Section 6 - Accidental Release Measures

---

#### PROCEDURE TO BE FOLLOWED IN CASE OF LEAK OR SPILL

Evacuate area.

#### PROCEDURE(S) OF PERSONAL PRECAUTION(S)

Wear self-contained breathing apparatus, rubber boots, and heavy rubber gloves.

#### METHODS FOR CLEANING UP

Sweep up, place in a bag and hold for waste disposal. Avoid raising dust. Ventilate area and wash spill site after material pickup is complete.

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### Section 7 - Handling and Storage

---

#### HANDLING

User Exposure: Do not breathe dust. Do not get in eyes, on skin, on clothing. Avoid prolonged or repeated exposure.

#### STORAGE

Suitable: Keep tightly closed.  
Store at -20°C

---

### Section 8 - Exposure Controls / PPE

---

## ENGINEERING CONTROLS

Use only in a chemical fume hood. Safety shower and eye bath.

## PERSONAL PROTECTIVE EQUIPMENT

Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator.

Hand: Compatible chemical-resistant gloves.

Eye: Chemical safety goggles.

## GENERAL HYGIENE MEASURES

Wash contaminated clothing before reuse. Wash thoroughly after handling.

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## Section 9 - Physical/Chemical Properties

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Appearance	Color: White Form: Powder	
Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
SG/Density	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	Solubility in Water: 1 mg/ml H <sub>2</sub> O clear, very faintly yellow	

N/A = not available

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## Section 10 - Stability and Reactivity

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### STABILITY

Stable: Stable.

Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS

Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide.

HAZARDOUS POLYMERIZATION

Hazardous Polymerization: Will not occur

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Section 11 - Toxicological Information

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ROUTE OF EXPOSURE

Skin Contact: May cause skin irritation.  
Skin Absorption: Toxic if absorbed through skin.  
Eye Contact: May cause eye irritation.  
Inhalation: Material may be irritating to mucous membranes and upper respiratory tract. Toxic if inhaled.  
Ingestion: Toxic if swallowed.

TARGET ORGAN(S) OR SYSTEM(S)

Reproductive system.

SIGNS AND SYMPTOMS OF EXPOSURE

Exposure can cause sensitivity reactions, abdominal discomfort, ovarian rupture, and intraperitoneal hemorrhage.

TOXICITY DATA

Oral  
Mouse  
120 mg/kg  
LD50

CHRONIC EXPOSURE - TERATOGEN

Species: Rat Rat  
Dose: 11700 UG/KG 11700 UG/KG  
Route of Application: Intraperitoneal Intraperitoneal  
Exposure Time: (5-7D PREG) (5-7D PREG)  
Result: Effects on Embryo or Fetus: Fetal death. Effects on Embryo or Fetus: Fetal death.

Species: Rat Rat  
Dose: 10 MG/KG 10 MG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (1D PRE) (1D PRE)  
Result: Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus). Effects on Embryo or Fetus: Extra embryonic structures (e.g., placenta, umbilical cord). Effects on Embryo or Fetus: Extra embryonic structures (e.g., placenta, umbilical cord). Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus).

Species: Mouse Mouse  
Dose: 1 GM/KG 1 GM/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (1D PRE) (1D PRE)  
Result: Specific Developmental Abnormalities: Craniofacial (including nose and tongue). Effects on Embryo or Fetus: Fetal death. Specific Developmental Abnormalities: Musculoskeletal system. Effects on Embryo or Fetus: Fetal death. Specific Developmental Abnormalities: Craniofacial (including nose and tongue). Specific Developmental Abnormalities: Musculoskeletal system.

Species: Hamster Hamster  
Dose: 37500 UG/KG 37500 UG/KG  
Route of Application: Intraperitoneal Intraperitoneal  
Exposure Time: (4-6D PREG) (4-6D PREG)  
Result: Effects on Embryo or Fetus: Fetal death. Effects on Embryo or Fetus: Fetal death.

#### CHRONIC EXPOSURE - MUTAGEN

Species: Mouse Mouse  
Route: Intraperitoneal Intraperitoneal  
Dose: 750 UG/KG 750 UG/KG  
Mutation test: Cytogenetic analysis Cytogenetic analysis

#### CHRONIC EXPOSURE - REPRODUCTIVE HAZARD

Result: Overexposure may cause reproductive disorder(s) based on tests with laboratory animals.

Species: Rat Rat  
Dose: 188 MG/KG 188 MG/KG  
Route of Application: Intraperitoneal Intraperitoneal  
Exposure Time: (1-3D PREG) (1-3D PREG)  
Result: Effects on Fertility: Other measures of fertility  
Effects on Fertility: Other measures of fertility

Species: Rat Rat  
Dose: 188 MG/KG 188 MG/KG  
Route of Application: Intraperitoneal Intraperitoneal  
Exposure Time: (5-7D PREG) (5-7D PREG)  
Result: Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea). Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).

Species: Rat Rat  
Dose: 18750 UG/KG 18750 UG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (1D PRE) (1D PRE)  
Result: Maternal Effects: Parturition. Maternal Effects: Parturition.

Species: Rat Rat  
Dose: 37500 UG/KG 37500 UG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (18D PREG) (18D PREG)  
Result: Effects on Fertility: Abortion. Effects on Fertility: Abortion.

Species: Rat Rat  
Dose: 12500 UG/KG 12500 UG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (5D PREG) (5D PREG)  
Result: Effects on Fertility: Other measures of fertility  
Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants). Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants). Effects on Fertility: Other measures of fertility

Species: Rat Rat

Dose: 50 MG/KG 50 MG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (1D PRE) (1D PRE)  
Result: Maternal Effects: Ovaries, fallopian tubes. Maternal  
Effects: Uterus, cervix, vagina. Maternal Effects: Uterus,  
cervix, vagina. Maternal Effects: Ovaries, fallopian tubes.

Species: Rat Rat  
Dose: 12500 UG/KG 12500 UG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (2D PREG) (2D PREG)  
Result: Effects on Fertility: Female fertility index (e.g., #  
females pregnant per # sperm positive females; # females  
pregnant per # females mated ). Effects on Fertility: Female  
fertility index (e.g., # females pregnant per # sperm positive  
females; # females pregnant per # females mated ).

Species: Rat Rat  
Dose: 453 MG/KG 453 MG/KG  
Route of Application: Intramuscular Intramuscular  
Exposure Time: (7D PRE) (7D PRE)  
Result: Maternal Effects: Uterus, cervix, vagina. Maternal  
Effects: Ovaries, fallopian tubes. Maternal Effects: Uterus,  
cervix, vagina. Maternal Effects: Ovaries, fallopian tubes.

Species: Rat Rat  
Dose: 1120 IU/KG 1120 IU/KG  
Route of Application: Unreported Unreported  
Exposure Time: (2W MALE) (2W MALE)  
Result: Paternal Effects: Testes, epididymis, sperm duct.  
Paternal Effects: Testes, epididymis, sperm duct.

Species: Mouse Mouse  
Dose: 10 MG/KG 10 MG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (2D PREG) (2D PREG)  
Result: Effects on Fertility: Female fertility index (e.g., #  
females pregnant per # sperm positive females; # females  
pregnant per # females mated ). Effects on Fertility: Female  
fertility index (e.g., # females pregnant per # sperm positive  
females; # females pregnant per # females mated ).

Species: Mouse Mouse  
Dose: 50 MG/KG 50 MG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (8-11D PREG) (8-11D PREG)  
Result: Effects on Fertility: Post-implantation mortality (e.g.,  
dead and/or resorbed implants per total number of implants).  
Effects on Fertility: Abortion. Effects on Fertility:  
Post-implantation mortality (e.g., dead and/or resorbed implants  
per total number of implants). Effects on Fertility: Abortion.

Species: Mouse Mouse  
Dose: 50 MG/KG 50 MG/KG  
Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (12-15D PREG) (12-15D PREG)  
Result: Maternal Effects: Parturition. Maternal Effects:  
Postpartum. Maternal Effects: Postpartum. Maternal Effects:  
Parturition.

Species: Mouse Mouse  
Dose: 50 MG/KG 50 MG/KG

Route of Application: Subcutaneous Subcutaneous  
Exposure Time: (3D PREG) (3D PREG)  
Result: Effects on Fertility: Pre-implantation mortality (e.g.,  
reduction in number of implants per female; total number of  
implants per corpora lutea). Effects on Fertility:  
Pre-implantation mortality (e.g., reduction in number of  
implants per female; total number of implants per corpora lutea).

Species: Hamster Hamster  
Dose: 300 MG/KG 300 MG/KG  
Route of Application: Intraperitoneal Intraperitoneal  
Exposure Time: (1-3D PREG) (1-3D PREG)  
Result: Effects on Fertility: Pre-implantation mortality (e.g.,  
reduction in number of implants per female; total number of  
implants per corpora lutea). Effects on Fertility:  
Pre-implantation mortality (e.g., reduction in number of  
implants per female; total number of implants per corpora lutea).

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## Section 12 - Ecological Information

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## Section 13 - Disposal Considerations

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### APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION

Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

---

## Section 14 - Transport Information

---

### DOT

Proper Shipping Name: Toxic solids, organic, n.o.s.  
UN#: 2811  
Class: 6.1  
Packing Group: Packing Group III  
Hazard Label: Toxic substances.  
PIH: Not PIH

### IATA

Proper Shipping Name: Toxic solid, organic, n.o.s.  
IATA UN Number: 2811  
Hazard Class: 6.1  
Packing Group: III

---

## Section 15 - Regulatory Information

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### EU ADDITIONAL CLASSIFICATION

Symbol of Danger: T  
Indication of Danger: Toxic.  
R: 25  
Risk Statements: Toxic if swallowed.  
S: 45  
Safety Statements: In case of accident or if you feel unwell,  
seek medical advice immediately (show the label where possible).

### US CLASSIFICATION AND LABEL TEXT

Indication of Danger: Toxic.  
Risk Statements: Toxic if swallowed.  
Safety Statements: In case of accident or if you feel unwell,  
seek medical advice immediately (show the label where possible).

US Statements: Target organ(s): Reproductive system.

UNITED STATES REGULATORY INFORMATION

SARA LISTED: No

CANADA REGULATORY INFORMATION

WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.

DSL: No

NDSL: No

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Section 16 - Other Information

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DISCLAIMER

For R&D use only. Not for drug, household or other uses.

WARRANTY

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale. Copyright 2007 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only.

**1. PRODUCT AND COMPANY IDENTIFICATION**

Product name : **beta -Estradiol**  
 Product Number : E8875  
 Brand : Sigma  
 Company : Sigma-Aldrich Canada, Ltd  
 2149 Winston Park Drive  
 OAKVILLE ON L6H 6J8  
 CANADA  
 Telephone : +1 9058299500  
 Fax : +1 9058299292  
 Emergency Phone # : 800-424-9300

**2. COMPOSITION/INFORMATION ON INGREDIENTS**

Synonyms : 1,3,5-Estratriene-3,17beta -diol  
 17beta -Estradiol  
 3,17beta -Dihydroxy-1,3,5(10)-estratriene  
 Dihydrofolliculin  
 Formula : C18H24O2  
 Molecular Weight : 272.39 g/mol

CAS-No.	EC-No.	Index-No.	Concentration [%]
<b>Estradiol</b>			
50-28-2	200-023-8	-	-

**3. HAZARDS IDENTIFICATION**

**Emergency Overview**

**Target Organs**

Female reproductive system., Male reproductive system.

**WHMIS Classification**

Not Rated

Not Rated

**HMIS Classification**

Health Hazard: 0

Chronic Health Hazard: \*

Flammability: 0

Physical hazards: 0

**Potential Health Effects**

**Inhalation**

May be harmful if inhaled. May cause respiratory tract irritation.

**Skin**

May be harmful if absorbed through skin. May cause skin irritation.

**Eyes**  
**Ingestion**

May cause eye irritation.  
May be harmful if swallowed.

#### 4. FIRST AID MEASURES

**General advice**

Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

**If inhaled**

If breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

**In case of skin contact**

Wash off with soap and plenty of water. Consult a physician.

**In case of eye contact**

Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

**If swallowed**

Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

#### 5. FIRE-FIGHTING MEASURES

**Flammable properties**

Flash point no data available

Ignition temperature no data available

**Suitable extinguishing media**

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

**Special protective equipment for fire-fighters**

Wear self contained breathing apparatus for fire fighting if necessary.

#### 6. ACCIDENTAL RELEASE MEASURES

**Personal precautions**

Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

**Environmental precautions**

Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

**Methods for cleaning up**

Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

#### 7. HANDLING AND STORAGE

**Handling**

Avoid exposure - obtain special instructions before use. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

**Storage**

Keep container tightly closed in a dry and well-ventilated place.

## 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

### Personal protective equipment

#### Respiratory protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N99 (US) or type P2 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

#### Hand protection

Handle with gloves.

#### Eye protection

Safety glasses

#### Skin and body protection

Choose body protection according to the amount and concentration of the dangerous substance at the work place.

#### Hygiene measures

Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

## 9. PHYSICAL AND CHEMICAL PROPERTIES

### Appearance

Form powder

Colour white

### Safety data

pH no data available

Melting point 176 °C (349 °F)

Boiling point no data available

Flash point no data available

Ignition temperature no data available

Lower explosion limit no data available

Upper explosion limit no data available

Water solubility no data available

## 10. STABILITY AND REACTIVITY

### Storage stability

Stable under recommended storage conditions.

### Materials to avoid

Strong oxidizing agents

### Hazardous decomposition products

**Hazardous decomposition products formed under fire conditions.**

Carbon oxides

## 11. TOXICOLOGICAL INFORMATION

### Acute toxicity

no data available

### Irritation and corrosion

no data available

### Sensitisation

no data available

### Chronic exposure

The National Toxicology Program (Tenth Report on Carcinogens) has determined that steroidal estrogens are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans, which indicates a causal relationship between exposure to steroidal estrogens and human cancer. This is or contains a component that has been reported to be carcinogenic based on its IARC, OSHA, ACGIH, NTP, or EPA classification.

Genotoxicity in vitro - rat - Other cell types  
DNA damage

Genotoxicity in vivo - rat - Oral  
Morphological transformation.

May cause reproductive disorders.

### Signs and Symptoms of Exposure

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

### Potential Health Effects

<b>Inhalation</b>	May be harmful if inhaled. May cause respiratory tract irritation.
<b>Skin</b>	May be harmful if absorbed through skin. May cause skin irritation.
<b>Eyes</b>	May cause eye irritation.
<b>Ingestion</b>	May be harmful if swallowed.
<b>Target Organs</b>	Female reproductive system., Male reproductive system.,

## 12. ECOLOGICAL INFORMATION

### Elimination information (persistence and degradability)

no data available

### Ecotoxicity effects

Toxicity to fish            LC50 - *Oryzias latipes* - 0.002 µg/l - 96 h

### Further information on ecology

no data available

## 13. DISPOSAL CONSIDERATIONS

### Product

Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

### Contaminated packaging

Dispose of as unused product.

**14. TRANSPORT INFORMATION**

**DOT (US)**

Not dangerous goods

**IMDG**

Not dangerous goods

**IATA**

Not dangerous goods

**15. REGULATORY INFORMATION**

**TSCA Status**

Not On TSCA Inventory

Estradiol

CAS-No.

50-28-2

**DSL Status**

All components of this product are on the Canadian DSL list.

**WHMIS Classification**

Not Rated

Not Rated

**16. OTHER INFORMATION**

**Further information**

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## Cell Line Designation: **MCF-7** ATCC® Catalog No. **HTB-22™**

### Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

### Cell Line Description

**Organism:** *Homo sapiens* (human)

**Tissue:** mammary gland; breast adenocarcinoma; derived from metastatic site: pleural effusion

**Age:** 69 years

**Gender:** female

**Ethnicity:** Caucasian

**Morphology:** epithelial

**Doubling time:** about 29 hours

**Growth Properties:** adherent

**Oncogene:** wnt7h +

**Antigens Expressed:** Blood Type O; Rh+

**Products:** insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5

**DNA profile** (STR analysis)

Amelogenin: X  
CSF1PO: 10  
D13S317: 11  
D16S539: 11,12  
D5S818: 11,12  
D7S820: 8,9  
TH01: 6  
TPOX: 9,12  
vWA: 14,15

**Depositor:** C.M. McGrath

**Comments:** The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.

Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.

**Karyology:** modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases.

No DM were detected. Chromosome 20 was nullisomic and X was disomic.

**Note:** Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

**Purified DNA** from this line is available as ATCC® HTB-22D™ (10µg)

**Total RNA** from this line is available as ATCC® HTB-22R™ (100µg)

### Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at [www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm).

### Use Restrictions

**These cells are distributed for research purposes only.** ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

### Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

**SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.**

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at



## Product Information Sheet for ATCC® HTB-22™

approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.

4. Transfer the cell pellet to an appropriate size vessel. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

**Note:** Present batches of MCF7 cells are exhibiting the following growth pattern:

The cells usually attach as three-dimensional clusters and eventually grow to a 80-90% confluent monolayer. However, we are finding that most of the clusters remain in suspension until after the 2nd subculture.

After first subculture all the cells will not attach. There will be clusters in suspension. Break up the clusters the best you can by gently pipetting with a small bore pipette (5 ml or smaller). After a few days incubation, the cells should reattach as three-dimensional islands (there will be some clusters that do not reattach). Growth will eventually spread out from the islands and the culture should, after the second subculture, flatten and become 70-80% confluent.

### Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO<sub>2</sub> in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm<sup>2</sup> flask. Incubate at 37°C in a 5% CO<sub>2</sub> in air atmosphere until cells are ready to be subcultured.

### Subculturing Procedure

Volumes used in this protocol are for 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

**Note:** if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum, which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually with 5 to 10 minutes).

**Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from Step #1 and spin at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Maintain cultures at a cell concentration between 2x10<sup>4</sup> and 2 x 10<sup>5</sup> cells/cm<sup>2</sup>.  
**Subcultivation Ratio:** 1:3 to 1:6.
7. Place culture vessels in incubators at 37°C.

**Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

### Medium Renewal

Two to three times weekly

### Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium:

- 0.01mg/ml bovine insulin
- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO<sub>2</sub> in air atmosphere.



## Product Information Sheet for ATCC<sup>®</sup> HTB-22<sup>™</sup>

ATCC tested fetal bovine serum is available as ATCC<sup>®</sup> Catalog No. 30-2020 (500ml) or ATCC<sup>®</sup> Catalog No. 30-2021 (100ml).

### Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.  
Cell culture tested DMSO is available as ATCC<sup>®</sup> Catalog No. 4-X.

### Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at [www.atcc.org](http://www.atcc.org), or by e-mail at [tech@atcc.org](mailto:tech@atcc.org).

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(additional references may be available in the catalog at [www.atcc.org](http://www.atcc.org))

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**Cell Line Designation: 293 (HEK293)****ATCC® Catalog No. CRL-1573™****Table of Contents:**

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

**Cell Line Description****Organism:** *Homo sapiens* (human)**Tissue:** kidney; transformed with adenovirus 5 DNA**Age:** fetus**Morphology:** epithelial**Growth properties:** adherent**Doubling time:** about 19 hours**Tumorigenic:** tumors developed within 21 days at 100% frequency (5/5) in nude mice inoculated subcutaneously with 10(7) cells.**Receptors expressed:** vitronectin**Virus susceptibility:** human adenoviruses**DNA profile (STR analysis)**

Amelogenin: X  
 CSF1PO: 11,12  
 D13S317: 12,14  
 D16S539: 9,13  
 D5S818: 8,9  
 D7S820: 11,12  
 TH01: 7,9.3  
 TPOX: 11  
 vWA: 16,19

**Depositors:** F.L. Graham**Comments:** Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome, it is now clear that only left end sequences are present. The line is excellent for titrating human adenoviruses.

The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached. The cells will re-attach to the flask over a period of several days in culture at 37°C.

The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit.

The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2).

**Karyotype:** This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %.

The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired.

There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

**Note:** Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

**Purified DNA:** from this line is available as ATCC Catalog No. CRL-1573D™ (10 µg).

**Biosafety Level: 2**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at [www.cdc.gov/od/ohs/biosfty/bml44/bml44toc.htm](http://www.cdc.gov/od/ohs/biosfty/bml44/bml44toc.htm).

**Use Restrictions**

**These cells are distributed for research purposes only.** 293 cells, their products, or their derivatives may not be distributed to third parties. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement.

**Handling Procedure for Frozen Cells**

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

**SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials.**

*It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this*

*point on should be carried out under strict aseptic conditions.*

- Transfer the vial contents to a centrifuge tube containing 9.0 ml complete growth medium and spin at approximately 125 xg for 5 to 7 minutes.
- Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm<sup>2</sup> or a 75 cm<sup>2</sup> culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*
- Incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

### Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

**The cell line does not adhere to the substrate when left at room temperature for any length of time**, therefore, live cultures may be received with the cells detached. The cells will re-attach to the flask over a period of several days in culture at 37°C.

- Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
- If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO<sub>2</sub> in air atmosphere until they are ready to be subcultured.
- If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm<sup>2</sup> flask. Incubate at 37°C in a 5% CO<sub>2</sub> in air atmosphere until cells are ready to be subcultured.

### Subculturing Procedure

Volumes used in this protocol are for 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

- Remove and discard culture medium.
- Add 2.0 to 3.0 ml of 0.25% (w/v) Trypsin-0.53mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 10 minutes).

**Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

- Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
- Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 2 X 10<sup>3</sup> to 6 X 10<sup>3</sup> viable cells/cm<sup>2</sup> is recommended.  
**Subcultivation Ratio;** 1:10 to 1:20 weekly.
- Incubate cultures at 37°C.
- Subculture when cell concentration is between 6 and 7 X 10<sup>4</sup> cells/cm<sup>2</sup>

**Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

### Medium Renewal

Two to three times weekly.

### Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO<sub>2</sub> in air atmosphere.

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 (500ml) and ATCC Catalog No. 30-2021 (100ml).

### Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

### Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at [www.atcc.org](http://www.atcc.org), or by e-mail at [tech@atcc.org](mailto:tech@atcc.org).

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(additional references may be available in the catalog description at [www.atcc.org](http://www.atcc.org))

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RCB1154 : <b>KGN</b>	
Comment	Granulosa cell tumor. Possible to produce progesterone after HCG addition. Cell growth is slow.
Animal	human
Sex	Female
Age	69 years
Tissue	ovary, peritoneum
Morphology	other
Anchored	Yes
Medium	(DMEM:HamF12=1:1)+10%FBS
Antibiotics	Free
Growth temp	37°C
CO <sub>2</sub> concentration	5%
Passage method	0.25% trypsin
Split ratio	1:8 split
Subculture frequency	once/2 weeks
Cloned	No
Lifespan	infinite
Mycoplasma	-
Virus	HIV-1(-)
Isozyme analysis	LD, NP
Chr distrib	43-46(50):43(1),44(1),45(45),46(3)
Originator	Nishi, Yoshihiro & Nawata, Hajime
Depositor	Nishi, Y. & Nawata, H.
Approver	Yanase, Toshihiko  <a href="#">Other resources</a>
Restriction	A prior written permission of the depositor is necessary for any kind of use including academic use and for-profit use. <span style="float: right;">b</span>
User's Publication	3293

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## Cell Line Designation: **PANC-1** ATCC<sup>®</sup> Catalog No. CRL-1469

### Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

### Cell Line Description

**Organism:** *Homo sapiens* (human)

**Tissue:** pancreas; duct; epithelioid carcinoma

**Age:** 56 years

**Gender:** male

**Ethnicity:** caucasian

**Morphology:** epithelial

**Growth properties:** adherent

**DNA profile (STR analysis)**

Amelogenin: X

CSFIPO: 10,12

D13S317: 11

D16S539: 11

D5S818: 11,13

D7S820: 8,10

TH01: 7,8

TPOX: 8,11

vWA: 15

**Depositors:** M. Lieber

**Comments:** Growth is inhibited by 1 unit/ml L-asparaginase.

The cells will grow in soft agar.

**Karyotype:** Chromosome studies indicate a modal number of 63 with 3 distinct marker chromosomes and a small ring chromosome. This is a hypertriploid human cell line. The modal chromosome number was 61, occurring in 32% of cells. However, cells with 63 chromosomes also occurred at a high frequency (22%). The rate of cells with higher ploidies was 8.5%.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

### Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. ATCC recommends that appropriate safety procedures be used when

handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al., 1995) the ATCC manual on quality control (Hay et al., 1992), the *Journal of Tissue Culture Methods* (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories**, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at [www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm)

### Use Restrictions

**These cells are distributed for research purposes only.** ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

### Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

**SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.**

1. Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125  $\times$ g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25  $\text{cm}^2$  or a 75  $\text{cm}^2$  culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

**Handling Procedure for Flask Cultures**

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO<sub>2</sub> in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm<sup>2</sup> flask. Incubate at 37°C in a 5% CO<sub>2</sub> in air atmosphere until cells are ready to be subcultured.

**Subculturing Procedure**

Volumes used in this protocol are for 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-053mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).  
**Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.  
**Subcultivation Ratio:** 1:2 to 1:4.
6. Incubate cultures at 37°C.

**Note:** For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in *Culture of*

*Animal Cells, a manual of Basic Technique* by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

**Medium Renewal**

2 to 3 times weekly.

**Complete Growth Medium**

The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002.

To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO<sub>2</sub> in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO<sub>2</sub> in air atmosphere is then recommended).

ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

**Cryoprotectant Medium**

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

**Additional Information**

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at [www.atcc.org](http://www.atcc.org), or by e-mail at [tech@atcc.org](mailto:tech@atcc.org).

**References**

(additional references are available in the catalog at [www.atcc.org](http://www.atcc.org))

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6FSHR-17

Rapid Paper

Establishment of steroidogenic granulosa cell lines expressing follicle stimulating hormone receptors

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(Received 7 June 1993; accepted 18 June 1993)

**Key words:** Follicle stimulating hormone; Ovary; Progesterone, Ha-ras oncogene; Immortalized cell line

**Summary**

Follicle stimulating hormone (FSH) plays an important role in the regulation of oogenesis, spermatogenesis and production of steroid hormones. Receptors to FSH, which are uniquely expressed in ovarian granulosa and testicular Sertoli cells, are rapidly lost in tissue culture conditions and upon cell transformation. We have succeeded, by triple transfection of primary rat granulosa cells with SV40 DNA, Ha-ras oncogene and an FSH receptor expression plasmid, to establish stable steroidogenic cell lines expressing FSH receptors. The cell lines respond to rat, ovine and bovine FSH, which stimulate progesterone production at levels comparable to primary granulosa cells obtained from preovulatory follicles. No steroidogenic response is detected upon stimulation with ovine luteinizing hormone or human chorionic gonadotropin. The steroidogenic response is accompanied by de novo appearance of adrenodoxin which serves as a marker for the mitochondrial steroidogenic enzyme system. These cells express approximately 27,000 receptors per cell with a  $K_d$  of 100–115 pM. This  $K_d$  is close to the value calculated for the native receptor. The  $ED_{50}$  for the steroidogenic response to ovine FSH is 200 pM, suggesting a tight coupling between receptor activation and the steroidogenic response. FSH induces pronounced morphological changes in the established cell lines, which are also characteristic of primary granulosa cells. These FSH responsive cell lines can serve as a useful model for the study of the structure and function of the FSH receptor and the effect of oncogenes on its expression.

**Introduction**

The gonadotropic glycoprotein hormones play a major role in controlling ovarian function. Follicle-stimulating hormone (FSH) stimulates the development of ovarian follicles and steroidogenesis, while luteinizing hormone (LH) initiates ovulation and luteinization (see reviews by Hsueh et al., 1984; Amsterdam and Rotmensch, 1987; Knobil and Hotchkiss, 1988). These two hormones, which are released from the anterior pituitary, exert their effects on ovarian cells via binding to specific membrane glycoprotein receptors, containing 7 transmembrane domains characteristic of G protein-coupled receptors, which exert their major effect by activation of the hormone stimulated adenylate cyclase

(Johnson and Dhanasekaran, 1989; Loosfelt et al., 1989; McFarland et al., 1989; Sprengel et al., 1990). Interestingly, the gonadotropin receptors differ from other G protein-coupled receptors since they contain a large extracellular domain displaying amino terminal leucine rich sequence repeats which appear to form amphipathic peptide surfaces involved in protein-protein interaction. While the extracellular N-terminal region of the receptor is the site of high-affinity gonadotropin binding, low-affinity interactions with the ligand pocket formed by the seven transmembrane domains (assumed to occur following a conformational change in the receptor) is important for receptor activation and hence for G protein-coupled signal transduction (e.g. Braun et al., 1991).

The FSH receptor molecule (FSHR) and the gene coding for the receptor molecule are closely related to the LH receptor gene and its product, the LHR. These receptors display a 50% amino acid sequence similarity

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in their large extracellular domains and 80% identity across the seven trans-membrane segments (McFarland et al., 1989; Sprengel et al., 1990; Heckert et al., 1992). Nevertheless they are different in the size of the genes and the number of exons as well as in their polypeptide chain and in the glycosylation of the extracellular domain (McFarland et al., 1989; Sprengel et al., 1990; Heckert et al., 1992; Tilly et al., 1992). Moreover, FSH receptors are expressed at early stages of follicular development before the expression of LH receptors. Stimulation by FSH leads to the expression of LH receptors (Hsueh et al., 1984; Nimrod et al., 1977; Erickson et al., 1979).

In spite of the key role that FSHR plays in regulating the development and maturation of the ovarian follicle and thus controlling the reproductive cycle (for review see Hsueh et al., 1984; Amsterdam and Rotmensch, 1987; Richards and Hedin, 1988), very little is known about the signal transduction mechanisms by which FSH exerts its effects on growth and differentiation of granulosa cells *in vivo*.

Numerous efforts to study the mechanism of FSH action in primary cultures of granulosa cells revealed that although the major signal transduction mechanism of FSH is activation of the hormone sensitive adenylate cyclase (for example see Erickson et al., 1979; Richards and Hedin, 1988; Amsterdam et al., 1981; Knecht et al., 1981; Adashi and Resnick, 1984), other alternative mechanisms, such as mobilization of intracellular calcium may mediate FSH action (Flores et al., 1990, 1992; Grasso and Reichert, 1990; Grasso et al., 1991). Protein kinase C (PKC) was also implicated in modulation of FSH stimulation of adenylate cyclase (Nikula et al., 1990). Synergistic effects of FSH with steroid hormones, glucocorticoids and peptide growth factors were also observed in cultured granulosa cells, related to the effect of FSH on both growth and differentiation of these cells (Adashi et al., 1981; Amsterdam et al., 1988b). Nevertheless, the biochemical basis for such cross-talk between different signal transduction pathways has not yet been fully characterized.

The expression of FSHR is extremely sensitive to *in vitro* conditions and its life span in primary cultures is limited to a short period of 1-2 days. In fact, there are no stable steroidogenic cell lines derived from primary granulosa cells that express FSH receptor molecules. We have succeeded recently in establishing steroidogenic granulosa cell lines by cotransfection of primary cells with Simian virus 40 (SV40) DNA and Harvey-*ras* (*Ha-ras*) oncogene. These cells preserved their cAMP response to forskolin, but unfortunately lost their response to both FSH and LH (Amsterdam et al., 1984; Suh and Amsterdam, 1990). The establishment of the steroidogenic granulosa cell lines by oncogene transfection (Amsterdam et al., 1988a; Suh and Amsterdam, 1990), and the cloning of the gene coding for the

FSHR (Sprengel et al., 1990; Minegishi et al., 1991; Tilly et al., 1992) have opened the possibility of establishing steroidogenic cell lines which will be able to express the FSHR in transfected cells.

In this work we report that cotransfection of primary granulosa cells obtained from preovulatory follicles, by SV40 DNA, *Ha-ras* oncogene and an FSHR expression plasmid, yields cell lines which constitutively express FSHR. Moreover, in these cell lines, FSH can efficiently stimulate progesterone production at levels comparable to primary cells stimulated by gonadotropic hormone, and thus can serve as a useful model for the study of the molecular and the cellular events initiated by the interaction of FSH with its specific receptor.

## Materials and methods

**Reagents.** Highly purified ovine (o)FSH for iodination (NIDDK-oFSH-I-1), and for cell stimulation (NIAMDD-oFSH-14), bovine (b) FSH (NIH-FSH-B1), rat (r) FSH (NIAMD-rFSH-B-1) and ovine LH (NIAMDD-oLH-23) were kindly provided by the National Institutes of Health, Bethesda, MD. Highly purified hCG-CR-123 was kindly provided by Dr. E. Canfield of Columbia University, NY. Anti-adrenodoxin antibody and anti-progesterone antibody were kindly provided by Drs. I. Hanukoglu and F. Kohen of the Department of Hormone Research, The Weizmann Institute of Science.

**Plasmids.** pSVBam contains the entire SV40 genome (Michalovitz et al., 1986). PEJ.6.6 encodes activated human *Ha-ras* oncogene (Shih and Weinberg, 1982). pSV-FSHR encodes the complete coding sequence for rat FSHR. For the construction of the SV40 promoter based expression plasmid pSV-FSHR, the cytomegalovirus promoter/enhancer region (approx. 700 bp) of pRFHSR-3 (Braun et al., 1991) was replaced by the SV40 promoter/enhancer region (approx. 400 bp) of pSV2neo (Clontech). The expression plasmid pSV-FSHR thus contains the entire coding region of the testicular rat FSHR cDNA as an *EcoRI/BamHI* restriction fragment (nucleotides -71 to 2095) (Sprengel et al., 1990) downstream to the cytomegalovirus promoter/enhancer region of expression plasmid pRK5 (Schall et al., 1990).

**Transfection of granulosa cells.** Granulosa cells were isolated from 25-day old immature female rats, 48 h after injection with 15 IU pregnant mare serum gonadotropin (PMSG). The procedure for preparation of the cells has been described (Amsterdam et al., 1979).

Primary cultures on 100 mm dishes were transfected simultaneously with 2  $\mu$ g of pSVBam, 5  $\mu$ g pEJ.6.6 and 5  $\mu$ g of pSV-FSHR using the calcium phosphate precipitation procedure (Van der Eb and Graham, 1980).

Densely growing foci of transformed cells were visualized, selected and expanded into cell lines. The detailed procedures have been described previously (Amsterdam et al., 1988a; Suh and Amsterdam, 1990). Initial screening for lines expressing a functional FSHR was performed by stimulating the cells with FSH for 3 h and screening for cell rounding which occurs as a consequence of elevation of intracellular cAMP (Amsterdam et al., 1981; Knecht et al., 1981). The FSH responsive cell lines (named GFSHR lines) were further characterized by  $^{125}\text{I}$ -FSH binding and cAMP and steroidogenic responses to FSH.

**Cell cultures.** Cells were maintained on Nunc Petri dishes (100 mm) containing 8 ml DMEM/F12 medium (1:1) supplemented with penicillin (100 U/ml), streptomycin (100  $\mu\text{g}/\text{ml}$ ) and 5% fetal calf serum (FCS). For biochemical assays, cells were cultured in medium containing 5% FCS for 24 h on 35 mm Nunc Petri dishes ( $0.5 \times 10^6$  cells/dish) or on Nunc 24-well plates ( $0.25 \times 10^5$  cells/well), and the medium was replaced by serum-free medium containing 100 nM dexamethasone (Dex) and the appropriate stimulant. In some experiments, stimulation of cells was performed in the presence of 5% FCS, either with or without dexamethasone, as indicated.

**Steroid hormone, cAMP and protein assays.** Progesterone, accumulating in the culture medium of granulosa cells, was determined by radioimmunoassay (RIA) (Suh and Amsterdam, 1990; Amsterdam et al., 1979; Erlanger et al., 1959; Kohen et al., 1975). Antiserum for progesterone was a generous gift of Dr. F. Kohen of the Department of Hormone Research, the Weizmann Institute. cAMP in the medium and in cells was measured by a protein binding method (Amsterdam et al., 1988a; Amsterdam et al., 1979; Gilman, 1970). Cells were cultured in DMEM/F12 containing 0.1 mM 3-isobutyl-1-methylxanthine (IBMX). For measurement of intracellular cyclic adenosine 3'-5'-monophosphate (cAMP), cells were washed twice with phosphate-buffered saline, pH 7.3 (PBS), lysed with 1 ml of 50 mM sodium acetate (pH 4.5) containing 0.1 mM IBMX, harvested into Eppendorf tubes using a rubber policeman, and heated for 10 min at 90°C. The cell debris was precipitated by centrifugation for 5 min ( $12,000 \times g$ ), and cAMP in the supernatant was measured. Protein was determined by the Bradford method (Bradford, 1976). All measurements for each treatment were performed in triplicate.

**Iodination procedure.** Radioiodination of highly purified oFSH was carried out by the iodogen method (Fraker and Speck, 1978).  $\text{Na}^{125}\text{I}$  (0.5 mCi) was added to 10  $\mu\text{g}$  of FSH in an iodogen-coated tube and reacted for 3 min on ice. Radiolabeled FSH ( $^{125}\text{I}$ -FSH) was eluted from a Sephadex G-50 column by 1% bovine serum albumin (BSA) in PBS (BSA-PBS). 1 ml fractions were collected into plastic test tubes (10  $\times$  75

mm). The fraction containing most of the radiolabeled hormone ( $1.5\text{--}1.9 \times 10^8$  CPM) was used for the binding assay. Specific activity of the radiolabeled ligand ( $^{125}\text{I}$ -FSH) was between  $3 \times 10^4$  and  $4 \times 10^4$  cpm/ng protein.

**$^{125}\text{I}$ -oFSH binding assay.** FSHR expressed in GF-SHR-17 cells was measured by a displacement binding assay using radiolabeled ligand. For displacement binding, a fixed amount of  $^{125}\text{I}$ -FSH ( $2.5 \times 10^5$  cpm) were used for binding to  $2.5 \times 10^6$  cells in the presence of increasing concentrations of unlabeled FSH. Cells were removed from tissue culture plates by brief incubation (2 min) with calcium- and magnesium-free PBS containing 2 mM ethylene diamine tetra acetic acid (EDTA) pH 7.4. Medium was removed after 5 min centrifugation at  $600 \times g$  and cells were resuspended in 0.1% BSA-PBS. Samples of cells ( $2.5 \times 10^6$  in 50  $\mu\text{l}$ ) were lysed at room temperature by adding 250  $\mu\text{l}$  of 10 mM phosphate buffer, pH 7.4, containing 0.5% BSA and 0.5 mM phenylmethylsulfonylfluoride (PMSF) to inhibit any traces of proteolytic activity (lysis buffer) (Suh et al., 1992). Overnight incubation at 25°C of cell lysates with the hormone was carried out in a final volume of 0.30 ml, in Eppendorf tubes. Following incubation, bound and free ligand was separated by 5 min centrifugation in an Eppendorf centrifuge ( $12,000 \times g$ ). The pellets containing the cell membranes were washed three times by resuspension and recentrifugation with the lysis buffer. Radioactivity in the cell pellets was measured in a  $\gamma$ -counter (LKB 1277 GammaMaster). Non-specific binding was measured in the presence of 24 nM of unlabelled oFSH ( $\times 30\text{--}35$ -fold of the  $^{125}\text{I}$ -FSH) and amounts to 16% of total binding. This value was subtracted from the total binding to obtain specific binding.

**Phase contrast and immunofluorescence.** Cultures were fixed with 2.5% glutaraldehyde in PBS and photographed at  $400 \times$  magnification using a Zeiss photomicroscope III, equipped with a water immersion lens. For immunofluorescence studies, cells were grown and stimulated on square coverglasses ( $22 \times 22$  mm) in the presence of 5% FCS. The presence of the serum was essential to avoid detachment of the cells during the fixation, staining and rinsing of the cultures. The cells were fixed and reacted first with an anti-adrenodoxin antibody and then with rhodamine-labeled second antibody (Hanukoglu et al., 1990). The specimens were visualized using a Zeiss photomicroscope III equipped with a vertical fluorescence illuminator.

**Statistical analysis.** Analysis of progesterone, cAMP and number of cells was performed using the *t*-test for comparison of means. Differences between treatment groups were considered statistically significant at  $P < 0.05$ .

Data in Figs. 2-6 were from representative experiments which were performed at least twice in different

cell preparations; *n* refers to replicates in individual experiments.

## Results

Cotransfection of primary cultures of rat granulosa cells with SV40, Ha-*ras* and pSVFSHR yielded 57 distinct foci of growth that were expanded into cell lines, as described in Materials and Methods. The lines were tested for their response to 1.6 nM oFSH by their shape changes (rounding up) and production of progesterone during 48 h incubation with the hormone. Upon FSH stimulation, 8 lines were found to exhibit morphological changes, and showed at least 10-fold higher progesterone production than non-stimulated cells (20–400 ng progesterone/mg protein compared to 1–3 ng progesterone/mg protein, *n* = 3, *P* < 0.001). The average number of cells/mg protein was  $4 \times 10^6$  cells. In this paper, we describe the characteristics of a cell line (GFSHR-17) that yields the highest FSH-stimulated progesterone production among the 8 responsive lines.

**Morphological appearance of cells.** Non-stimulated cells appeared as elongated clusters which very often seemed to be in contact by elongated cell processes. Considerable rounding of the cells was already evident 30–60 min after stimulation with 1.6 nM oFSH, while the cells remained in contact through their long cell processes. This morphological change includes more than 95% of the cell population and persists at least 12 h after the onset of stimulation (Fig. 1A and 1B).

**Steroidogenic cellular response to gonadotropic hormones.** Non-stimulated GFSHR-17 cells produced only traces of progesterone during 24 h incubation at 37°C, either in the presence or absence of 5% FCS (Fig. 2). When cells were stimulated with 1.6 nM FSH, the production of progesterone increased 15-fold in serum-free medium and 26-fold in the presence of 5% FCS (*n* = 3, *P* < 0.001). Addition of dexamethasone (100 nM) further increased progesterone production by approximately 4-fold, compared to cultures stimulated by FSH alone, in both serum-free and serum-containing medium (*n* = 3, *P* < 0.001). In contrast, dexamethasone did not exert any effect on progesterone production in non-stimulated cultures. When cells were stimulated with 50 μM forskolin in the presence or absence of serum, progesterone production was increased by 4–8-fold compared to cells stimulated by FSH alone (*n* = 3, *P* < 0.001). The synergistic effect of dexametha-

none in forskolin-stimulated cells was similar to the effect that the glucocorticoid hormone exerted in FSH-stimulated cells.

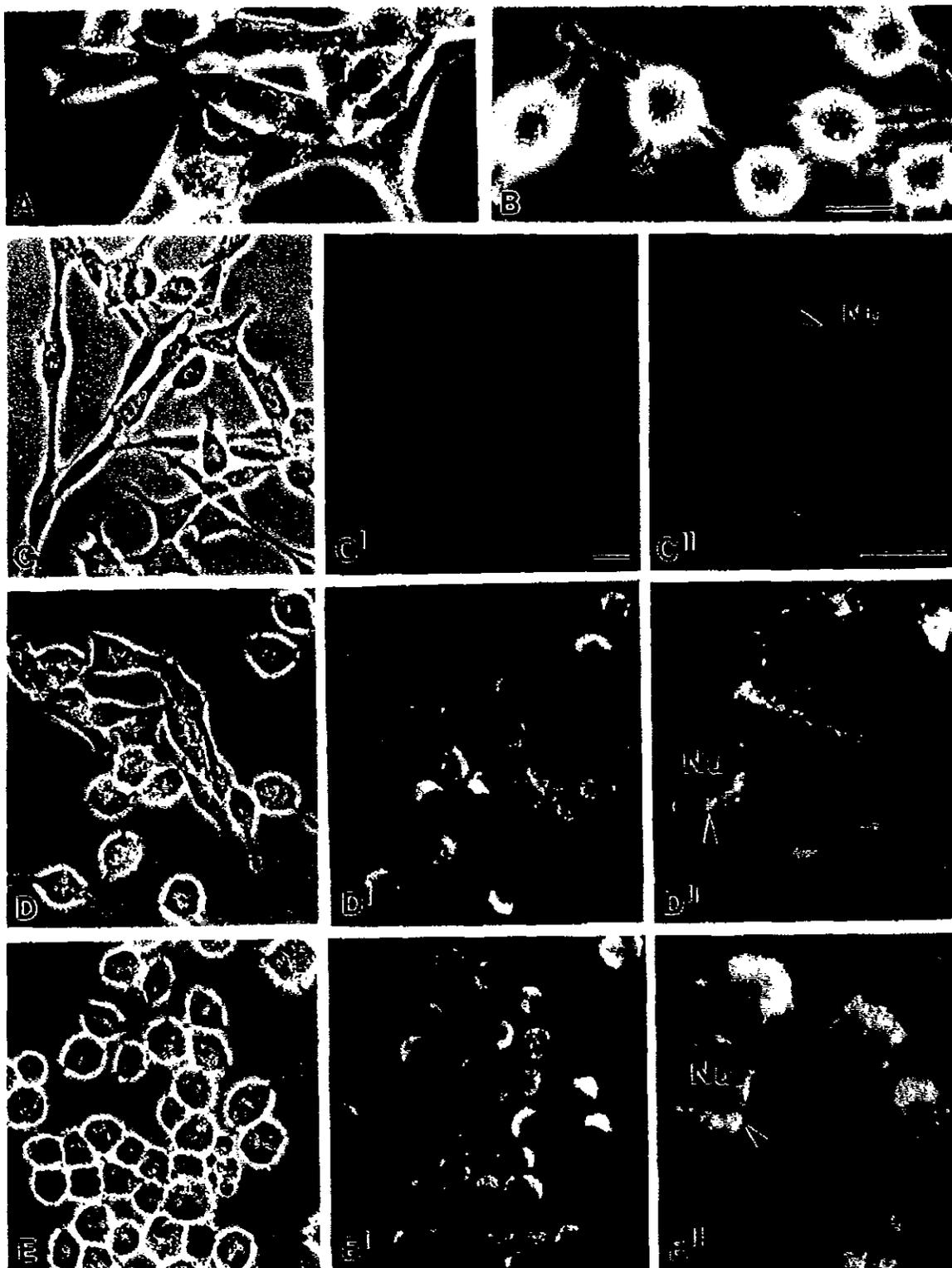
The specificity of the cellular response of GFSHR-17 to different gonadotropins were evaluated in comparison to primary preovulatory cells from which the line was derived (Fig. 3). GFSHR-17 cells and primary preovulatory cells (prepared according to Amsterdam, et al., 1979) were stimulated with FSH from different species as well as by oLH and human chorionic gonadotropin (hCG) which specifically binds to LH receptors (for review see Amsterdam and Lindner, 1984).

In GFSHR-17 cells there was a clear stimulation by oFSH (88-fold), bFSH (248-fold) and rFSH (47-fold) compared to non-stimulated cells (*P* < 0.001; *n* = 3). Primary cells showed a similar pattern of progesterone production in response to the FSH from the different sources. No significant difference in progesterone levels between GFSHR-17 and primary cells was found following stimulation with either oFSH or rFSH (*P* > 0.1). Saturating doses of LH or hCG (1.6 nM) did not cause any significant rise in progesterone levels in GFSHR-17 line. In contrast, primary cells demonstrated, as expected, a clear response to oLH (4.2-fold; *P* < 0.001; *n* = 3) and hCG (4-fold; *P* < 0.001; *n* = 3). The inability of the transformed cells to respond to LH and hCG is probably due to the loss of endogenous gonadotropin receptors (Suh and Amsterdam, 1990; Amsterdam et al., 1992).

**Characterization of FSH receptors.** Binding of <sup>125</sup>I-FSH to cell membranes could be displaced in a dose-dependent manner by increasing concentrations of unlabeled FSH with an ED<sub>50</sub> of 45 ± 5 pM (Fig. 4A). Scatchard analysis revealed high affinity binding sites to FSH (Fig. 4B). The K<sub>d</sub> calculated for the high affinity binding sites was 107 ± 9 pM (mean ± SEM, *n* = 4) and the number of FSH receptors per cell was 27,000 ± 2300 (mean ± SEM, *n* = 4).

**Dose and time dependence of FSH stimulated progesterone production.** The intracellular level of cAMP in cells stimulated by a saturating dose of FSH (3.0 nM) increased by 20-fold within 30 min after onset of stimulation (16.3 pM/10<sup>6</sup> cells compared to 0.8 pM/10<sup>6</sup> cells; *n* = 3, *p* < 0.001). The elevated level of cAMP returned to non-stimulated levels after 3 h of stimulation (not shown) due to desensitization to the stimulant characteristic also of primary granulosa cells and transformed cells which express the LH receptors (Suh et al., 1992). When progesterone production was mea-

Fig. 1. Changes in morphology and immunostaining for adrenodoxin in GFSHR-17 cells following FSH stimulation. Phase contrast images of non-stimulated cells (A) and cells stimulated for 3 h with FSH (B). Cells stained for adrenodoxin were control cells (C', C'') or cells treated with either 1.6 nM oFSH (D', D'') or 50 μM forskolin (E', E''). C, D, and E are phase contrast images of the identical areas of the cultures as in C', D', and E'. Individual mitochondria are visible in the high magnification images (arrowheads in C'', D'' and E''). Nuclei (Nu) remain unstained. Cell processes are marked by arrows (A–E). magnifications: A, B × 1200; C, D, E, C', D', E' × 500; C'', D'', E'' × 1400. Bar represents 10 μM.



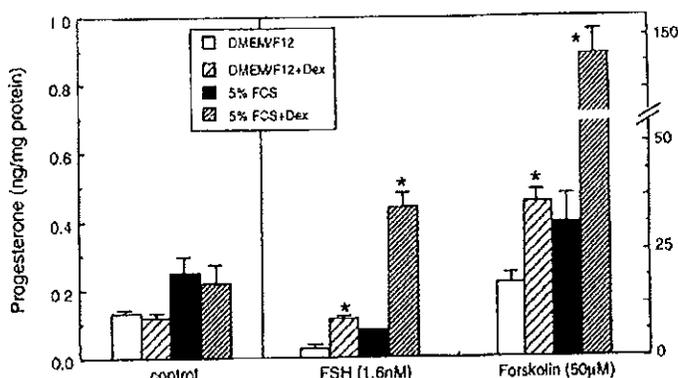


Fig. 2. Effect of dexamethasone and serum on progesterone production in GFSHR-17 cells. Cells ( $5 \times 10^4$ ) were incubated in DMEM:F12 medium in the presence or absence of 100 nM dexamethasone with or without 5% FCS, as indicated in the Fig. 2. 1.6 nM oFSH or 50  $\mu$ M forskolin were added to the culture medium. After 24 h, progesterone and protein were assayed. Results were calculated as the means  $\pm$  SEM in triplicate plates. Note different y axis scale for control and stimulated cells (FSH and forskolin). All FSH and forskolin stimulated cultures are significantly higher ( $p < 0.001$ ) compared to the parallel non stimulated cultures (control). Dexamethasone significantly elevated progesterone production in stimulated cultures (\*  $p < 0.001$ ).

sured as a response to increasing concentrations of FSH in GFSHR-17 cells, a typical saturation curve was observed with an  $ED_{50}$  of 200 pM of FSH reaching a plateau at 0.4 nM FSH (Fig. 5A).

When the development of the steroidogenic response with time was analyzed, it was found that stimulation of progesterone production was only detected after 12 h or longer incubation with forskolin or FSH (Fig. 5B). After 48 h, the amount of progesterone production in forskolin-treated cells was 3 times higher than in FSH stimulated cells ( $n = 3$ ,  $P < 0.001$ ).

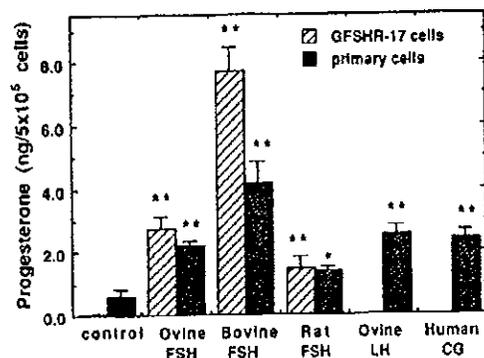


Fig. 3. Progesterone production in GFSHR-17 and primary cells stimulated by gonadotropins. GFSHR-17 cells and primary cultures obtained from PMSG treated immature rats (prepared according to Amsterdam et al., 1979) were stimulated for 48 h at 37°C. Data for each FSH hormone was obtained using concentration of  $10^{-3}$  U/ml which was within the linear portion of dose response curve obtained for each gonadotropin at concentration range of  $10^{-5}$ - $10^{-2}$  U/ml (not shown). LH and hCG did not cause any significant rise of progesterone production in GFSHR-17 cells above basal levels ( $< 0.02$  ng/ $5 \times 10^5$  cells). Data are mean  $\pm$  SEM ( $n = 3$ ) Stimulated cultures are significantly higher than controls \*  $p < 0.01$ ; \*\*  $p < 0.001$ .

The number of cells was determined during the 48 h period of incubation of the cells at 37° in a serum-free medium with no added hormone or in the presence of 1.6 nM FSH or 50  $\mu$ M forskolin (Fig. 6). The rate of growth was found to be inversely related to proges-

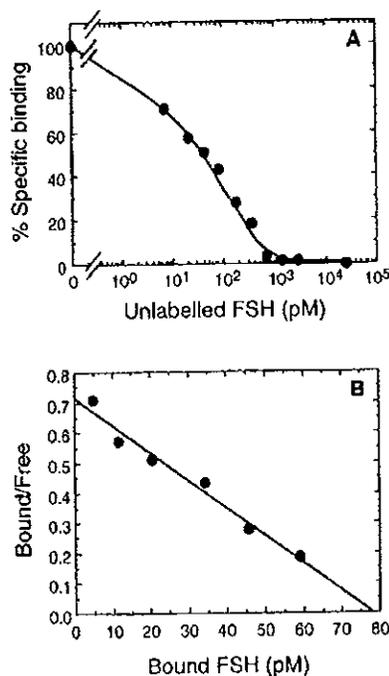


Fig. 4. Characterization of oFSH binding to GFSHR-17 cells. (A) Displacement of  $^{125}$ I-oFSH binding by increasing concentrations of unlabeled hormone. Data are plotted following subtraction of non-specific binding (see Materials and methods). (B) Scatchard plot of  $^{125}$ I-oFSH calculated from the displacement curve in A. Data are means of duplicate assays. Deviation from the mean did not exceed 7%.

terone production. In non-stimulated cultures the number of cells after 48 h increased 4.2-fold, compared to zero time, whereas in FSH and forskolin-stimulated cultures it increased only 3.3- and 2.4-fold respectively.

**Adrenodoxin appearance in FSH stimulated cells.** In order to examine whether FSH stimulated progesterone production is due to de novo synthesis of the mitochondrial steroidogenic enzymes, cells were immunostained for adrenodoxin, 24 h (Fig. 1) and 48 h (not shown) after onset of the FSH stimulation using specific antiserum to this enzyme and the indirect immunofluorescence technique. Location of intensive staining in mitochondria was evident both after 24 and 48 h of stimulation, leaving the nuclei of the cells unstained (Fig. 1D). In non-stimulated cells, only weak staining of mitochondria was evident (Fig. 1C). Cells stained after stimulation with 50  $\mu$ M forskolin showed even more intense mitochondrial staining, than FSH-stimulated cells (Fig. 1E).

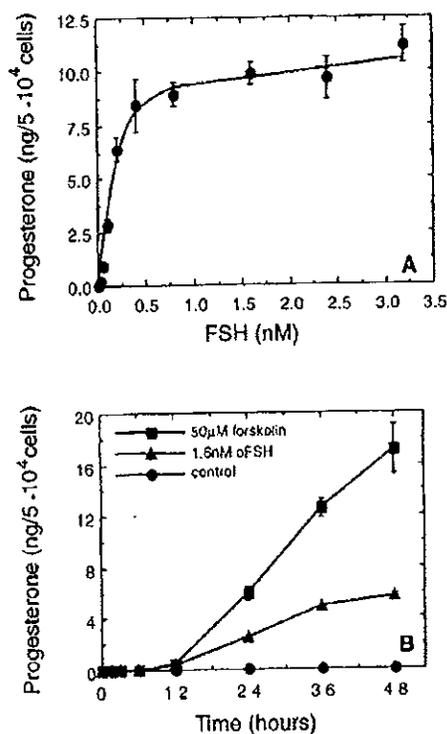


Fig. 5. Dose and time response for stimulation by oFSH of progesterone production by GFSHR-17 cells. (A) Cells ( $5 \times 10^4$ ) were incubated for 48 h at 37°C in the presence of increasing concentration of oFSH and progesterone was assayed in triplicate. Values in nonstimulated cells were  $< 4.0$  pg. (B) Cells ( $5 \times 10^4$ ) were incubated (1½–48 h) with no added hormone or in the presence of 1.6 nM oFSH or 50  $\mu$ M forskolin. Progesterone values are means  $\pm$  SEM ( $n = 3$ ). Progesterone values of all stimulated cultures in A and at 24 h–48 h in B are significantly higher compared to non stimulated cells ( $p < 0.001$ ).

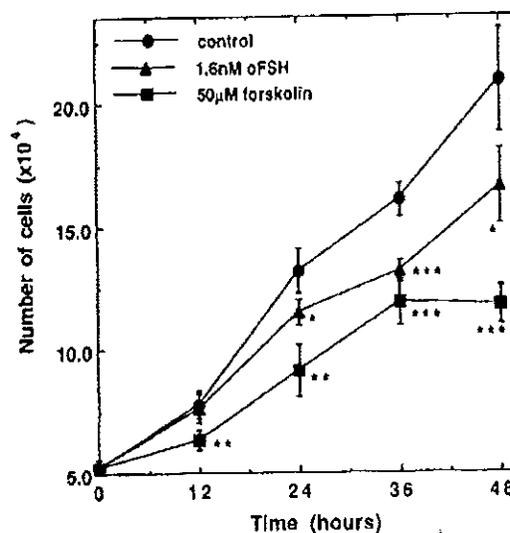


Fig. 6. GFSHR-17 growth in the presence of oFSH and forskolin. Cells ( $10^5$ ) were incubated with no added hormone or in the presence of 1.6 nM oFSH or 50  $\mu$ M forskolin. The number of cells at 12, 24, 36 and 48 h after onset of stimulation was counted twice on quintuplicate plates. Data are means  $\pm$  SEM ( $n = 5$ ). \*  $p < 0.01$ ; \*\*  $p < 0.05$ ; \*\*\*  $p < 0.001$  compared to control at the same time point.

## Discussion

In this work, we succeeded in establishing oncogene-transformed granulosa cell lines that express constitutively FSH receptors. This could be achieved only if the cells, which had lost the expression of the native receptors (see below), were transfected with a plasmid DNA coding for the receptor molecule which was expressed under control of the SV40 early promoter. The loss of expression of the native FSH receptor was demonstrated in our early studies by lack of response to gonadotropins in established cell lines transfected with SV40 DNA and the Ha-ras oncogene alone (Amsterdam et al., 1988a; Suh and Amsterdam, 1990). In fact, no permanent steroidogenic cell line derived from granulosa cells expressing the FSH receptors had been characterized until the present work.

The introduction of the plasmid coding for the FSHR receptor into a steroidogenic cell allows us to examine characteristics of the newly synthesized receptor molecules and their ability to transduce signals which lead to stimulation of steroidogenesis. We found that the transfection of the cells with SV40 DNA and the Ha-ras oncogene did not interfere with the signal transduction mechanism leading to enhancement of steroidogenesis by FSH (Figs. 2, 3, 5). Moreover, the specific response to oFSH, bFSH and rFSH demonstrate a characteristic pattern which was similar to primary granulosa expressing the native receptors (Fig. 3). The extremely low basal levels of progesterone production in GFSHR-17 compared to primary granu-

losa cells is probably due to almost complete loss of the steroidogenic enzymes upon transformation. These enzymes are dramatically upregulated upon stimulation (see also Hanukoglu et al., 1990; Suh et al., 1992). Thus, the magnitude of stimulation of steroidogenesis in the newly established cells is even higher than in primary cells.

The synergistic effect of a glucocorticoid hormone, dexamethasone, on progesterone production induced by FSH, characterized in primary cultures (Adashi et al., 1981), was completely preserved in our cell line (Fig. 2). This synergistic effect, which is also characteristic of forskolin-stimulated transfected cells bearing or lacking the FSH receptor (Plehn-Dujowich et al., 1991) may be accomplished by the modulation of the glucocorticoid receptor function by protein kinase A (Rangarajan et al., 1992).

From Scatchard analysis of  $^{125}\text{I}$ -FSH binding to the new lines (Fig. 4), we calculated that the high affinity binding sites for FSH have a  $K_d$  value of 100–115 pM. This value falls in the range of  $K_d$  values calculated for the native receptor molecules in rat granulosa cells obtained from different stages of follicular development (32–180 pM, Nimrod et al., 1976). A similar range of  $K_d$  was also found for ovarian and testicular tissue in other mammals using FSH from different sources (Combarrous et al., 1978; Zhang et al., 1991; Xiao et al., 1992; Yarney et al., 1990). In contrast to the affinity, we found that FSH receptor density on the transformed cells was 15–20 times higher than on primary granulosa cells (800–1500/cell Nimrod et al., 1976). Nevertheless, the steroidogenic response to FSH was about 50% lower than to forskolin, known to elevate intracellular cAMP levels in these and other cells (Seamon and Daly, 1986). This suggests that the cellular response to gonadotropic hormone may not be mediated exclusively via activation of adenylate cyclase and that the receptor molecule might be coupled to additional G inhibitory proteins interacting with other signalling pathways in the cells such as protein kinase C mediated processes. Indeed, the phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA) which activate PKC was recently found to decrease the cAMP response to FSH in nontransformed granulosa cells (Nikula et al., 1990). Thus the oncogene transformed cell system can lend itself to a more detailed analysis of alternative pathways associated with FSH stimulation.

The steroidogenic response to FSH in the newly established cell lines was found to have an  $\text{ED}_{50}$  of 200 pM, which is comparable to the  $K_d$  value calculated for FSH-binding to GFSHR-17 cells. A similar  $\text{ED}_{50}$  value was found for oFSH stimulated steroidogenesis in primary rat granulosa cells (380 pM, Adashi and Resnick, 1984). This further supports the view that coupling between FSH stimulation and steroidogenesis remains intact in the oncogene transformed cells.

The kinetics of the steroidogenic response to FSH is different in the oncogene transformed cells compared to primary granulosa cells. While in primary cells, stimulation of progesterone was evident already after 3 and 6 h (Adashi and Resnick, 1984; Suh and Amsterdam, 1990), in the transformed cells it was not evident before 12 h (Fig. 5). These differences are probably due to de novo synthesis of the steroidogenic enzymes whose levels in nonstimulated cells were low, as can be judged from the immunostaining of adrenodoxin (Fig. 1) and from our previous studies in recently established lines established by cotransfection with SV40 DNA, Ha-*ras* oncogene and LHR expression plasmid (Suh et al., 1992). A substantial appearance of adrenodoxin 24 and 48 h after stimulation confirms the de novo synthesis of this electron carrier protein. This induction of adrenodoxin synthesis is most likely accompanied by de novo synthesis of adrenodoxin reductase and the cytochrome P450 side chain enzyme as was demonstrated previously in SV40 and Ha-*ras*-transformed cells stimulated by substances which elevate intracellular cAMP levels (Hanukoglu et al., 1990).

The lag period in the steroidogenic response is also characteristic of forskolin stimulation in SV40 and Ha-*ras*-transfected cells (Suh and Amsterdam, 1990), as well as of hCG stimulation in the LH/hCG responsive cell lines (Suh et al., 1992). Staining of all stimulated cells for adrenodoxin in FSH responsive cell lines is also a good indication of the homogeneity of the cell lines (Fig. 1).

An inverse relationship between growth, and differentiation stimulated by FSH or forskolin, was evident in the FSH responsive cell line (Figs. 5 and 6). This is not surprising in view of similar relationships in SV40-Ha-*ras*-transformed cells lacking or expressing LH receptors (Amsterdam et al., 1988a; Suh and Amsterdam, 1990; Suh et al., 1992). However, in this study cells were derived from preovulatory follicles that show a high degree of maturation. It is still unknown, whether transfection of cells obtained from earlier stages of follicular development, would yield cell lines in which FSH would be able to stimulate cell growth, as is believed to occur in undifferentiated normal granulosa cells of preantral follicles (for review see Richards and Hedin, 1988).

Comparing the signal transduction mechanisms associated with FSH and LH stimulation revealed interesting differences: (i) FSH receptors appear earlier in follicular development than do LH receptors and in fact FSH, in synergism with oestradiol, serves as the major physiological trigger for the expression of LH receptors (for review see Richards and Hedin, 1988). (ii) While stimulation of FSH receptors is associated with both growth and differentiation of granulosa cells, depending on the maturity of the cells, LH action always involves enhanced steroidogenesis associated

with depression of growth. (iii) While the LH response includes opening of calcium channels (Davis et al., 1987; Gudermann et al. 1992) FSH does not seem to elicit such a phenomenon (Shibata et al., 1992), although modulation of calcium fluxes in FSH stimulated Sertoli cells was recently suggested (Grasso and Reichert, 1990; Grasso et al., 1991). Unfortunately, the cellular function of the two related gonadotropin receptors is complicated to compare, especially in early stages of follicular development, mainly because the receptors for these hormones appear at different stages of granulosa cell maturation. Recently, the cellular response to the two related gonadotropins (FSH and LH) was compared in human granulosa cells and it was found that cAMP can mediate divergent gonadotropic effects on growth and differentiation of these cells (Yong et al., 1992). Since we have established lines expressing either LH (Suh et al., 1992) or FSH receptors (the present work) which are derived from cells at an identical stage of maturation, that express exclusively either LH or FSH receptors, we can now compare the LH and FSH signalling in homologous cell systems. Cotransfection of granulosa cells obtained from selected stages of maturation with plasmids expressing both LH and FSH receptors or a chimeric receptor is also not beyond reach.

Upon completion of this work it was reported that the human FSHR gene was successfully introduced into mouse adrenal tumor Y-1 cell lines whose parental adrenal cells do not express the gonadotropin receptor (Kelton et al., 1992). In contrast, the newly established cell lines, characterized in this work, display a more physiological model since they were derived from primary granulosa steroidogenic cells which naturally express the FSHR.

It is hoped that this novel cell system can provide a valuable tool for the investigation of the regulation of the FSHR and the signal transduction mechanisms leading to growth and differentiation evoked by such a stimulus. Since FSH also affects the expression of cellular oncogenes such as *c-fos*, *c-jun*, *Jun B* and *c-myc* (Hall et al., 1991; Pennybacker and Herman, 1991), it is hoped that this system will lend itself to a detailed analysis of the role of such oncogenes and other transcription factors in modulation of the expression and the function of the FSH receptors.

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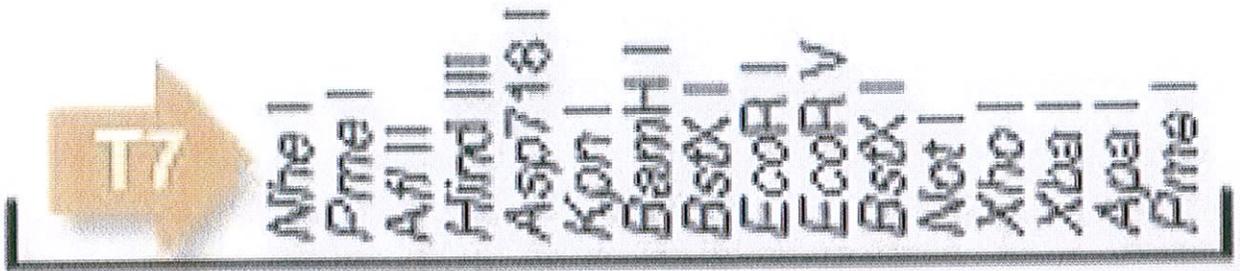
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