Visual evaluation of early (~ 4-cell) mammalian embryos.
How well does it predict subsequent viability?

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APPENDICES

1) Glossary (alphabetical)

(From the Aberdeen Fertility Centre website except as indicated:
http://www.aberdeenfertility.org.uk/page.php?p=glossary.php?letter=f)

Blastocyst

The early stage of embryonic development during which implantation occurs, approximately eight days after fertilisation

Cryopreservation

Storage of eggs, embryos and /or semen at temperatures well below zero in liquid nitrogen

Embryo

The early stages of fetal growth, from conception to the eighth week of pregnancy

Embryo Transfer (http://www.fert.org.uk/intro.htm**)**

Embryos are placed gently inside the womb (uterus) on the second or third day after egg retrieval, when they usually consist of two to six cells. They are transferred via a thin plastic tube (catheter) which is inserted through the neck of the womb (cervix) into the cavity of the uterus. Embryo transfer is an outpatient procedure which takes only a few minutes to perform and is similar to having a cervical smear taken. No pain relief, therefore, is required. Couples will have a chance to see their embryos on the closed-circuit monitor and may have a photograph of them, if they wish.

The number of embryos transferred depends upon several factors, including the number that are available, their quality, the woman's age and the couple's wishes. The Human Fertilisation and Embryology Authority (HFEA) restricts transfer to a maximum of three embryos. Experience has shown that the chance of becoming pregnant is very similar, regardless of whether two or three embryos are transferred (in cases where there are more than three embryos to choose from). What is also clear, however, is that if three embryos

are transferred then there is a risk of a triplet pregnancy occurring. Triplet pregnancies are associated with a number of major complications the most serious being miscarriage, pregnancy complications, premature delivery and handicap.

Fallopian Tubes

Tubes, which lead from the uterus and end in finger like projections near the ovaries. Fertilization usually takes place in the fallopian tubes

Fertilization

Fusion of an egg (oocyte) and a sperm

Fetus

The embryo in the uterus from the third month of pregnancy

Follicle

Sac which contains the egg during growth and maturation

ICSI Intracytoplasmic sperm injection (http://www.fert.org.uk/intro.htm)

Carried out to assist the process of fertilisation for patients who are unlikely to achieve fertilisation with conventional IVF by micro-manipulation of the egg and sperm. ICSI involves injecting a single sperm into the centre of the egg, using a very fine needle. Fertilisation rates for ICSI are comparable to those for conventional IVF. Success is dependent on the severity of the sperm disorder, and on the number of good quality, mature eggs that are available for injection. ICSI is a relatively new technique and in many countries, the outcome of ICSI pregnancies has been closely followed. Although there is no evidence to date that children born as a result of ICSI face a higher incidence of ill health or handicap it may be the case that baby boys born following the ICSI procedure could inherit the male factor infertility suffered by their fathers.

***In vitro* fertilisation (IVF) (**http://www.fert.org.uk/intro.htm**)**

There are times when the normal processes of fertilisation cannot take place in the body; in

other words it is difficult or impossible for the sperm and egg to meet in the fallopian tube. The commonest reasons are that the tubes are blocked or that the sperm are too few or of too poor a quality to fertilise an egg. IVF offers an opportunity to avoid such problems by allowing fertilisation to occur outside the body in a glass dish; hence the use of the Latin words "*in vitro*" which literally mean "in glass".

Although IVF was originally used to treat women with blocked tubes, it has since been used successfully to treat couples with other problems. These include endometriosis, sperm-mucus problems, and 'unexplained' infertility - those couples who have been extensively investigated without any obvious cause for their infertility being discovered.

The main steps in IVF are to: -

- give drugs to suppress the natural cycle and prevent ovulation (Down-regulation)
- give drugs to encourage the development of several follicles in the woman's ovaries (Ovarian stimulation)
- remove the eggs from the developed follicles and mix them with the man's sperm (Egg retrieval)
- transfer usually two, but a maximum of three, resulting embryos into the uterus (Embryo transfer)

Infertility (Primary)

The inability of a couple to achieve a pregnancy after one year of regular unprotected sexual relations, or the inability of the woman to carry a pregnancy to term

Infertility (Secondary)

The inability to conceive or carry a pregnancy after having successfully conceived and carried one or more pregnancies

Oocyte Retrieval

Recovery of the eggs (oocytes) contained within the ovarian follicles for in-vitro or in-vivo fertilization, as is done in assisted reproduction technologies such as IVF or GIFT. Nowadays, ultrasound guided egg retrieval has become the most commonly used technique

Oocyte/Ovum

The female egg cell which contains chromosomes from the woman and is surrounded by cells of the follicle

Sterility

The total inability to reproduce. Not to be confused with infertility

Uterus

The woman's womb that holds and nourishes the foetus until the time of birth

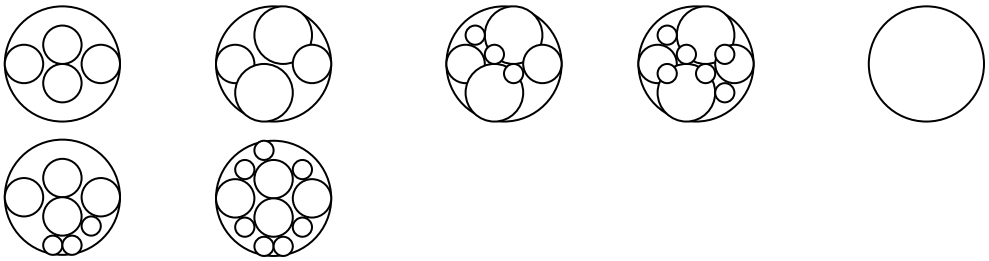
Zygote

An embryo in the early stages of development

2) Embryo grading system currently used at the Aberdeen Fertility Centre

Embryos are graded at the time of transfer. The system is used is as follows for fresh embryos:

Grade 1	Equal size of cells	+	no or few fragments
Grade 2	Unequal size of cells	+	no fragments
	Equal size of cells	+	lots of fragments
Grade 3	Unequal size of cells	+	few fragments
Grade 4	Unequal size of cells	+	lots of fragments
Grade 5	Undivided embryo		



Grade 1 | Grade 2 | Grade 3 | Grade 4 | Grade 5

Note: If the embryo has an odd number of cells (eg. 3), and has uneven sized cells, it is treated as being equal sized in the above grading system.

To complete the grading structure, the number of cells present is included. Thus a 4-cell embryo with equal sized cells and no fragments present would be defined as 4c1 where the "c" stands for cell.

Information provided by Dr. A. Srikantharajah (Aberdeen Fertility Centre)

3) Media compositions

G1 / G2 and G1.2 / G2.2 Culture Media Composition according to Gardner and Lane (1999) and Lane *et al* (2003)

	G1	G2
Component	**miliMolar**	**miliMolar**
NaCl	85.16	85.16
KCl	5.5	5.5
Na_2HPO_4	0.5	0.5
$MgSO_4*7H_2O$	1	1
$NaHCO_3$	25	25
$CaCl_2.2H_2O$	1.8	1.8
Sodium pyruvate	0.32	0.1
Sodium Lactate L	10.5	5.87
Glucose	0.5	3.15
Alanine	0.1	0.1
Aspartic acid	0.1	0.1
Arginine	0	0.6
Asparagine	0.1	0.1
Cystine	0	0.1
Glutamate	0.1	0.1
Glutamine	1	1
Glycine	0.1	0.1
Histidine	0	0.2
Isoleucine	0	0.4
Leucine	0	0.4
Lysine	0	0.4
Methionine	0	0.1
Phenylalanine	0	0.2
Proline	0.1	0.1
Serine	0.1	0.1
Taurine	0.1	0
Threonine	0	0.4
Tryptophan	0	0.5
Tyrosine	0	0.2
Valine	0	0.4
EDTA	0.01	0
HSA (Hum.Ser.Alb)	5 mg/ml	5 mg/ml
Phenol Red	0.001 g/l	0.001 g/l

	G1.2	G2.2
Component	**miliMolar**	**miliMolar**
NaCl	90.08	90.08
KCl	5.5	5.5
NaH_2PO_4	0.25	0.25
$MgSO_4$	1	1
$NaHCO_3$	25	25
$CaCl_2$	1.8	1.8
Pyruvate	0.32	0.1
Lactate	10.5	5.87
Glucose	0.5	3.15
Alanine	0.1	0.1
Aspartate	0.1	0.1
Arginine	0	0.6
Asparagine	0.1	0.1
Cystine	0	0.1
Glutamate	0.1	0.1
Alanyl-glutamine	0.5	1
Glycine	0.1	0.1
Histidine	0	0.2
Isoleucine	0	0.4
Leucine	0	0.4
Lysine	0	0.4
Methionine	0	0.1
Phenylalanine	0	0.2
Proline	0.1	0.1
Serine	0.1	0.1
Taurine	0.1	0
Threonine	0	0.4
Tryptophan	0	0.5
Tyrosine	0	0.2
Valine	0	0.4
EDTA	0.01	0
Ca pantothenate	0	0.0042
Choline chloride	0	0.0072
Folic acid	0	0.0023
Inositol	0	0.01
Niacinamide	0	0.0082
Pyridoxal	0	0.0049
Riboflavin	0	0.0003
Thiamine	0	0.003

SOF Culture Medium – Composition according to Tervit *et al*, 1972.

Basic SOF	
Component	**Molarity=mmol/L**
NaCl	107.67
KCl	7.16
KH_2PO_4	1.19
$NaHCO_3$	25.009
Phenol Red	0.028
Pyruvate	0.981
$CaCl_2.2H_2O$	1.714
Na Lactate	3.291
$MgCl_2.6H_2O$	0.4564
Glucose	1.498
Glutamine	0.999
pH 7.4	
mOsmo 270-280	

SOFaaBSA: To basic SOF, add:	
FAF BSA (Sigma, cat. No A8806)	150 mg/50 mL SOF
L Arginine*HCl	0.1
L-Cystine*2HCl	0.04
L-Histidine (free base)	0.05
L- Isoleucine	0.2
L-Leucine	0.2
L-Lysine*HCl	0.2
L-Methionine	0.05
L-Phenylalanine	0.1
L-Threonine	0.2
L-Tryptophan	0.02
L-Tyrosine	0.01
L-Valine	0.2
L-Alanine (free base)	0.1
L-Asparagine*H_20	0.11
L-Aspartic Acid	0.1
L-Glutamic Acid	0.1
Glycine	0.1
L-Proline	0.1
L-Serine	0.1
pH 7.4	
mOsmo 270-280	

P1 Culture Medium – Commercially Available Composition with SSS (Synthetic Serum Substitute) and without (to be added in the lab) according to Irvine Scientific (2003) (http://www.irvinesci.com/web1/website/techinfo/index.cfm)

P1

Component	**miliMolar**
NaCl	101.6
KCl	4.69
$MgSO_4$	0.2
$CaCl_2.2H_2O$	2.04
$NaHCO_3$	25
Na Pyruvate	0.33
Na Lactate	21.4
Taurine	0.05
Sodium Citrate	0.15 mg/L
Phenol Red	0.005 g/L
Gentamicin	10ug/ml

P1 with SSS

Component	**miliMolar**
NaCl	101.6
KCl	4.69
$MgSO_4$	0.2
$CaCl_2.2H_2O$	2.04
$NaHCO_3$	25
Na Pyruvate	0.33
Na Lactate	21.4
Taurine	0.05
Sodium citrate	0.0005
Phenol red	5 mg/l
Gentamicin sulfate	10ug/ml
Human Serum Albumin	5mg/ml
Globulins	1 mg/ml

Complete Blastocyst Medium – Commercially Available Composition with SSS (Synthetic Serum Substitute) and without (to be added in the lab) according to Irvine Scientific (2003) (http://www.irvinesci.com/web1/website/techinfo/index.cfm)

Blastocyst medium with SSS

Component	**miliMolar**
NaCl	104.9
KCl	3.4
Potassium phosphate	0.5
$CaCl_2$	0.3
Magnesium Sulfate	1
Sodium bicarbonate	18
Glucose	5.5
Phenol Red	0.009
Potassium bicarbonate	4.5
Pyruvic Acid	0.9
Calcium Lactate	0.9
Alanine	0.09
Arginine	0.9
Asparagine	0.09
Aspartic Acid	0.09
Choline Chloride	0.0045
Riboflavin	0.0009
Serine	0.09
Thiamine	0.0027
Thymidine	0.0027
Thioctic Acid	0.0009
Ferrous sulfate	0.0027
Pantothenic Acid	0.0027
Sodium phosphate	0.99
Cysteine	0.18
Folic Acid	0.0027
Glutamine	0.09
Glutamic Acid	0.09
Glutathione	0.9
Glycine	0.09
Histidine	0.09
I-Inositol	0.0027
Isoleucine	0.018
Leucine	0.09
Lysine	0.09
Methionine	0.027
Nicotinamide	0.0045
Tryptophan	0.0027
Phenylalanine	0.027
Proline	0.09
Pyridoxine	0.0009

Valine	0.027
Culpric Sulphate	0.000009
Threonine	0.027
Zinc Sulphate	0.00009
Biotin	0.00009
Vitamine B-12	0.0009
Tyrosine	0.009
Human serum albumin	5 mg/ml
Globulins	5 mg/ml
Antibiotic: Gentamicin Sulphate	10 ☒g/ml

Blastocyst medium

Component	miliMolar
NaCl	116.6
KCl	3.8
Potassium phosphate	0.6
$CaCl_2$	0.3
Magnesium Sulfate	1.1
Sodium bicarbonate	20
Glucose	6.1
Phenol Red	0.001
Potassium bicarbonate	5
Pyruvic Acid	1
Calcium Lactate	1
Alanine	0.1
Arginine	1
Asparagine	0.1
Aspartic Acid	0.1
Choline Chloride	0.005
Riboflavin	0.001
Serine	0.1
Thiamine	0.003
Thymidine	0.003
Thioctic Acid	0.001
Ferrous sulfate	0.003
Pantothenic Acid	0.003
Sodium phosphate	1.1
Cysteine	0.2
Folic Acid	0.003
Glutamine	1
Glutamic Acid	0.1
Glutathione	1
Glycine	0.1
Histidine	0.1
I-Inositol	0.003
Isoleucine	0.02
Leucine	0.1
Lysine	0.1
Methionine	0.03
Nicotinamide	0.005
Tryptophan	0.003
Phenylalanine	0.003
Proline	0.1
Pyridoxine	0.001
Valine	0.03
Culpric Sulphate	0.00001
Threonine	0.03
Zinc Sulphate	0.0001

Biotin	0.0001
Vitamine B-12	0.001
Tyrosine	0.01
Antibiotic: Gentamicin Sulphate	10 ug/ml