

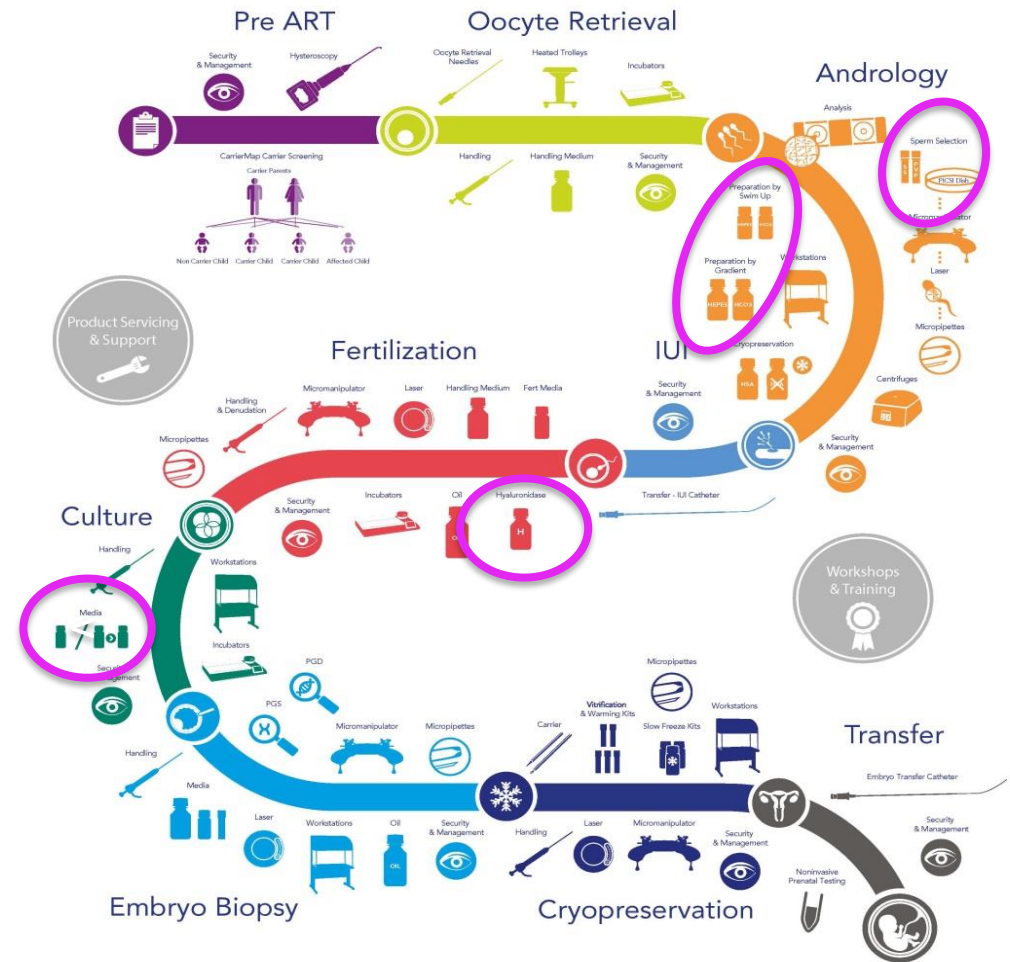
Embryo Culture and Related Products

Dave Morroll, Director of Clinical Support








Jerusalem, 28 February 2019



Products and Solutions for every step in the ART process

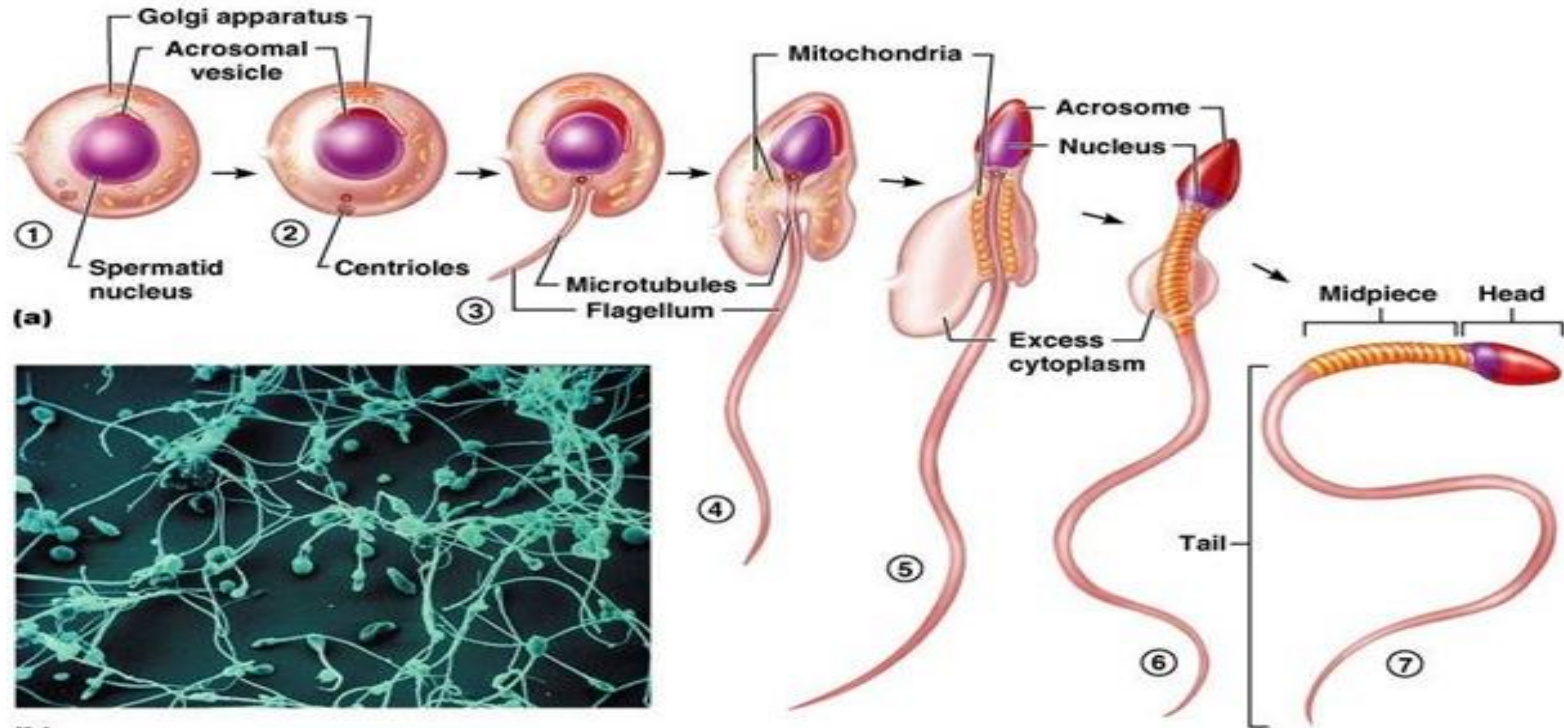


Andrology Products for Every Protocol

Analysis	Preparation by swim-up	Preparation by Gradient	Sperm Selection	Oocyte Denudation
HBA [®] Assay				
ORIGIO [®] Sperm Wash	 NEW			
ORIGIO [®] Gradients™		 NEW		
Sperm Prep Medium (IUI)				
PureCeption™				
PVP				
PICSI [®] dish & SpermSlow™				
ICSI Cumulase [®]				
SynVibro Hyadase				

Hyaluronan
HEPES + Bicarbonate
HEPES + Bicarbonate
Bicarbonate
HEPES
NO Hyaluronan
Hyaluronan
Recombinant
Animal derived

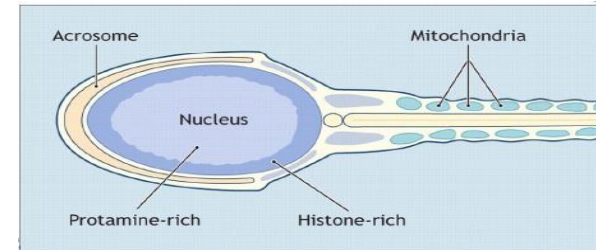
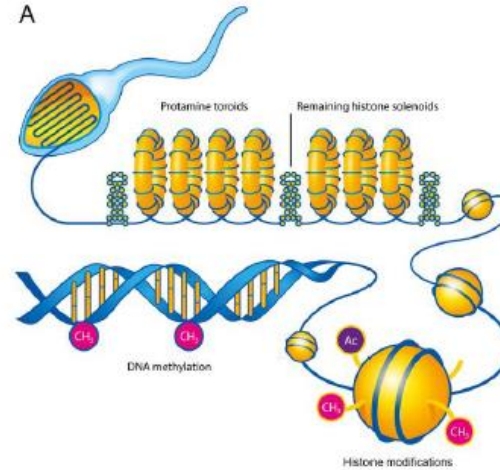
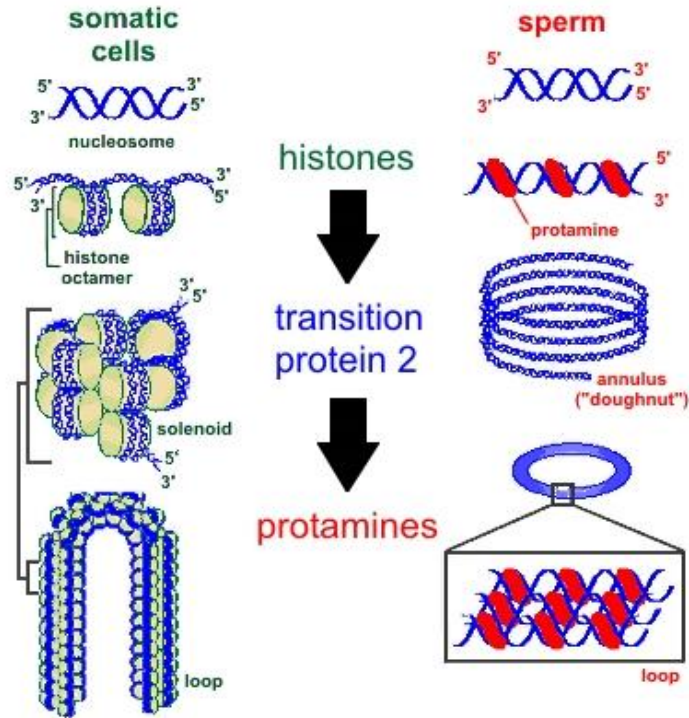
Spermiogenesis



(b)

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Repackaging of chromatin



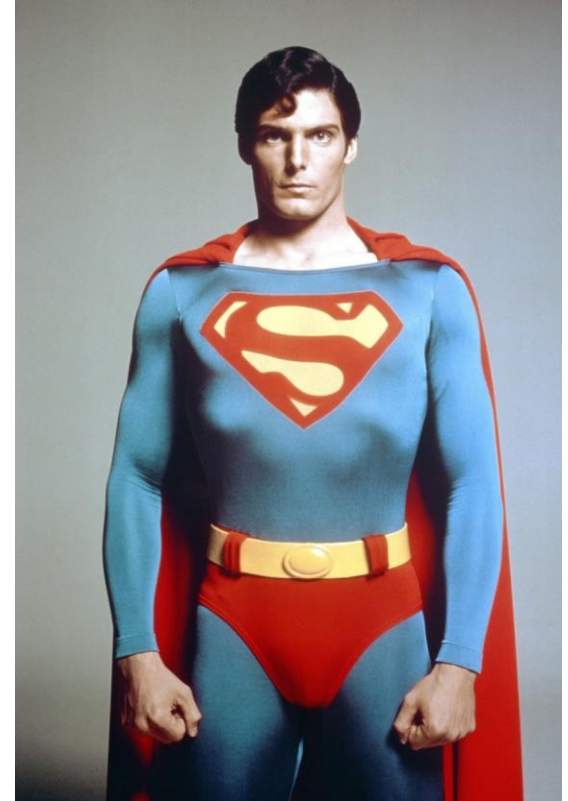
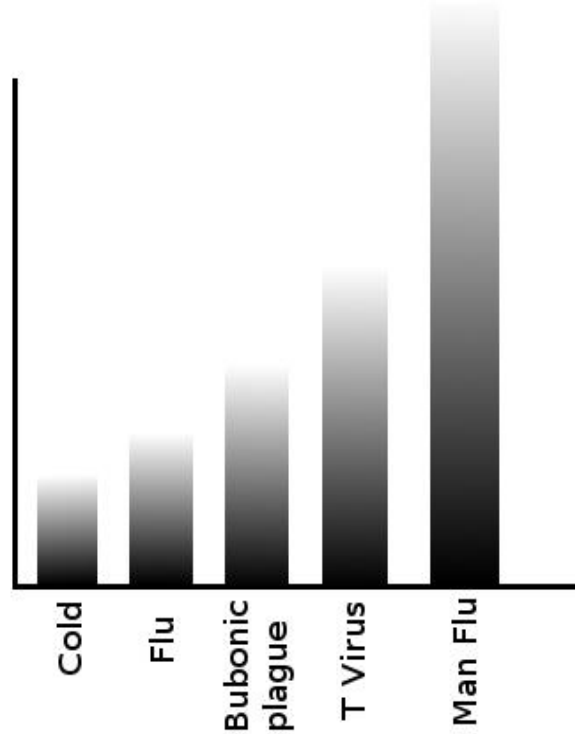
What causes elevated sperm DNA damage?



**STEP ASIDE
COFFEE
THIS IS A JOB FOR
ALCOHOL**



What causes elevated sperm DNA damage?



Impact of DNA damage on fertility

Author	ART	Patient no.	Assay	Association with ART	Prognostic threshold (%)
Tomsu et al. (2002)	IVF	40	COMET	Pregnancy	–
Morris et al. (2002)	IVF	20	COMET	Embryo cleavage	–
Simon et al. (2010)	IVF	230	COMET	Fertilisation, Pregnancy	–
Simon et al. (2010)	ICSI	130	COMET	Fertilisation, Pregnancy	–
Simon et al. (2011)	IVF	75	COMET	Fertilisation, Pregnancy	25
Zini et al. (2005)	ICSI	60	SCSA	Pregnancy loss	30
Boe-Hansen et al. (2006)	IVF	139	SCSA	Pregnancy	27
Boe-Hansen et al. (2006)	ICSI	47	SCSA	No association	27
Benchaib et al. (2007)	IVF	88	SCSA	Fertilisation, Pregnancy loss	15
Benchaib et al. (2007)	ICSI	234	SCSA	Fertilisation, Pregnancy loss	15
Burgum et al. (2007)	IVF	388	SCSA	No association	30

- varying tests and reference values
- impact seen at numerous stages of reproductive process
- most compelling is the association with pregnancy loss

Muriel et al. (2006b)	IUI	100	SCD	Semen parameters	–
Velez de la calle et al. (2008)	IVF/ICSI	622	SCD	Embryo quality Fertilisation	18
Evenson et al. (1999)	Natural	165	SCSA	Pregnancy	30
Giwerzman et al. (2010)	Natural	273	SCSA	Pregnancy	20

Sperm quality and its relationship to natural and assisted conception: British Fertility Society Guidelines for practice

Human Fertility, 2013; 16(3): 175–193

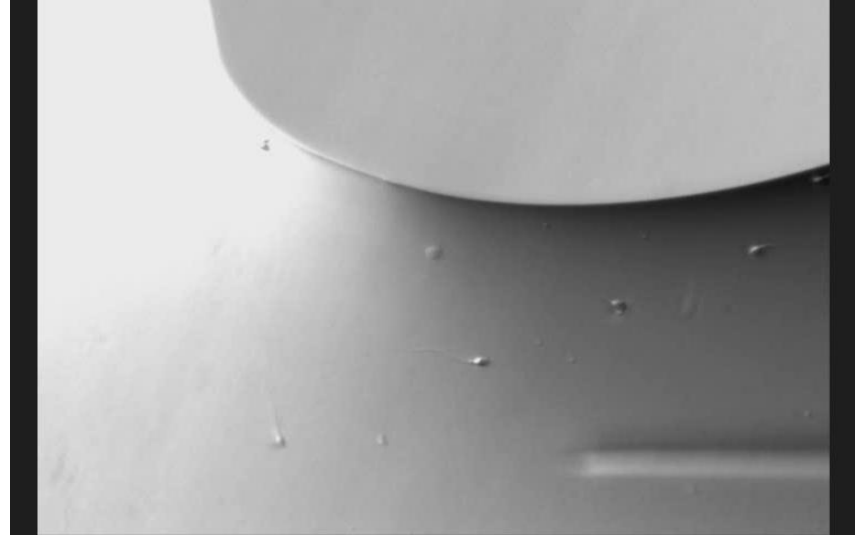
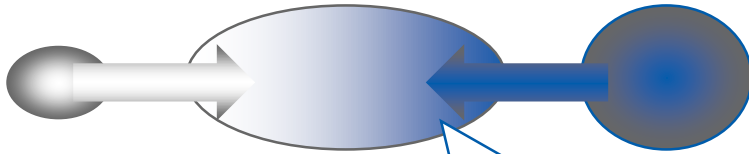
MATHEW TOMLINSON¹, SHEENA LEWIS² & DAVID MORROLL³

HA binding & ICSI: SpermSlow

sperm
suspension

clean
medium

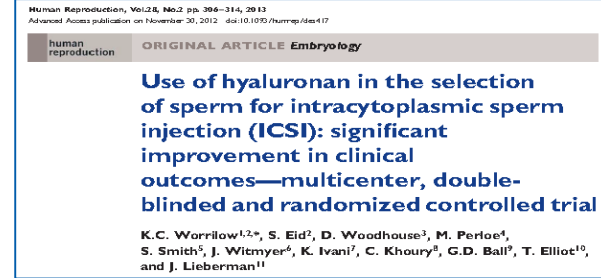
SpermSlow



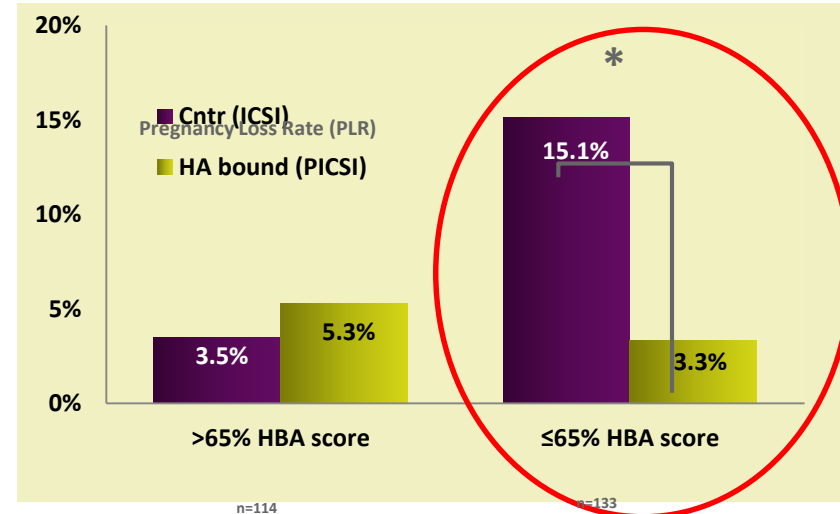
spermatozoa bound to HA in the area where they meet SpermSlow
can be selected and easily picked up in the injection pipette

Selection using HA binding: PICSI

- combination of diagnostic HBA test and sperm selection using PICSI gives significant reduction in pregnancy loss rate
- unexplained pregnancy loss may be due to sperm selected for ICSI being immature with compromised DNA integrity
- 15% of ICSI patients has $\leq 65\%$ HBA score and will benefit from PICSI



802 ICSI patients participating—Multi-center trial (10 center) - Randomized, double blinded, controlled.



Statistical significance (*) is indicated at $P > 0.05$

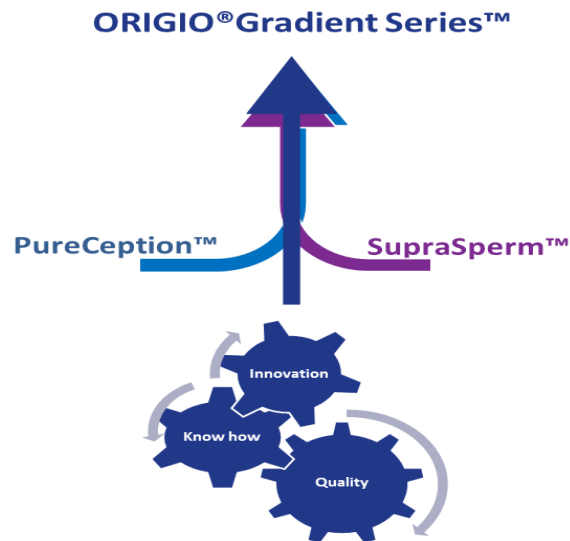
ICSI + HBA: UK multi-centre study



Preliminary conclusions (as presented at Fertility 2018, Liverpool Jan 2018):

- PICS I had no effect on LBR (OR 1.12; 0.95-1.34)
- PICS I gave a significant reduction in miscarriage rate per couple treated (OR 0.61; 0.43-0.84)
- PICS I gave an overall increase in live birth per clinical pregnancy (OR 1.47; 1.02-1.19)
- PICS I especially benefited older women by reducing miscarriage rate

New Andrology products: rationale & design

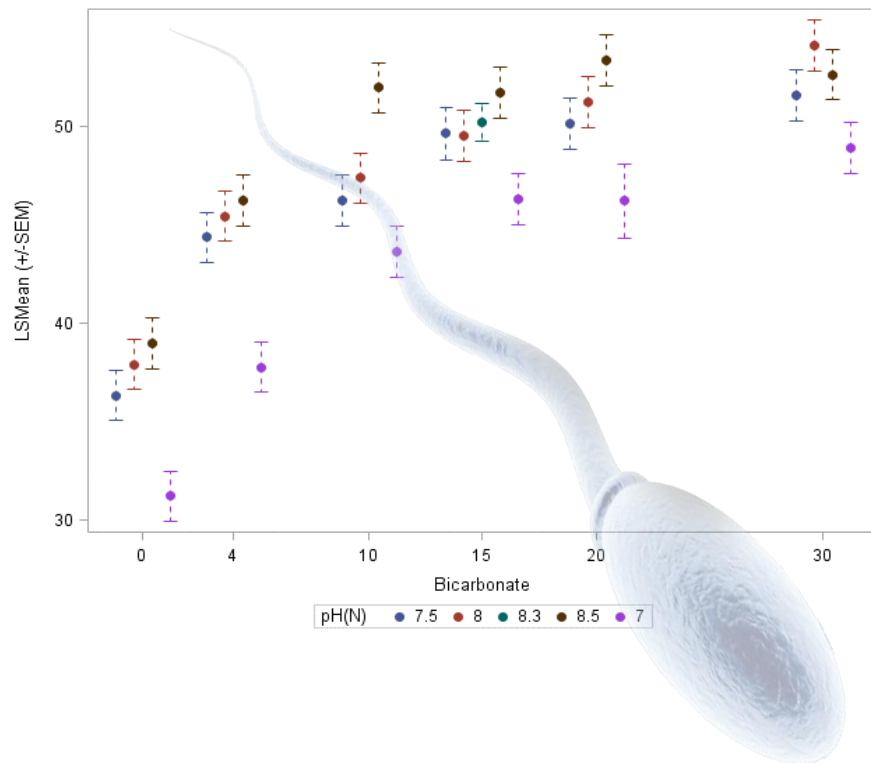


- combined the best from ORIGIO and SAGE systems
- looked toward natural conception for inspiration:
 - alkaline pH
 - elevated bicarbonate
 - higher HSA
 - antioxidants
- combines features from SupraSperm & PureCeption

New Andrology products: rationale & design

pH (8.0 – 8.5 @ RT°C)

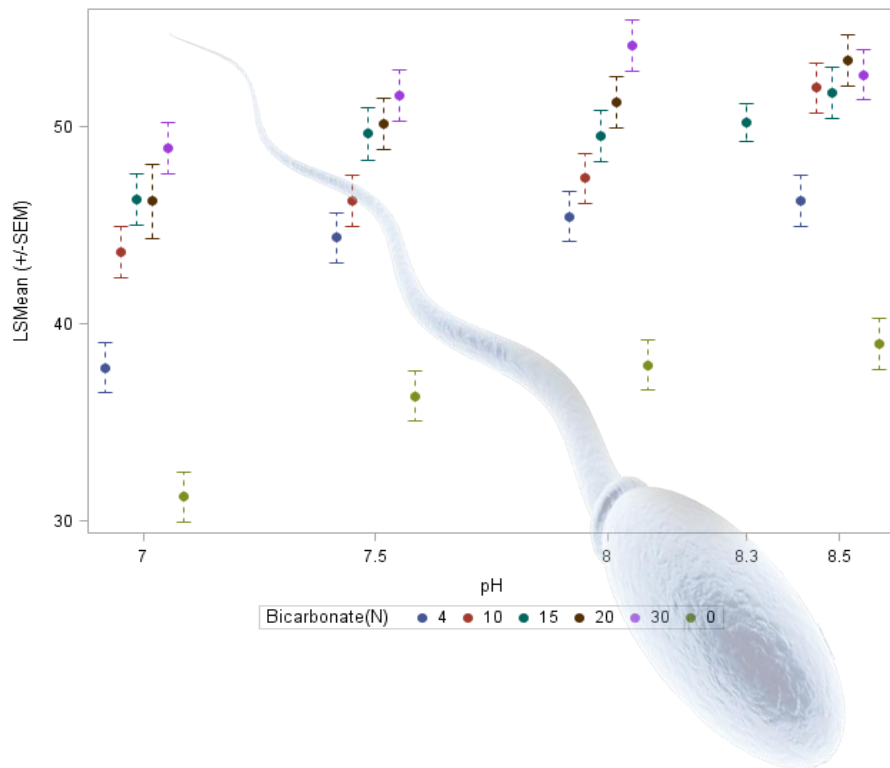
- alkaline pH in the sperm's natural environment (seminal plasma and the cervical mucus at time of ovulation)
- required for the sperm to undergo capacitation
- initiates a range of intracellular reactions in the tail and the acrosome



New Andrology products: rationale & design

bicarbonate

- active component - not for buffer (HEPES does that job)
- found in high concentration in seminal plasma and the oviduct
- required for the sperm to undergo capacitation
- initiates a range of intracellular reactions in the tail and the acrosome



New Andrology products: rationale & design

HSA

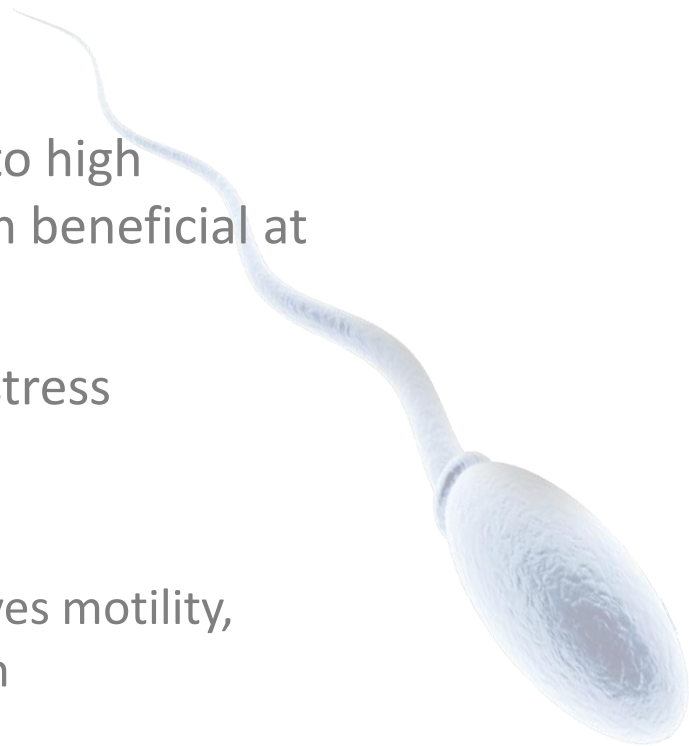
- found in high concentrations in the oviduct (30mg/ml)
- needed for capacitation process
- supports sperm motility
- antioxidant effects
- protects from DNA damage
- prevents sperm from sticking to surfaces



New Andrology products: rationale & design

antioxidants

- ROS a major cause of sperm damage due to high polyunsaturated fatty acid content (though beneficial at physiological levels)
- prepared sperm more prone to oxidative stress
- systems uses:
 - EDTA – chelates heavy metals
 - taurine – found in seminal plasma, improves motility, necessary for capacitation and fertilisation
 - albumin



New Andrology products: sperm function

HSA

- Found in high concentrations in the oviduct
- Needed for capacitation process
- Supports sperm motility
- Antioxidant effects
- Protects from DNA damage
- Prevents sperm from sticking to surfaces

Bicarbonate

- Added as an active component, not because of its buffering capacity (HEPES does that job)
- Found in high concentration in seminal plasma and the oviduct
- Required for the sperm to undergo capacitation
- Initiates a range of intracellular reactions in the tail and the acrosome

Alkaline pH

- Alkaline pH in the sperms natural environment (seminal plasma and the cervical mucous at time of ovulation).
- Required for the sperm to undergo capacitation
- Initiates a range of intracellular reactions in the tail and the acrosome

Alteration of membrane leads to later acrosome reaction

Bicarbonate oxidizes cholesterol in the cell membrane which is transferred to the surrounding albumin molecules. This prepares the sperm membrane for the acrosome reaction.

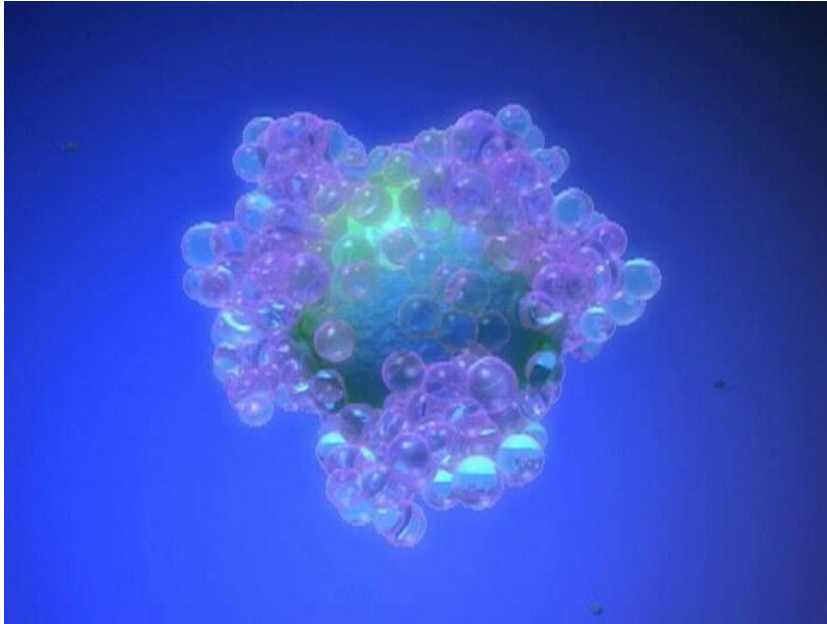
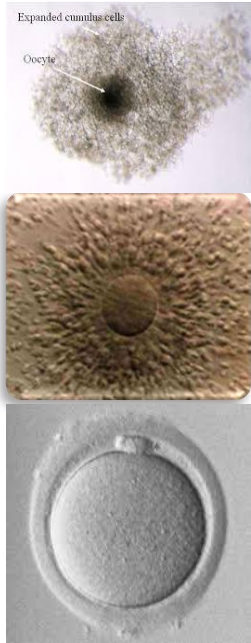
Bicarbonate and alkaline pH support hyperactivation

Bicarbonate and alkaline external pH drives an efflux of H^+ and increase in intracellular Ca^{2+} resulting in an increased intracellular pH which activates the "motors" of the sperm

New Andrology products: optimised composition

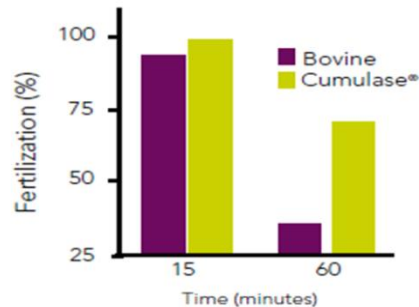
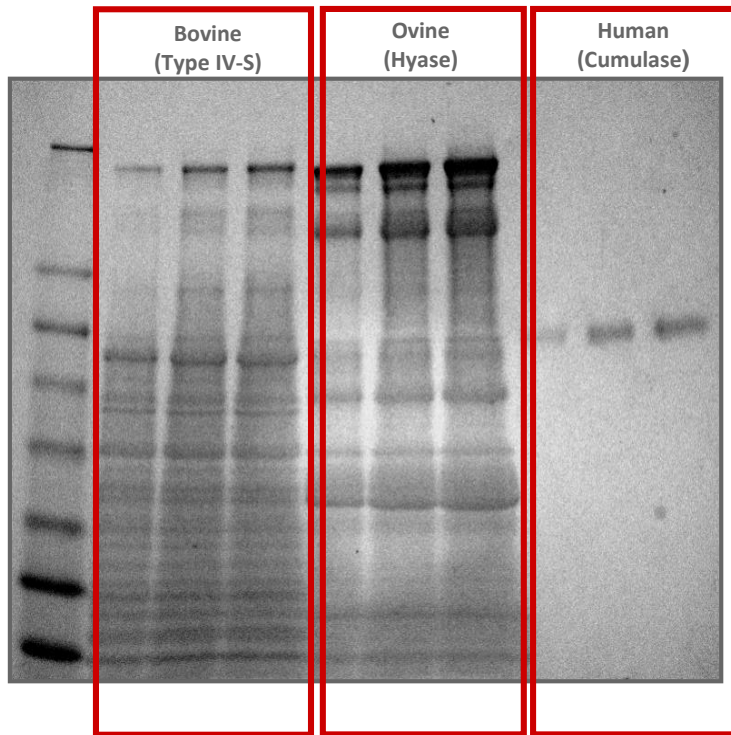
	ORIGIO® Sperm Wash	ORIGIO® Gradient™100	ORIGIO® Gradient™90	ORIGIO® Gradient™80	ORIGIO® Gradient™40
pH	8-8.5	8-8.5	8-8.5	8-8.5	8-8.5
Osmolality (mOsm/kg)	272-288	297-313	297-313	297-313	317-333
Endotoxin (EU/ml)	<0.15	≤0.8	≤0.8	≤0.8	≤0.8
HSA (mg/ml)	10	-	5	5	5
Gentamicin	√	-	√	√	√
Shelf life (weeks)	52	104	52	52	52
Open bottle shelf life (days)	28	28	28	28	28
HEPES buffered for use outside incubator	√	√	√	√	√
Bicarbonate	√	√	√	√	√
Energy substrates	Glucose/Pyruvate	Glucose/Pyruvate	Glucose/Pyruvate	Glucose/Pyruvate	Glucose/Pyruvate
Antioxidants	Citrate, Taurine, EDTA	Citrate, Taurine, EDTA	Citrate, Taurine, EDTA	Citrate, Taurine, EDTA	Citrate, Taurine, EDTA

Preparation of oocytes: 'stripping' or 'denudation'



Preparation of oocytes

Contents of typical products



Behr et al., (2005) showed significantly higher fertilization rates in mouse oocytes after long time exposure (60 min) to Cumulase® as compared to bovine hyaluronidase.

RBM Online - Vol 18 No 6, 2009 811-814 Reproductive BioMedicine Online; www.rbmonline.com/Article/3781 on web 20 April 2009

Article

Human recombinant hyaluronidase (Cumulase®) improves intracytoplasmic sperm injection survival and fertilization rates

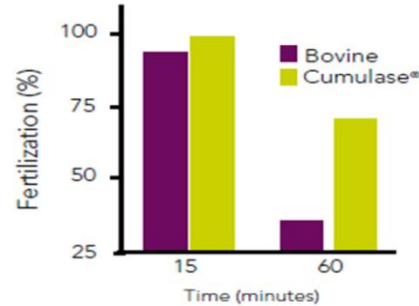
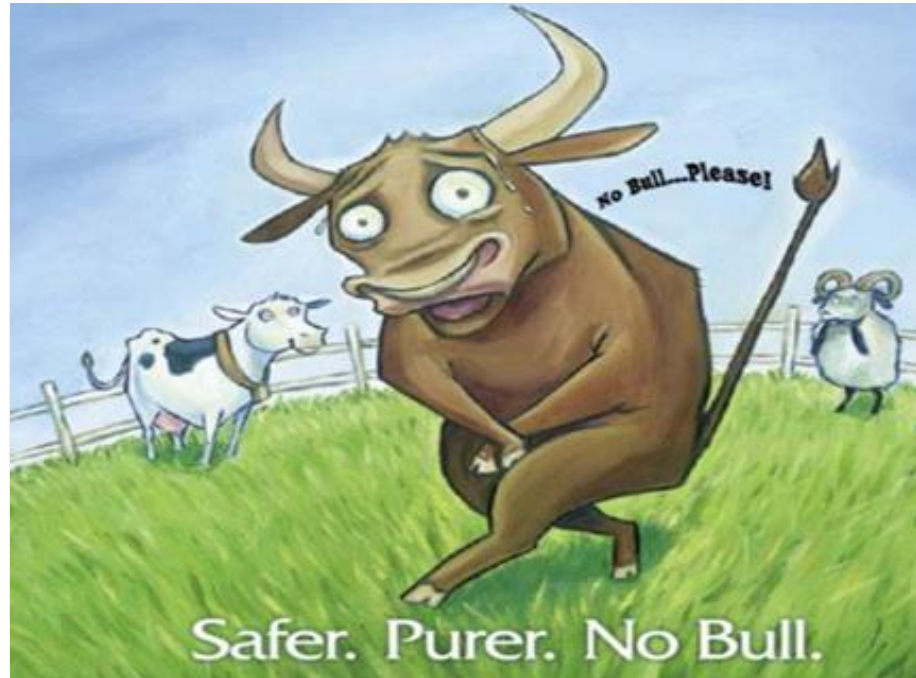


Mrs Melanie Evison

Melanie Evison, Catherine Pretty, Elaine Taylor, Caroline Franklin

Melanie Evison is a senior embryologist at the Woking Nuffield Hospital in Surrey, England. She has been an embryologist since 1997 and is a State Registered Clinical Scientist. She has had considerable experience with ICSI. Her unit is presently gaining much success with their elective single embryo transfer policy and Melanie has used her data analysis skills to validate this and other protocols.

Preparation of oocytes



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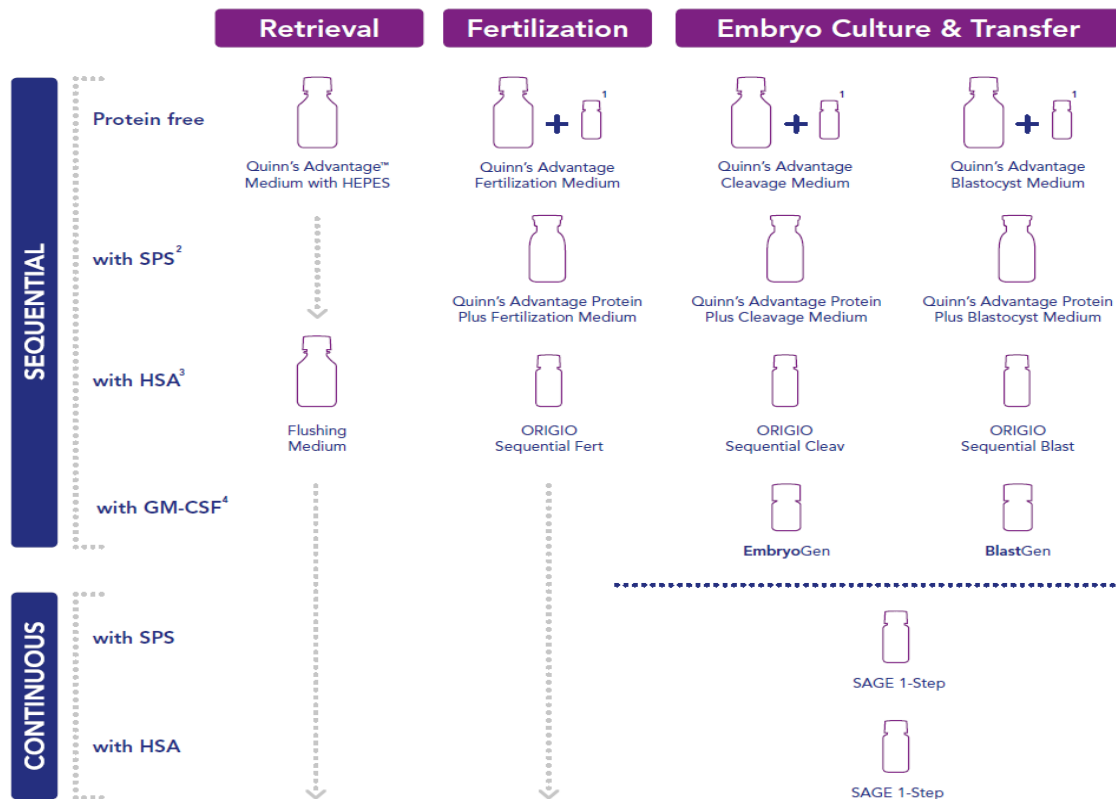


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Culture media for every protocol



1. Protein supplement
2. Serum Protein Supplement
3. Human Albumin Solution
4. The cytokine Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)

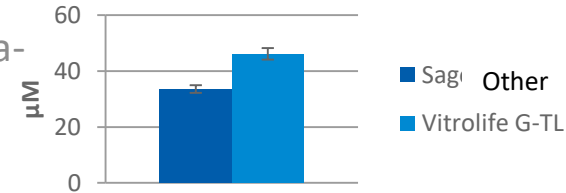
SAGE 1-Step™

SAGE 1-Step™ designed as a true continuous culture medium:

- optimized design for uninterrupted continuous culture (ionic composition and energy substrates)
- hyaluronan – improved embryo development, implantation rates and cryo survival rates
- high Mg^{2+} to Ca^{2+} ratio to limit detrimental stress-induced calcium influx into the embryo
- reduced ammonium build-up via use of a stable glutamine source (Ala-Gln) and increased removal of ammonium through adjusted alanine concentration (pyruvate + ammonium \leftrightarrow Alanine)
- choice of protein depending on preference and regulatory access



Ammonium concentration



SAGE 1-Step™: variants

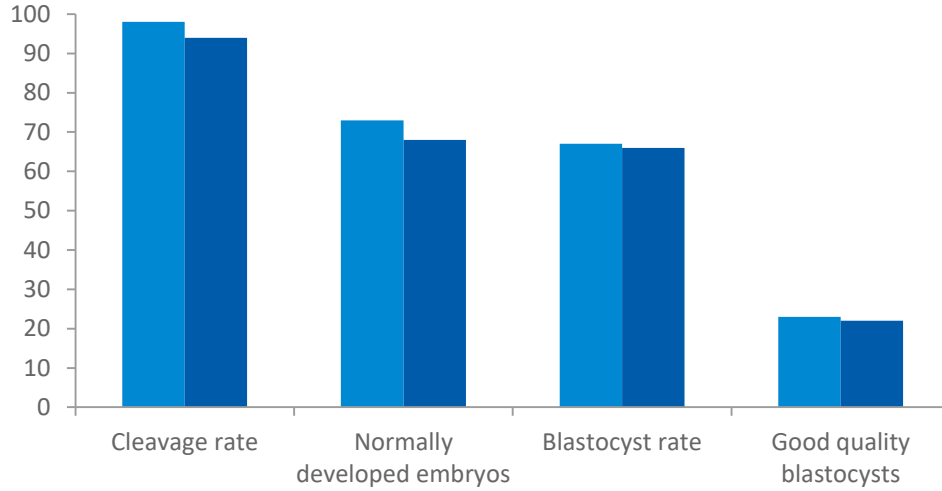
SAGE 1-Step™ comes with two options:

- SPS – HSA + α - and β -globulins
- HSA – HSA + hyaluronan – improved embryo development, implantation rates and cryo survival rates
- base medium the same

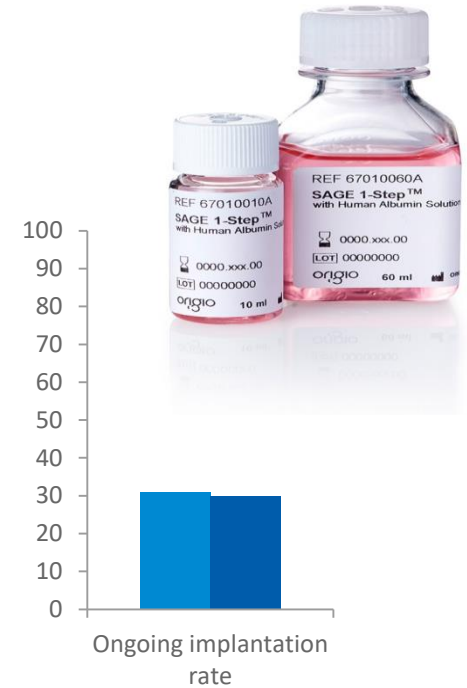


Reference	Description	Size
67010010	SAGE 1-Step™, with HSA and phenol red	10 ml
67010060	SAGE 1-Step™, with HSA and phenol red	60 ml
67020010	SAGE 1-Step™, with SPS and phenol red	10 ml
67020060	SAGE 1-Step™, with SPS and phenol red	60 ml

SAGE 1-Step™: clinical results

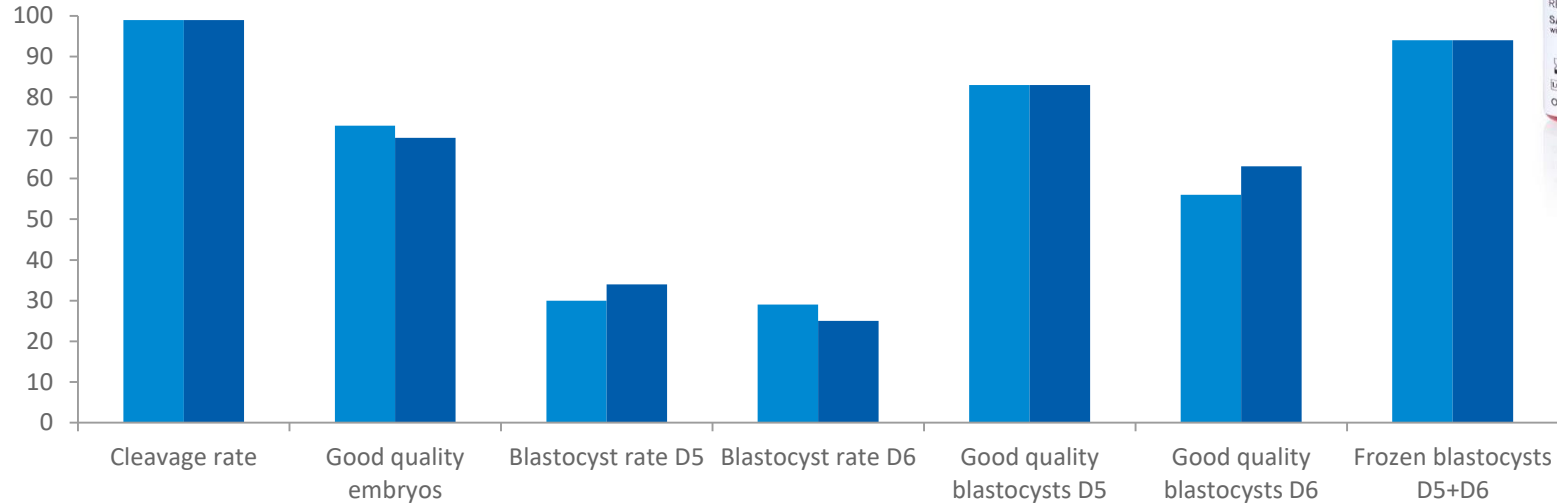


■ Global®Tot...



Conclusion: single culture, uninterrupted v d3 refresh in LG -> same outcomes

SAGE 1-Step™: clinical results

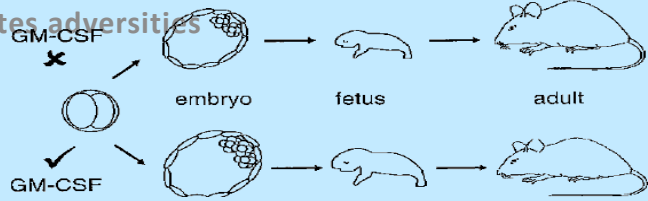


Conclusion: group culture (5 per 50ul drop), uninterrupted v d3 refresh in LG -> same outcomes

GM-CSF- media including a growth factor

- **Recommended for patients who have**
- Experienced recurrent clinical and biochemical pregnancy loss
- Experienced recurrent implantation failure
- Unexplained infertility
- Advanced maternal age

GM-CSF alleviates impact of *in vitro* culture

	(+)GM-CSF	(-)GM-CSF
Robertson et al 2001 Wang et al 2002 Behr et al 2001, 2005 Shapiro et al 2003	<ul style="list-style-type: none"> • enhanced blastocyst formation • enhanced hatching and attachment • enhanced inner cell mass 	control
Papayannis et al 2007	<ul style="list-style-type: none"> • higher blastomere number • better post thaw re-expansion 	control
Imakawa et al 1993, 1997	<ul style="list-style-type: none"> • increased implantation 	control
Sjöblom et al 2005	<ul style="list-style-type: none"> • alleviates adversities 	<ul style="list-style-type: none"> • lower fetal growth • excess compensatory growth after birth • adult obesity
Robertson et al 1999 (GM-CSF gene knockout)	control	<ul style="list-style-type: none"> • retarded blastocyst development • fewer blastomeres • decreased fetal size • increased fetal resorption • higher postnatal mortality

GM-CSF upregulates maternal immune system for implantation

Human Reproduction Vol.16, No.10 pp. 2219–2226, 2001

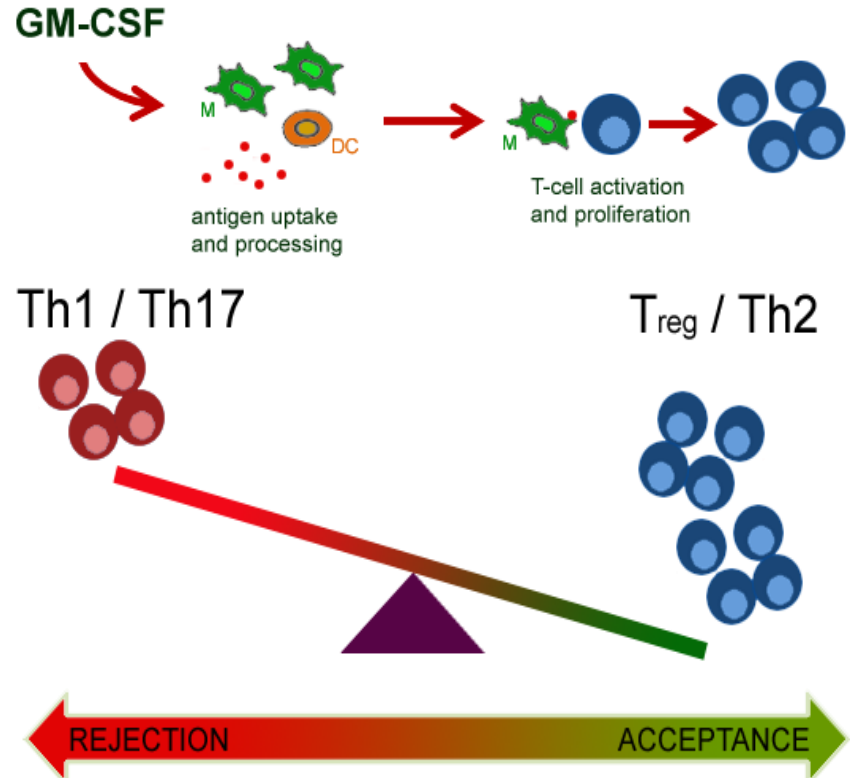
Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions

M.Makhseed^{1,4}, R.Raghupathy², F.Azizieh², A.Omu¹, E.Al-Shamali³ and L.Ashkanani³

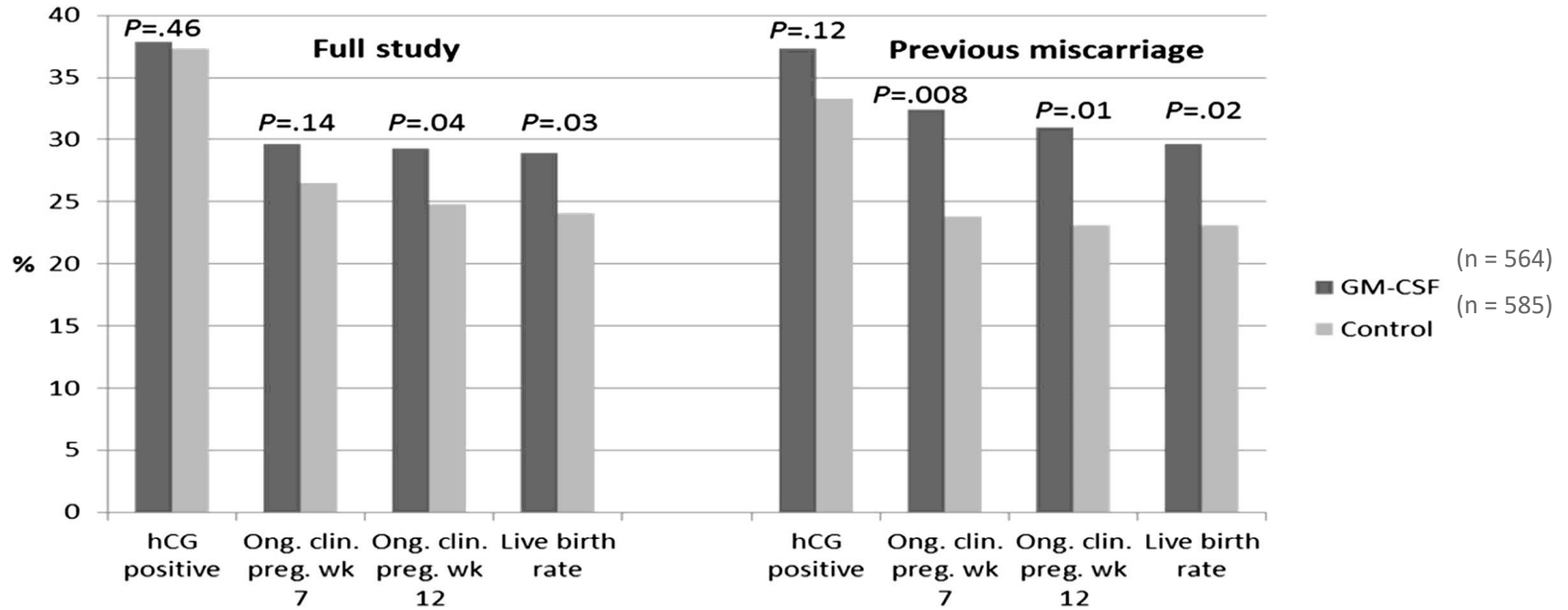
Departments of ¹Obstetrics & Gynecology and ²Microbiology, Faculty of Medicine, Kuwait University and ³Maternity Hospital, Kuwait

⁴To whom correspondence should be addressed at: Department of Obstetrics & Gynecology, Faculty of Medicine, Kuwait University, P.O.Box 24923, Kuwait 13110.

BACKGROUND: This study compared Th1–Th2 cytokine profiles in a subgroup of recurrent aborters who had an abortion with those in a subgroup of recurrent aborters who had a successful pregnancy. **METHODS:** Fifty-four women with a history of at least three normal pregnancies, 24 women with a history of recurrent spontaneous abortion (RSA) followed by abortion (RSA→A) and 39 women with a history of RSA followed by normal pregnancy (RSA→N) were studied. Blood samples and placentas were obtained at the time of delivery or abortion; peripheral blood mononuclear cells were stimulated separately with phytohaemagglutinin and with autologous placental cells, and the secreted cytokines estimated. **RESULTS:** Peripheral blood mononuclear cells from the RSA→N subgroup secreted higher concentrations of Th1-type cytokines as compared with normal pregnant women, indicating a higher Th1 bias in these women. However, women in the RSA→N subgroup had significantly higher concentrations of Th2 cytokines as compared with women in the RSA→A subgroup. A comparison of Th1:Th2 cytokine ratios indicated a higher Th2 bias in RSA→N women as compared with RSA→A women. **CONCLUSIONS:** We conclude that abortion-prone women who proceed to have successful pregnancy are more Th2-biased than abortion-prone women who abort, and that recurrent aborters who undergo spontaneous abortion have a stronger Th1 bias than aborters who have normal pregnancy.



GM-CSF: RCT to evaluate effects in culture media



Ziebe *et al* (2013) *F&S* 99: 1600-1609

EmbryoGen® and BlastGen™

EmbryoGen® and **BlastGen™** are highly innovative and unique culture and transfer media suite which aim to improve clinical outcome, especially in patients with a history of IVF failure

- **EmbryoGen®** and **BlastGen™** make up the only media suite containing the recombinant human GM-CSF cytokine
- A low-cost, non-invasive alternative to specialized clinical treatments for IVF failure/miscarriage
- A high-profile treatment option to generate clinic visibility
- The next step towards a more physiological in vitro environment



Oil overlay

- prevents evaporation
 - humidified vs dry incubators
- reduces rate of temperature and pH change outside incubator
- bacteriostat

Liquid Paraffin

For the overlay of culture media during IVF and ICSI procedures.

- Pure and sterile filtered light paraffin oil of pharmacopoeia and foodstuff grade
- Pre-washed with HSA-containing medium
- Very low endotoxin levels



Ref. No.	Description	Size	Shelf life
10100040	Liquid Paraffin	400 ml	Min. 14 weeks
10100050	Liquid Paraffin	500 ml	Min. 14 weeks
10100060	Liquid Paraffin	500 ml	Min. 14 weeks

Composition

- Liquid Paraffin, Ph. Eur., which has been pre-washed with Universal IVF Medium.

Quality control testing

- Sterility tested (Ph. Eur., USP)
- Endotoxin tested <0.1 EU/ml (Ph. Eur., USP)
- Mouse Embryo Assay (MEA)

Oil for Tissue Culture

Intended for use in laboratory procedures that may require small volumes of tissue culture medium to be covered with a relatively inert, nonmiscible liquid component that allows diffusion of gases.

REF#	Description	Size
ART-4008	Oil for Tissue Culture in Glass Bottle	100 mL
ART-4008-S	Oil for Tissue Culture In Glass Bottle	500 mL
ART-4008P	Oil for Tissue Culture in PET Plastic Bottle	100 mL
ART-4008-SP	Oil for Tissue Culture In PET Plastic Bottle	500 mL
ART-4009W	Washed Oil for Tissue Culture in Glass Bottle	100 mL
ART-4009-SW	Washed Oil for Tissue Culture in Glass Bottle	500 mL

Contains liquid petroleum/paraffin oil/mineral oil with an optimal viscosity for embryo handling and culture. 0.2 µm sterile filtered.



SAGE 1-Step + GM-CSF

- Contain 2 ng/mL GM-CSF
- Base medium formulation same as SAGE 1-Step™
- Contain either 5 mg/mL HSA or 5 mg/mL SPS (non-CE mark)
- HSA version contains Hyaluronan
- Only available with Phenol Red
- Bottle size: 3 mL



Product Code	Brand	Description	Bottle Size	
77010003	SAGE 1-Step GM-CSF	with HSA and phenol red	3 mL	
77020003	SAGE 1-Step GM-CSF	with SPS and phenol red	3 mL	

Oil – minimize environmental impact

Stable hydrocarbon chains, minimal peroxidation

origio

Liquid Paraffin

- Pure and sterile filtered light paraffin oil of pharmacopoeia and foodstuff grade
- **Pre-washed** with HSA containing medium
- 60ml, 5*60ml and 500ml
- Endotox <0.1EU/ml (ph. Eur., USP)



SAGE

Oil for tissue culture

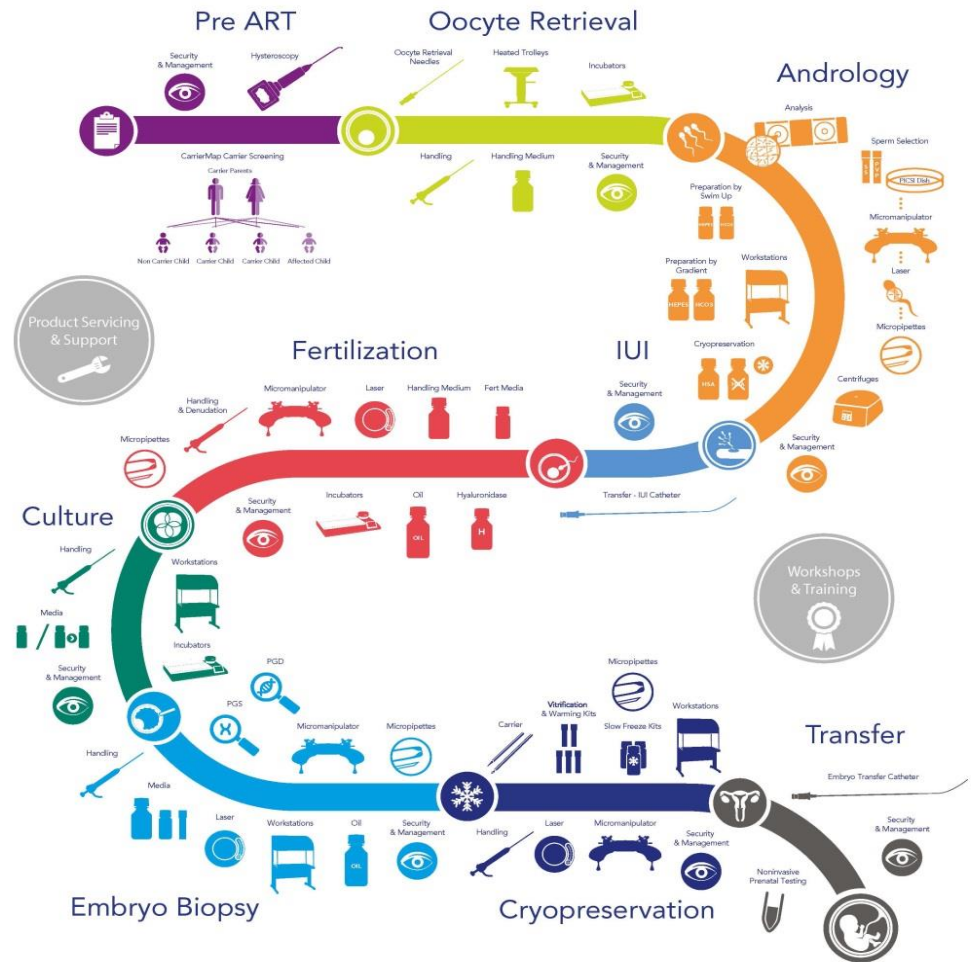
- Pharmaceutical grade light mineral oil
- **Unwashed**
- 100 or 500ml plastic or glass containers
- Atomic Fingerprint



Oil overlay - testing

Features	ORIGIO
Washed with HSA/ medium	✓
Atomic Fingerprint test	✓
Extended MEA	✓
POV test	✓
Endotoxin test	<0.1 EU/mL
Sterility Test	✓
MEA Test	✓
Broad bottle size selection	60, 100, 5x60 and 500 mL
Glass & Plastic Bottles	Plastic / glass
Shelf Life	1 year / 2 years
Open bottle shelf life	✓

Products and Solutions for every step in the ART process



CooperSurgical

A Proven
Portfolio of
Products and
Technologies

Expertise in
Medical Devices,
Fertility and
Genomic Solutions

Trusted by Clinicians
and Health Care
Professionals
Worldwide

Supporting Professional
Development Through
a Global Network of Cutting
Edge Training Centers

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