CooperSurgical Fertility Companies

CooperGenomics
a CooperSurgical company

INDIAN FERTILITY SOCIETY

Indian Fertility Society &
ORIGIO India Initiative

How to improve my ART outcome
Quality Control / Quality Assurance (QA/QC)

Infertility is a disease of the reproductive system characterized by inability to achieve pregnancy after 12 or more months of regular unprotected sexual intercourse.

In this competitive era, IVF clinics maintaining high success rates can only flourish and one thing, which is central to this idea, is quality. Clinics, which can maintain quality in all aspects of patient care, laboratory and clinical practices will set an example for others to follow. It not only detects flaws in the system but also inspires us towards continuous improvement in results and patient satisfaction.

Keeping this in mind we have designed panel of Three Pan India workshops to create awareness about the quality management in infertility.

I am sure you would enjoy the meetings and reading the manual.

Dr M Gouri Devi
President - IFS

According to the World Health Organization, infertility is a disease of the reproductive organs and is defined, as the inability of sexually active couples taking no contraceptives to achieve pregnancy within 1 year.

The treatment of infertility involves many variables many of which are beyond our control. One aspect in our practice, which can help us control these variables, is the practice of total quality management. It not only consists of quality control and assurance but also involves taking appropriate corrective actions and adding value to the existing practice.

I sincerely hope that these programs on "How to improve ART practice " will be of immense use to ART practitioners.

I am sure you would enjoy these programs, which we have put up with great hardwork and sincerity. I am grateful to Origio India for their support in organizing these events.

Prof (Dr) Pankaj Talwar
Secretary General -IFS

I feel honored to be handed over the responsibilty of conducting series of 3 meetings in Kolkata, Patna and Ahmedabad on "How to Improve my ART outcome (QA/QC)".

I am extremely delighted to present before you this handbook on Quality Control and Assurance in Assisted Reproduction Technology.

"Quality is a Lousy Idea – if it is Only an Idea"

Quality management should be an integral part of any ART program's DNA and good practices should be imbibed at spinal level. After achieving the said level of practice, one never feels burdened and quality becomes a way of life. The book is a compilation of series of lectures by renowned experts in the field where we focus on the art of ovarian stimulation and nitty gritty of techniques such as ovum retrieval, embryo transfer and vitrification.

This will be followed by session on proper documentation and audits, the information we derive from these and how to troubleshoot if reports are not satisfactory. We also look at practical aspects such as what patients go through while undergoing an IVF cycle and how we can improve ART dropout rate. In the end, we touch upon what we dread that is how to anticipate and be prepared for dealing with unexpected events in the IVF lab.

I am confident this book will be handy for anyone looking to improve clinical and lab practices.

I would like to thank President and Secretary, IFS for conceiving, organizing and overseeing the whole program. I would like to thank the contributors for taking time off and sending such comprehensive presentations.

I would also like to thank the Origio team for their support in organising the meetings and without which this would have been difficult to achieve. In the end, my sincere thanks to the local chapter secretaries for the extraordinary effort which they have put in.

Dr Sarabpreet Singh
National Coordinator
IFS & Origio QA/QC Initiative

- **MD (AIIMS)**
- **Senior Consultant & Chief Embryologist at the Artemis Health Institute since 2009**
- **IFS and ESHRE-certified Senior Clinical Embryologist**
- **Convenor, IFS SIG (Clinical Embryology)**
- **Executive member of Academy of Clinical Embryologists, India**
- **Area of Interest: Cryobiology and Male Infertility**

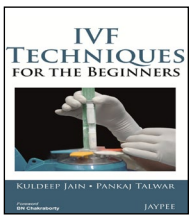
Dr M Gouri Devi
M.D

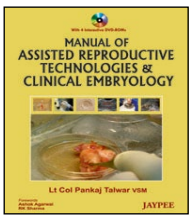
- Director, Gouri Hospitals Ltd.
- Director, Ridge IVF Group.(Runs a chain of IVF centres)
- President, Indian fertility society
- Ex-Secretary General, Indian Fertility Society
- Executive, AOGD governing council
- Member, Executive Board, NARCHI, DGES, FPSI
- Ex Vice President, NARCHI
- Chairperson, Advocacy &Ethics Committee, IFS.
- State Quality Assurance Committee (SQAC)Govt of NCT of Delhi.
- Member: MTP advisory committee, Govt Of NCT of Delhi
- Member Advisory committee on ethical practices in the field of obstetrics, Govt of NCT, Delhi
- Recipient of Kanak Goel Award 1995-1996 from IMA.
- Chairman's Appreciation Award by IMA AMS – 2002
- Dr. APJ Abdul Kalam Excellence Award – 2017
- Economic Times Award one of the Most Inspiring Gynecologists of India

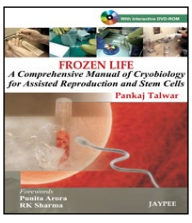
She is a keen academician, has organized many conferences, has been a speaker in many national and international conferences. Has many publications to her credit

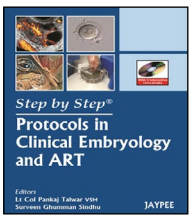
Col Pankaj Talwar, VSM
Professor and HOD
ART Centre, Army Hospital, New Delhi

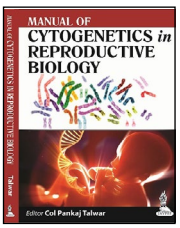
- Sec IFS.
- Secretary Fertility preservation society of India.
- Editorial board of multiple Infertility journals.
- Member Advisory committee ICMR
- Member Infertility committee FOGSI
- Editor Nexus / Artext – E bulletin of IFS
- Awarded Vishisht seva medal by the President of India for working in field of infertility
- Associate Editor FSR
- Set up four centres for Armed forces .
- Experience of 10,000 and ET cycles .
- Member International society of fertility preservation.
- Trained Human Embryonic Stem Cell Derivation – Israel
- Trained in ovarian cortex freezing (fertility preservation) - Paris
- Trained in PGD – Germany, Spain
- Trained in QA/QC-Spain
- Edited 6 books

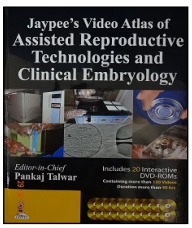


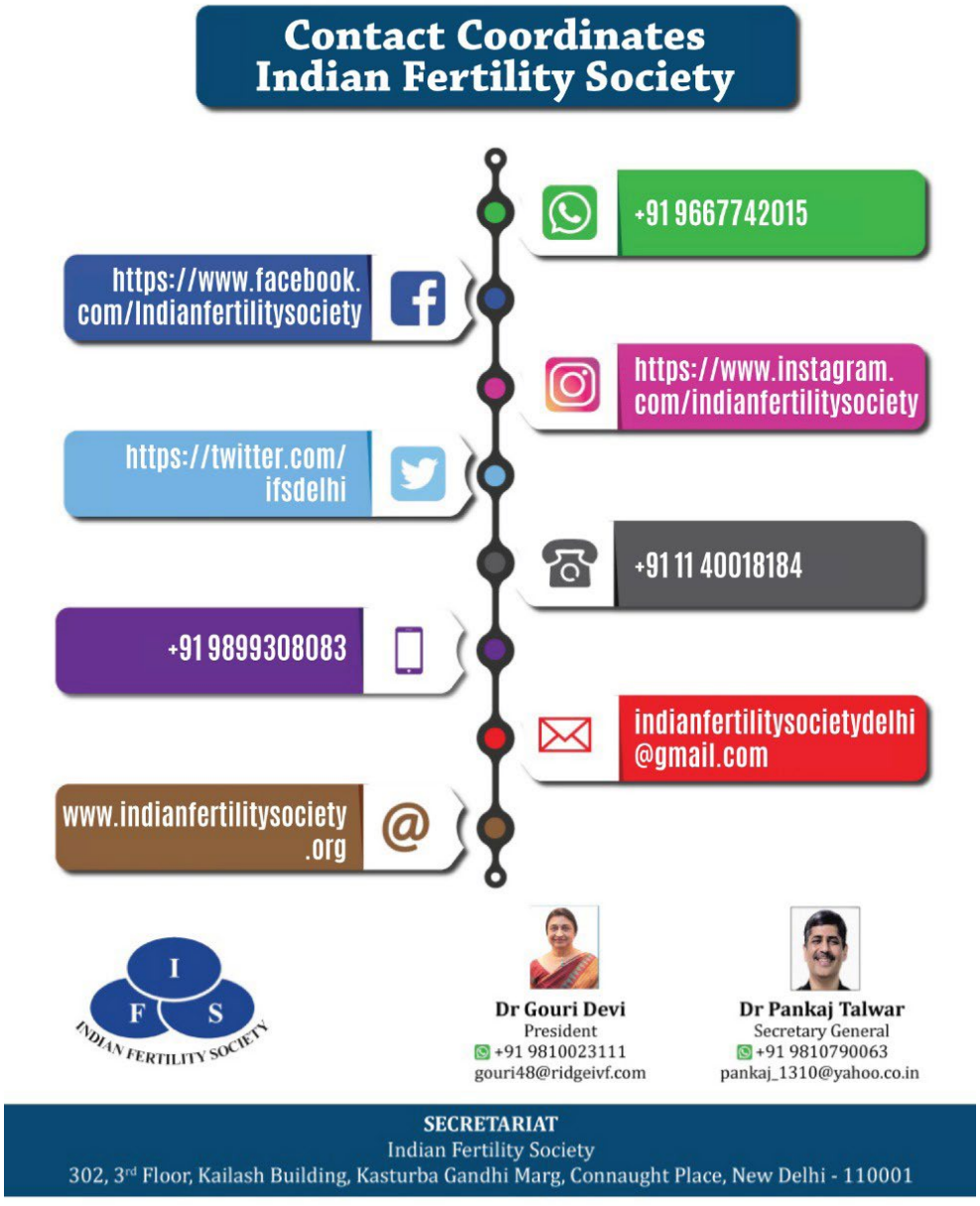








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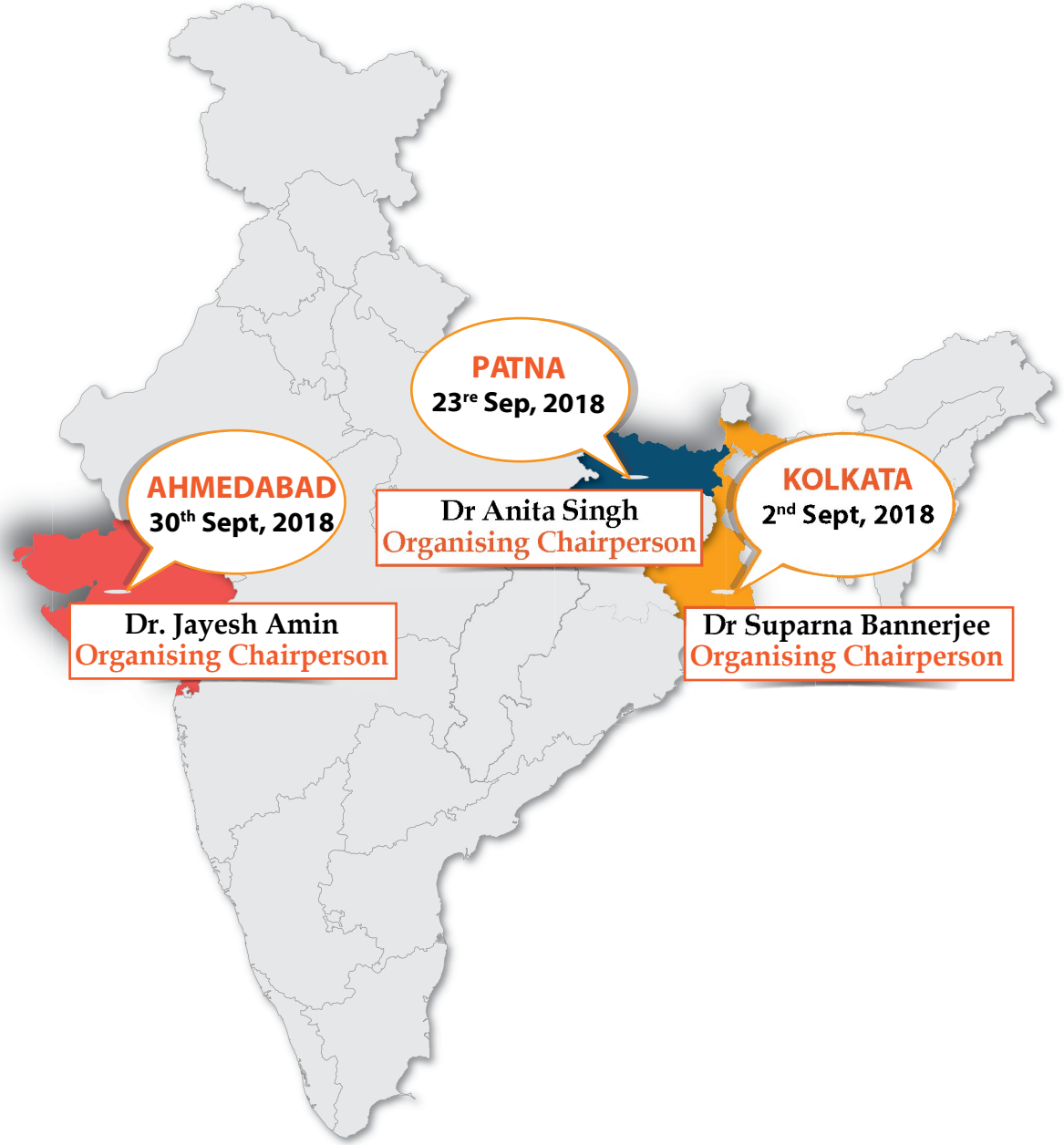
Venue and Dates



Organising Chairpersons

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National Coordinator

Dr Suparna Banerjee
Chapter Secretary
IFS, Bengal

Dr Anita Singh
Chapter Secretary
IFS, Bihar

Dr Jayesh Amin
Chapter Secretary
IFS, Gujrat

List of contributors

Topic	Contributed by
Individualized ovarian stimulation for IVF: finding a balance between cost, safety and outcome	Dr Jayesh Amin
Errorless OPU and ET: Is it possible?	Dr Sonu Blahara Dr Reeta Mahey
Is 100% outcome achievable in Vitrification?	Dr Sarabpreet Singh
KPIs in clinical practice: what is ideal?	Dr Suparna Banerjee
Why patients don't turn up for repeat IVF?	Dr Kalpana Singh
Expecting the unexpected in IVF lab?	Dr Sarabpreet Singh Dr Pranay Ghosh
Panel discussion: What lies between an average and successful IVF program	Dr Piya Ray Dr Saroj Agarwal

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Secretary IFS Bihar chapter
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Programme for the day

Time	Topic
08:30 - 09:00	Registration
09:00 - 09:30	Welcome address
09:30 - 09:50	Individualized ovarian stimulation for IVF: finding a balance between cost, safety and outcome
09:50 - 10:10	Errorless OPU and ET: Is it possible?
10:10 - 10:30	Is 100% outcome achievable in Vitrification?
10:30 - 11:00	Tea & discussion
11:00 -11:20	KPIs in clinical practice: what is ideal?
11:20 - 11:40	Why patients don't turn up for repeat IVF?
11:40 - 12:00	Expecting the unexpected in IVF lab?
12:00 - 12:30	Tea & discussion
12:30 - 13:30	Panel discussion: What lies between an average and successful IVF program
13:30 - 13:40	Vote of Thanks
Lunch	

INDEX

1. Individualized ovarian stimulation for IVF: finding a balance between cost, safety and outcome

INDIVIDUALIZED OVARIAN STIMULATION FOR IVF FINDING A BALANCE BETWEEN COST, SAFETY & OUTCOME

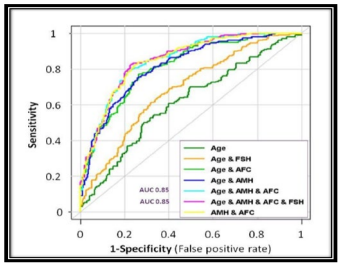
Why individualise?

- Obtain good number & quality egg to produce good euploid embryos
- Avoid OHSS
- Avoid cycle cancellation
- Maximise our success / transfer

Which two groups of patients can be divided ?

- GOOD PROGONOSIS
- LOW PRONOGOSIS

HOW CAN WE DEFINE ?

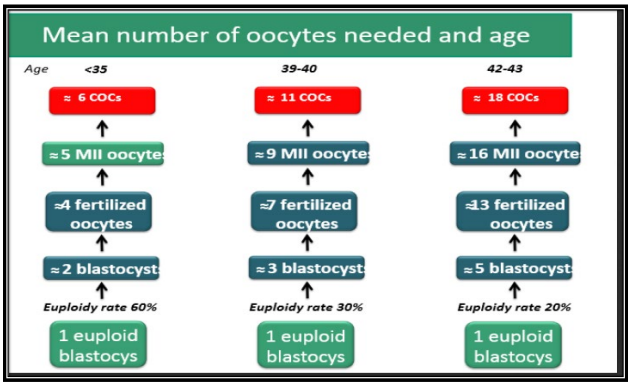


AMH predicts ovarian response

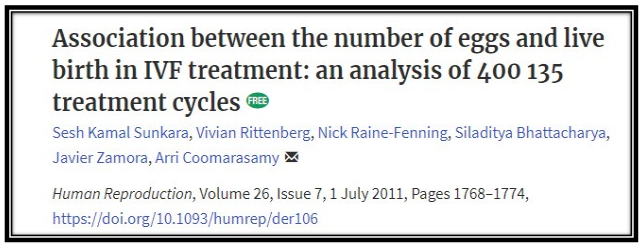
Value of AMH (ng/ml)	Ovarian response		
<0.4	Extreme Poor Responder		
0.4 – 1	0 to 5 AFC	Poor Responder	low prognosis
1 – 2	5 to 10 AFC	Poor Responder	
2 – 3.5	10 to 20 AFC	Normal Responder	
3.5 – 6	> 20 AFC	Hyper Responder	good prognosis
> 6	> 20 AFC	Hyper Responder	

La Marca A. Hum Reprod 2007 ; 22:766-71

HOW MANY OOCYTES ARE NEEDED FOR ATLEAST ONE EUPLOID EMBRYO?



HOW MANY EGGS WERE IVF SUCCESS RATE IS MORE?

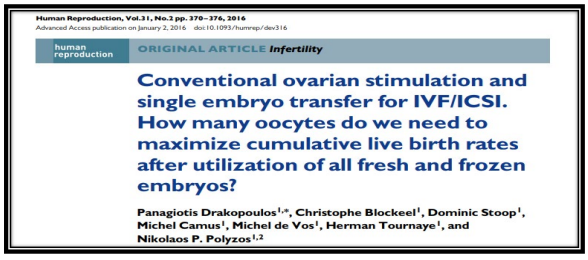


Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles FREE

Sesh Kamal Sunkara, Vivian Rittenberg, Nick Raine-Fenning, Siladitya Bhattacharya, Javier Zamora, Arri Coomarasamy

Human Reproduction, Volume 26, Issue 7, 1 July 2011, Pages 1768–1774, https://doi.org/10.1093/humrep/der106

- 2011-Association between number of eggs & live birth in IVF treatment: analysis of 400135 cycles; Sunkara , et.al concluded 15 eggs can maximise LBR
- 2013 Optimum number of oocytes in IVF treatment: analysis of 2455 cycles; Ji J, et.al concluded oocytes number should be 6-15 for achieving a live birth



Human Reproduction, Vol.31, No.2 pp. 370–376, 2016
Advanced Access publication on January 2, 2016 doi:10.1093/humrep/dev316

human reproduction ORIGINAL ARTICLE *Infertility*

Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos?

Panagiotis Drakopoulos[1,*], Christophe Blockeel[1], Dominic Stoop[1], Michel Camus[1], Michel de Vos[1], Herman Tournaye[1], and Nikolaos P. Polyzos[1,2]

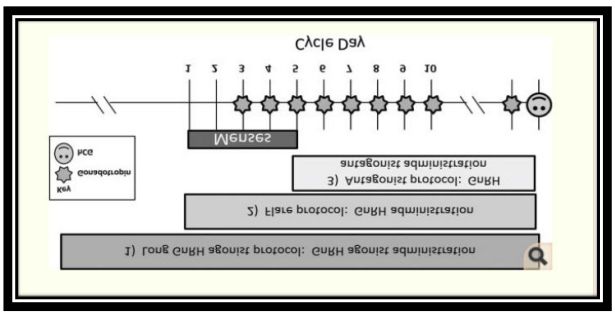
- Women undergoing COS for their first IVF/ICSI cycle and planned SET should be informed that, although the number of oocytes retrieved does not affect LBR in the fresh cycle, the **higher the oocyte yield the higher the probability to achieve a live birth after utilization of all cryopreserved embryos.**

What are the key concerns before starting the stimulations ?

- WHICH PROTOCOL ?
- WHICH GONADOTROPHINS ?
- WHAT IS THE STARTING DOSE ?
- IS ONE SIZE FIT FOR ALL ?
- IS BOUTIQUE APPROACH (individualize) WILL HELP AND WHEN ?

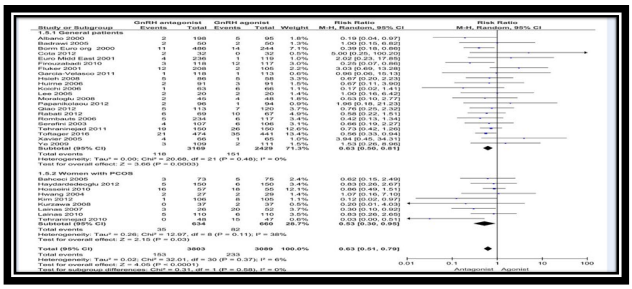
WHICH PROTOCOL ?

General approaches to COS in IVF



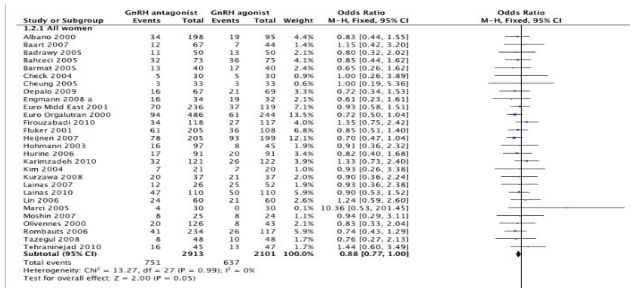
GnRH antagonist versus long agonist protocols in IVF: a systematic review and meta-analysis accounting for patient type.

Cornelis B. Lambalk, Franyke R Banga, +5 authors Madelon van Wely • Published 2017 in Human reproduction update •



Same live birth rate

Gonadotrophin-releasing hormone antagonists for assisted reproductive technology



WHICH GONADOTROPHINS ?

A randomised trial (MEGASET) comparing HP-hMG and r-hFSH in a GnRH antagonist cycle with single blastocyst transfer

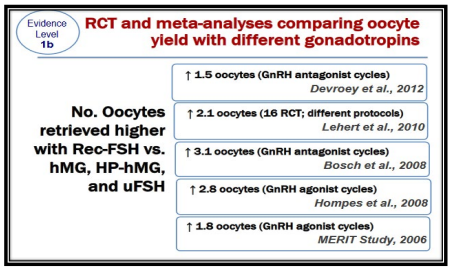
A. Nyboe Andersen[1], A. Pellicer[2], P. Devroey[3], J.C. Arce[4]

Conclusions

Controlled ovarian stimulation with HP-hMG in a GnRH antagonist cycle provides ongoing pregnancy rates that are at least similar to that achieved with rFSH. rFSH yields more oocytes than HP-hMG, but results in more interventions due to excessive responses.

ESHRE, Hum Reprod – suppl. 1, 2011

[1]Rigshospitalet Copenhagen University, Fertility Clinic, Copenhagen, Denmark; [2]Institut Universitari IVI-Valencia, Reproductive Endocrinology, Valencia, Spain; [3]UZ Brussel, Centre for Reproductive Medicine, Brussels, Belgium;[4]Ferring Pharmaceuticals A/S, Clinical R&D (Reproductive Health), Copenhagen, Denmark

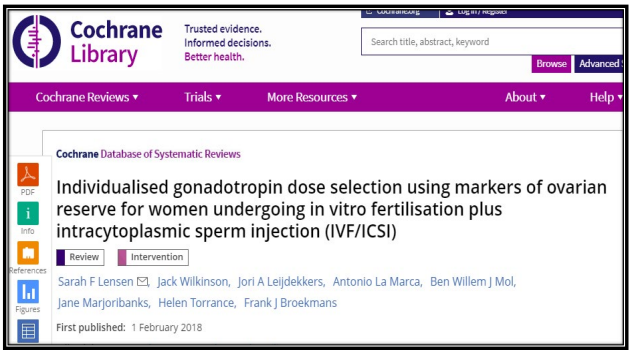


- **For the comparison HMG or HP-HMG versus FSH-P there was also no difference in the evidence on live birth rate (OR 1.36, 95% CI 0.58 to 3.18, 3 trials, 138 women, I^2 = 0%, low-quality evidence).**
- **This suggests that for a woman with a live birth rate of 18% with HMG or HP-HMG, the chance of live birth following uFSH is between 9% and 37%.**

Good Prognosis

- **NORMAL RESPONDER**
- **HYPER RESPONDER**

What is the starting dose One size fit for all ?



CONCLUSIONS: When using a starting dose of 225 IU rhFSH combined with the multiple dose of 0.25 mg cetrorelix from stimulation day 6, significantly more oocytes were obtained than with a starting dose of 150 IU rhFSH.

Live birth or ongoing pregnancy

- **200 versus 100 IU** (OR 0.88, 95% CI 0.57 to 1.36; N = 522; 2 studies; I2 = 0%). This suggests that if the chance of live birth or ongoing pregnancy with 100 IU is 20%, then the chance with 200 IU would be 13% to 26%.
- **225/200 versus 150 IU** (OR 1.03, 95% CI 0.57 to 1.86; N = 277; 1 study). This suggests that if the chance of live birth or ongoing pregnancy with 150 IU is 19%, then the chance with 200/225 IU would be 12% to 31%.
- **300 IU versus 225 IU** (OR 0.65, 95% CI 0.32 to 1.32; N = 135, 1 study). This suggests that if the chance of live birth with 225 IU is 40%, then the chance with 300 IU would be 17% to 47%.
- In the third comparison, the confidence interval remains wide, and it is not clear whether there is any effect from 300 IU versus 225 IU.

- **Cancellation** due to low ovarian response to **stimulation in comparison A (100 versus 200 IU/day recFSH) was observed to be more frequent in the 100 IU/day recFSH dose group** [OR 5.02 (calculated pooled estimates 16.4 and 3.8%, respectively); 95% CI 2.19–11.51; P = 0.0001].
- There was **no difference in cancellation rate** for low response in comparison B (150 versus 200–250 IU/day recFSH) [OR 1.10 (calculated pooled estimates 4.4 and 4.0%, respectively); 95% CI 0.59–2.05; P = 0.76].

Nice recommendations

- **Use an individualised starting dose of gonadotropin based on**

- Age
- BMI
- Ovarian reserve
- Presence of PCO

- **Never use gonadotropin dose >450IU**

Good responder

WHAT ABOUT HYPER RESPONDER?

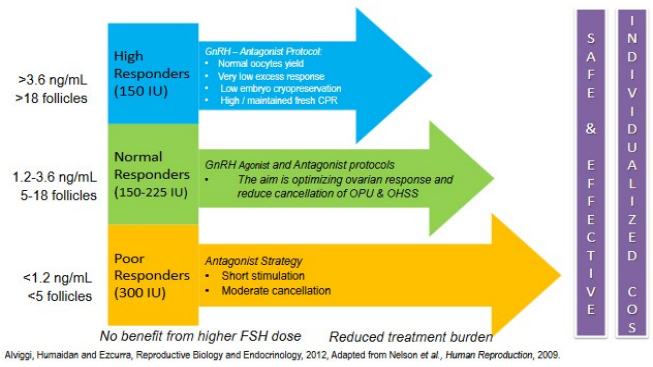
OHSS FREE CLINIC – A RELAITY

Predicted hyper responder

- **We randomized 255 women to a daily FSH dose of 100 IU and 266 women to a daily FSH dose of 150 IU.**
- The **cumulative live birth** rate was **66.3%** (169/255) in the reduced versus **69.5%** (185/266) in the standard group (relative risk (RR) 0.95 [95%CI, 0.85–1.07], P = 0.423).
- The occurrence **of any grade of OHSS** was lower after a lower FSH dose (5.2% versus 11.8%, RR 0.44 [95%CI, 0.28–0.71], P = 0.001), but the occurrence of **severe OHSS did not differ** (1.3% versus 1.1%, RR 1.25 [95%CI, 0.38–4.07], P = 0.728).
- As dose reduction was **not less expensive** (€4.622 versus €4.714, delta costs/woman €92 [95%CI, −479–325]), there **was no dominant strategy in the economic analysis.**

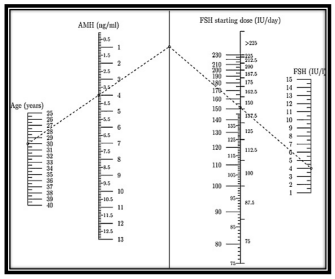
TAILOR APPROACH: IS IT POSSIBLE?

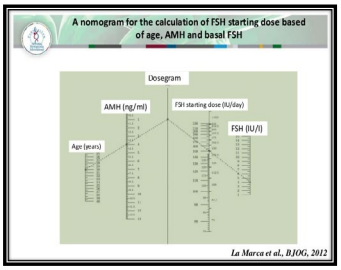
AMH/AFC for choosing protocol?



Alviggi, Humaidan and Ezcurra, Reproductive Biology and Endocrinology, 2012, Adapted from Nelson *et al.*, *Human Reproduction*, 2009.

Concept of individualized ovarian control on age, basal FSH, AMH & BMI





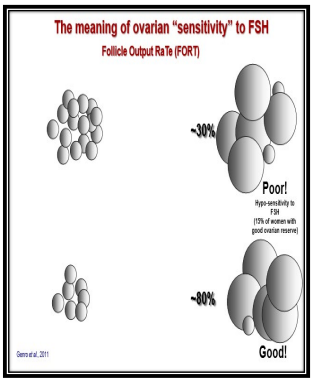
Concept of individualized ovarian control on age, basal FSH, AMH & BMI

AMH Level	Starting Dose FSH	Approx equivalent in µg
<5 pmol/l	210 IU	10-15 µg
5-14.9 pmol/l	180 IU	8-11 µg
>15-29.9 pmol/l	150 IU	6-9 µg
>30-44.9 pmol/l	120 IU	4-7 µg
>45 pmol/l	90 IU	2-5 µg
Not Available	120-180 IU	6-11 µg

LOW PROGNOSIS GROUP

Suboptimal
(4-9 oocytes)
means less than optimal
(10-15 oocytes)

So what is "hypo-response"?



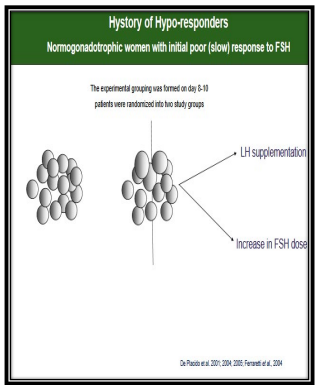
Hypo-response to rFSH

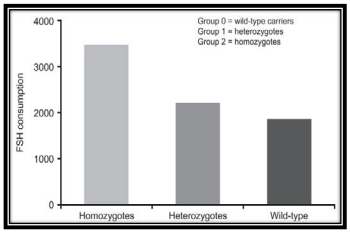
- Hypo-responders are women with normal ovarian reserve who can achieve 'adequate' number of oocytes retrieved and oestradiol production

BUT...

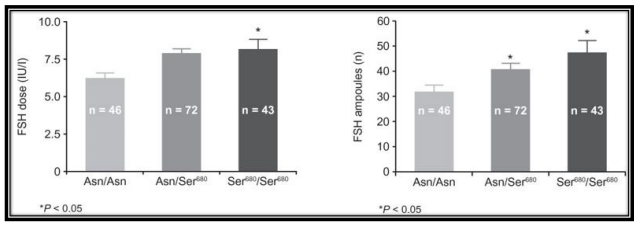
There is an increase in the cumulative rFSH dose (i.e. >3000 IU) and in the stimulation length

De Placido et. al, *Hum Reprod* 2001, *Clin Endocrinol* 2004, *Hum Reprod* 2005, *Drugs* 2008
Ferraretti et. al, *Fertil Steril* 2004, Kailasam et. al, *Hum Reprod* 2004
Alviggi et al., *RBMOnline* 2006, *RBMOnline* 2008, Devroey et al., *Hum Reprod Update* 2009





Concept of individualized ovarian control on age, basal FSH, AMH & BMI

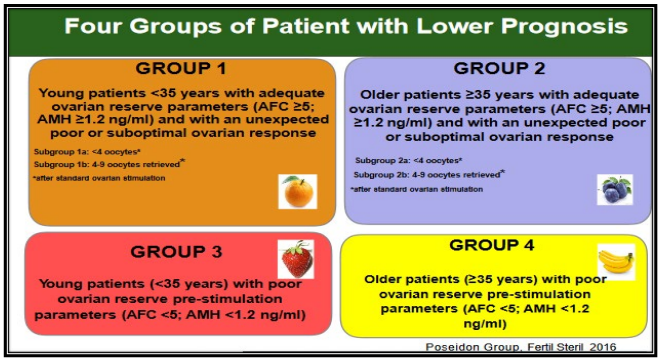


Basal FSH levels and ampoules of FSH used in COS for patients with variants of the FSH receptor. Basal FSH levels (left panel) and ampoules of FSH used in COS (right panel) for homozygote wild-type (Asn/Asn), heterozygote (Asn/Ser680) and homozygote (Ser680/Ser680) carriers of the Ser680 variant of the FSH receptor. ASN = asparagine; CPRs = clinical pregnancy rates; COS = controlled ovarian stimulation; FSH = follicle-stimulating hormone; r-FSH = recombinant follicle-stimulating hormone; Ser680 = Serine680. Adapted from Perez Mayorga et al. 2000

Patient genetic profiles: interpretation of physiology and biomarker levels

Genetic profile	Interpretation of genetic profile
Low AMH levels; low AFC	Suggests that even high doses of FSH would be ineffective and that LH would not improve results Patient would benefit from counselling to better understand her limited chances of success
FSH receptor variant (eg. Ser^{680}); good AMH levels; good AFC	Suggests a good prognosis, but it also predicts a genetic hyposensitivity to FSH that should be considered when formulating COS treatment
v-LH (variant in β subunit of LH receptor)	Suggest that a patient might benefit from LH supplementation during COS

AMH = anti-Müllerian hormone; AFC = antral follicle count.



Four group of patient with low prognosis

... But how many eggs we need?

GROUP 3

Young patients (<35 years) with poor ovarian reserve pre-stimulation parameters (AFC <5; AMH <1.2 ng/ml)

GROUP 4

Older patients (≥35 years) with poor ovarian reserve pre-stimulation parameters (AFC <5; AMH <1.2 ng/ml)

Poseidon Group, Fertil Steril 2016

Four group of patient with low prognosis

... So we need many eggs from women with low reserve
How can we do it if no Gn can compensate?

GROUP 3

Young patients (<35 years) with poor ovarian reserve pre-stimulation parameters (AFC <5; AMH <1.2 ng/ml)

GROUP 4

Older patients (≥35 years) with poor ovarian reserve pre-stimulation parameters (AFC <5; AMH <1.2 ng/ml)

Poseidon Group, Fertil Steril 2016

Characterization of Ovarian Follicular Wave Dynamics in Women[1]

Angela R. Baerwald,[1] Gregg P. Adams,[4] and Roger A. Pierson[2,3]

BIOLOGY OF REPRODUCTION 69, 1023–1031 (2003)
Published online before print 14 May 2003.
DOI 10.1095/biolreprod.103.017772

Documentation of major and minor follicular waves during the menstrual cycle challenges the traditional theory that a single cohort of antral follicles grows only during the follicular phase of the menstrual cycle.

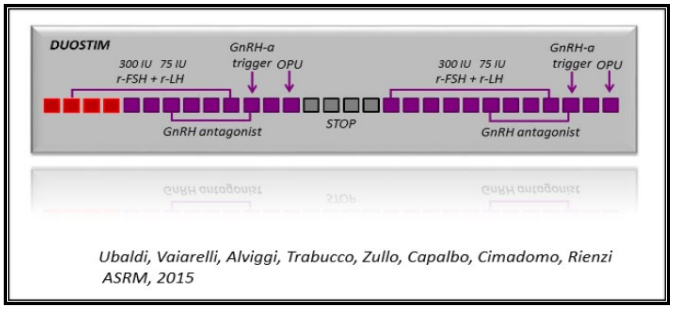
A new model for ovarian follicular development during the human menstrual cycle

FERTILITY AND STERILITY®
VOL. 80, NO. 1, JULY 2003

Angela R. Baerwald, B.Sc. Hon.,[a] Gregg P. Adams, D.V.M., M.S., Ph.D.,[b] and Roger A. Pierson, M.S., Ph.D.[a]

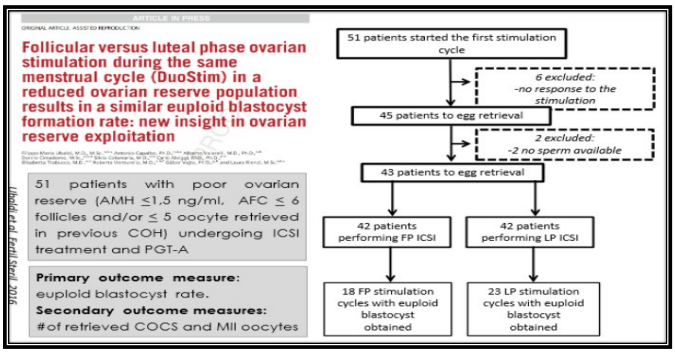
Sixty-eight percent of women exhibited two waves of follicle development during the IOI and 32 %exhibited three waves. Waves were characterized by an increase and subsequent decrease in the number of follicles 5 mm occurring in association with the growth of 2 follicles to 6 mm.

Duostim in low prognostic patient



Ubaldi, Vaiarelli, Alviggi, Trabucco, Zullo, Capalbo, Cimadomo, Rienzi
ASRM, 2015

Duostim in por/ poor prognosis patients



Take home message

- **In low prognosis patients (group 3 -4 Poseidon) duostim can maximize the number of oocytes per menstrual cycle increasing the chance of obtaining the embryo that can give a live birth and it could be applied in all patients with few " fertile time " left available**

Individualized versus standard FSH dosing in women starting IVF/ICSI: an RCT. Part 1: The predicted poor responder

Human Reproduction, Vol.32, No.12pp.2496-2505,2017

Summary:
In women with a predicted poor ovarian response(AFC<11) undergoing IVF/ICSI, an increased FSH dose(225/450 IU/day) does not improve cumulative live birth rates as compared to a standard dose(150 IU/day)

How to reduce the cost ?

IN IVF CYCLE THE MAJORITY OF COST IS OF HORMONES

- So for cost reduction we need to decrease total dose of hormone required and also the duration of stimulation
- **For that we need to compare the cumulative live birth rate in**

- Natural cycle
- Min to mild stimulation
- Conventional stimulation

Multiple Natural Cycles of IVF Required for Reasonable Success Rate

Nargund et al. Hum Reprod. 2001;16:259

4 cycles of natural cycle IVF = 1 cycle conventional IVF

Outcomes of Natural Cycle IVF

- **High cancellation rate** (premature LH surge, failed retrieval, etc)
- **Success rates remain consistently low**
- **Only multiple cycles for the same couples provide similar success to conventional IVF**
- **Per cycle costs 20 – 30% of conventional** (although IVF retrieval, laboratory and embryo transfer costs remain the same)

Comparison of Pregnancy rates for poor responders using IVF with mild ovarian stimulation versus conventional IVF: a guideline

American Society for Reproductive Medicine, Birmingham, Alabama

Fertil Steril June, 2018 by American Society for Reproductive Medicine.

CONCLUSION: Mild ovarian-stimulation protocols with IVF generally aim to use less medication compared with conventional IVF. In patients expected to be poor responders with IVF(based on poor response to a prior IVF cycle, age≥40 years, and /or Bologna criteria), pregnancy rates tend to be low regardless of protocol. There is fair to good evidence that clinical pregnancy rates are not substantially different using mild-stimulation protocols compared with conventional IVF in poor-responder populations. Based on one study, mild stimulation with CC was cost-effective compared to conventional IVF with high-dose gonadotropins.

Conclusions

- Flexible Antagonits for all is the right future.
- Adding LH in hyper responder and normal responder is controversial.
- Adding LH is beneficial in poor responder.
- Mild stimulation (150 IU to 200 IU r fsh) in compared to conventional stimulation gives the same cumulative live birth rates , with patient friendly approach and cost effective.
- Mild Simulation is the last option in poor responder.
- Duo stimulation is the new approach to treat poor responder.
- Pretreatment estradiol valerate is always beneficial
- Query Growth hormone is debatable

Conculsions

SAFETY:
1 : LUPERIDE TRIGGERING
2: OHSS FREE CLINIC

COST EFFECTIVE:
1 : HMG IS EQUALLY EFFECTIVE (DEBEAT STILL CONTIUES FOR ADDING LH)
2: 150 IU VS ANY HIGHER DOSE EQUAL EFFECTIVE

OUT COME:
CUMULATIVE LIVE BIRTH REMAINS SAME WITH ANY PROTOCOL OR ANY DOSE (150 IU IS THE LOWEST THRESHOLD DOSE)

THANK YOU

2. Errorless OPU and ET: Is it possible?

To Err Is Human

Human error means that something has been done that was "not intended by the actor; not desired by a set of rules or an external observer; or that led the task or system outside its acceptable limits".

Applies to the practice of ART

TECHNICAL ERRORS: can compound the problem

To err is human even in IVF

To ensure smooth and errorless operations:

- Reduce potential source of error (HAZARDS)
- Reduce possibility that something unpleasant happens (RISKS)
- A safety culture right from training should be part of program's DNA
- Reinforcement about how to avoid possible sources of errror is the key

Errors in oocyte retrieval – where we can go wrong

- **Treatment**
- **Operational**
- **Technical**
- **laboratory**

AIM

To get maximum yield of oocyte with minimum complications

Factors affecting oocyte yield

- Magnitude of ovarian stimulation
- Type of anesthesia (local, sedation or general)
- Type of aspiration needle (wide or narrow bore or single or double channel)
- Aspiration alone or aspiration with follicular flushing
- Experience and skill of the surgeon.

Ovarian stimulation

Choosing individualized starting dose of Gn acc to
- Age
- BMI
- Ovarian reserve markers(AFC, AMH)
- Previous IVF response

- Effective conscious sedation and analgesia for pain relief
- Any method can be used
- Simultaneous use of sedation combined with analgesia (opiates), further enhanced by paracervical block or acupuncture techniques :
- Better pain relief than occurred with one modality alone.
- Evidence insufficient to show conclusively whether any of the interventions influenced pregnancy rates.
- All techniques : a high degree of patient satisfaction.
- Women's preferences and resource availability for choice of pain relief merit consideration in practice.

Errors in oocyte retrieval

Treatment errors
Stimulation protocol
- Quantity and quality of oocytes

Trigger
- Time
- Dose (mixing it properly)
- Type of trigger

Errors in oocyte retrieval

Trigger
- Not given properly
- Time and dose of trigger (Ensure patient has taken on time)
- Donors make most mistakes
- HCG (only the saline is given and powder not mixed)

Errors in oocyte retrieval

Operational

- Haemorrage
- Bowel injury
- Bladder injury
- Infectious complications (rupture of endometrioma)
- Anesthetic

Errors in oocyte retrieval

Technical errors

- Identify the patient (time out)
- Pressure of suction pump (high pressure leads to empty follicles)
- Temperature (mobile nest, media, work station)
- Minimal duration of exposure to blood after retrieval (Rinsing, incubation)
- Duration of exposure to light (oocytes)

Ovarian stimulation

- Type of aspiration needle
- Suction pressure
- Follicular flushing or no flushing

Type of aspiration needle

- Any size between 15-18 G has been used in different studies
- Smaller size needles- cause less postoperative pain with similar yield of oocytes.

Suction pressure

- 100-120mmHg
- IVM – aspiration pressure should be further decreased
- High suction pressure:
  - oocytes become denuded of cumulus cells
  - Negative impact of increasing aspiration pressures is greater in larger-gauge needles

Follicular Flushing

In DOR patients – to maximise the oocyte yield

Benefits:
May increase the oocyte yield thus increasing pregnancy rate

Disadvantages:
- Time consuming
- More media use
- Risk of infection
- remove some follicular cells that could potentially serve an important endocrine luteal support function.

Errors in oocyte retrieval

Cochrane Database Syst Rev. 2018 Apr 26;4:CD004634. doi: 10.1002/14651858.CD004634.pub3.

Follicular flushing during oocyte retrieval in assisted reproductive techniques.

Georgiou EX[1], Melo P, Brown J, Granne IE.

- follicular flushing probably : little or no effect on live birth rates compared with aspiration alone.
- Data suggest little or no difference between follicular flushing and aspiration alone with respect to oocyte yield, total embryo number, or number of cryopreserved embryos.

- OCR : a safe procedure but is not without risks.
- Most important identifiable risk factors for occurrence of complications are:
  - High number of oocytes retrieved
  - Long duration of the procedure
  - Mean time per oocyte retrieved
  - Inexperience of the surgeon
  - Younger patients with a lesser BMI
  - H/0 prior abdominal or pelvic surgery or PID

Ovarian stimulation

Appraisal of clinical complications after 23,827 oocyte retrievals in a large assisted reproductive technology program

Paolo Emanuele Levi-Setti, M.D.,[a,b] Federico Cirillo, M.D.,[a] Valeria Scolaro, M.D.,[a] Emanuela Morenghi, Ph.D.,[c] Francesca Heilbron, M.D.,[d] Donatella Girardello, M.D.,[e] Elena Zannoni, M.D.,[a] and Pasquale Patrizio, M.D., M.B.E.[b]

[a] Division of Gynecology and Reproductive Medicine, Department of Gynecology, Humanitas Fertility Center, Humanitas University, Milan, Italy; [b] Department of Obstetrics, Gynecology and Reproductive Sciences, Yale University, School of Medicine, New Haven, Connecticut; [c] Biostatistics Unit, Humanitas University, Milan, Italy; [d] Humanitas University, Milan, Italy; and [e] Department of Anesthesiology and Intensive Care, Humanitas Research Hospital, Humanitas University, Milan, Italy

Conclusion(s): Oocyte retrieval can be considered a safe procedure but is not without risks. The most important, identifiable, risk fac- tors for the occurrence of complications are: [1] high number of oocytes retrieved, [2] a long duration of the procedure and mean time per oocyte retrieved, [3] inexperience of the surgeon, [4] younger patients with a lesser BMI, and [5] history of prior abdominal or pelvic surgery or pelvic inflammatory disease.

ESHRE guideline group on good practice in IVF lab (2015)

Oocyte retrieval is a particularly sensitive procedure and special attention should be given to temperature and pH as well as efficient and quick handling

- An identity check before the oocyte retrieval is mandatory.
- The time between oocyte retrieval and culture of washed oocytes should be minimal. Prolonged oocyte exposure to follicular fluid is not recommended

ESHRE guideline group on good practice in IVF lab (2015)

Appropriate equipment must be in place to maintain oocytes close to 37°C. Flushing medium, collection tubes and dishes for identifying oocytes should be pre-warmed.

Follicular aspirates should be checked for the presence of oocytes using a stereomicroscope and heated stage, usually at 8-60x magnification. Exposure of oocytes to light should be minimised

Timing of retrieval, number of collected oocytes and the operator should be documented.

EMBRYO TRANSFER

Embryo transfer

- One of the most critical steps in IVF is embryo transfer
- The technique has a great impact on the IVF results
- In a survey of 80 IVF practitioners, standardization of ET technique was considered the most important factor influencing the success rate

Embryo Transfer : Goal

To deliver the embryo(s) atraumatically to a location within the endometrial cavity that maximizes the chance of implantation

Standardization of Technique

Evaluation of uterine cavity

- Dummy ET
- Ultrasonographic evaluation

Avoiding the initiation of uterine contractions

- Avoid touching the uterine fundus
- Soft catheters
- Gentle manipultion
- Uterine relaxing substances

Standardization of Technique

- Removal of cervical mucus
- Ensure that the ET Catheter passed the internal cervical Os
- Prevention of embryo expulsion

Potential negative factors associated with ET

- Uterine contractions
- Failure to pass the Internal Cervical Os
- Cervical mucus

Important Points

- Mock ET should have been performed before the cycle and properly documented so that there is no problem while performing it
- Identify patient (staff should be aware of the time and identity of the patient)
- Identify the embryos

Technical errors in ET

- If the laboratory is some distance from the embryo transfer room, arrangements should be made to maintain temperature and pH whilst transporting embryos
- A double identity check of the patient, the patient file and the culture dishes is mandatory immediately before the transfer

ASRM practice guideline (2015)

The following interventions are supported by the literature for improving pregnancy rates:

- Abdominal ultrasound guidance for embryo transfer
- Removal of cervical mucus
- Use of soft embryo transfer catheters
- Placement of embryo transfer tip in the upper or middle (central) area of the uterine cavity, about 1-1.5cm proximal to the fundus
- Immediate ambulation once the embryo transfer procedure is completed

Disclosure: 2011 ASRM ethics committee report

- Ethical obligation
- Errors that affect the number of quality of embryos should be disclosed
- Obligatory to disclose errors where gametes or embryos are switched
- Promote culture of truth-telling
- Write procedures for disclosure
- Rigorous procedures for proper ID and prevention of loss

Disclosure

Difficult

- Hard to admit mistake has been made
- Reputation
- Legal fears
- Blame focused on individual, not system

Procedure for Identification

- Witnesses for every step
- Wristband checks for all egg retrievals and transfers
- Time out prior to egg retrieval
- At time of transfer, TV monitor displays pt. name and DOB on bottom of dish to pt., nurses and physician

Conclusions

- The practice of reproductive medicine can involve medical errors in which gametes and embryos are lost, degraded, or misdirected, as well as near misses in which errors are averted before producing any clinical impact.
- This should include written labeling as well as verbal identification at the initiation of embryo transfer.

Conclusions

- The best ethical practice is for programs to have in place rigorous procedures to prevent errors.
- To prepare for the possibility that errors may occur despite these procedures, programs should foster an environment of truth telling that will allow prompt identification and disclosure of errors to patients.
- It is recommended that clinics have written policies and procedures that outline how to reduce and disclose medical errors.

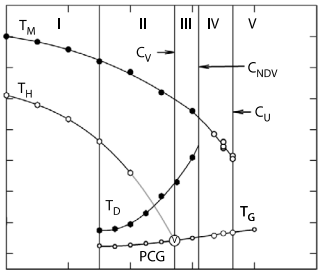
Final Thoughts

Errors are inevitable!

- We can ignore them or
- Use them to improve
- Acknowledge them
- Understand why they happened - get to the source of the problem
- Opportunities to improve processes

3. Is 100% outcome achievable in Vitrification?

Introduction



Melting point,TM,

glass transition temperature, TG,

homogeneous nucleation temperature, TH,

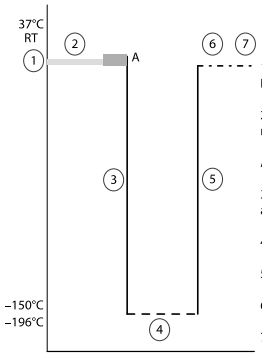
devitrification temperature, TD,

The fundamental issue in all vitrification methods is to achieve and maintain conditions within the cells that guarantee a glass like state and prevent the formation of ice crystals throughout the cooling, as well as during the warming process

- An optimal balance between:

- The speed of cooling, warming (time and temperature), and
- The optimal cell dehydration and penetration of CP when the cells are exposed to concentrated hypertonic solutions

Overview



- Step 1: Selection of embryos (top and good)
- Step 2: Exposure of embryos to the CPsol.
- Step 3: Loading on the carrier device and plunging it in liquid nitrogen (LN_2).
-
- Step 4: Storage in LN_2 containers.
-
- Step 5: The warming process.
-
- Step 6: Rehydration and removal of the intracellular cryoprotectant (CP).
- Step 7: Selection of warmed embryos before embryo transfer.

Step 1: Selection of Embryos

Human Reproduction, Vol.26, No.6 pp. 1270–1283, 2011
Advanced Access publication on April 18, 2011 doi:10.1093/humrep/der037

human reproduction ORIGINAL ARTICLE *ESHRE pages*

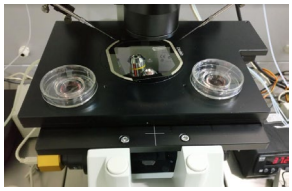
The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting†

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology

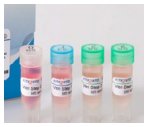
- **Transferable: Top, Good and Fair**
- **Freezable: Top and Good**

How to set-up Vitrification Cooling Media

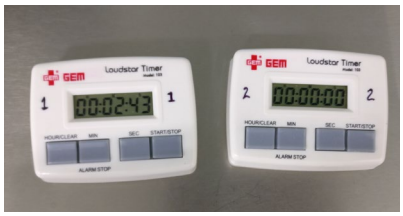
- Follow manufacturer's instructions
- Innovation is ok but experimentation is not



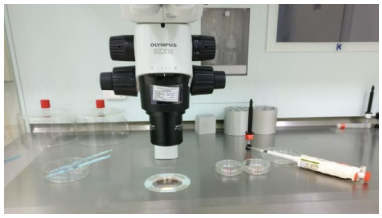
- should be warmed to RT for atleast 30-60 min



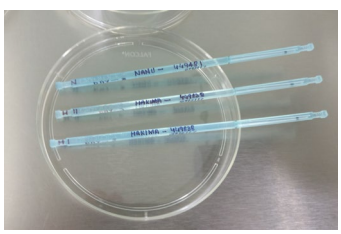
ES & VS

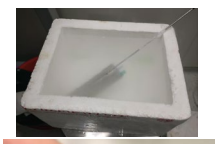


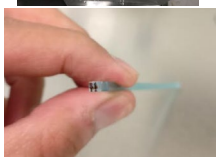
Written instructions explained and pasted



Labeling







Step 2: Exposure of Embryos

- CP toxicity decreases with **temperature:**

- Work on RT, ice

- CP toxicity is **time** dependent

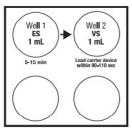
- Don't experiment with time

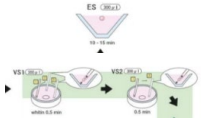
- CP toxicity is **concentration** dependent:

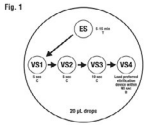
- **Two- cstep strategy** – preincubate in equilibrium solution (5-15 min): 20 – 50 % of the VS oncentration

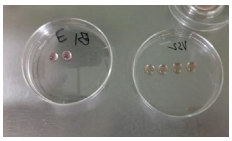
- Short exposure to VS – approx 1 min

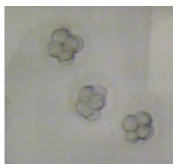
Well vs Drops





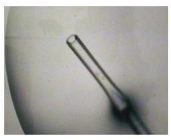




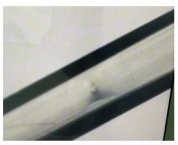


ES: 5-15 min
Stage of embryos

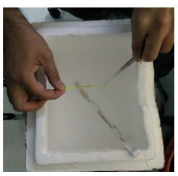
Step 3: Loading on the carrier device and plunging it in Liquid Nitrogen



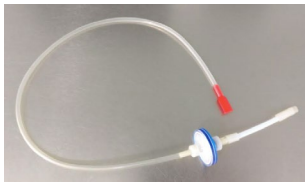
Aspirating embryos with minimal volume



Minimum Drop Size (MDS)

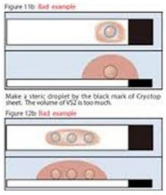


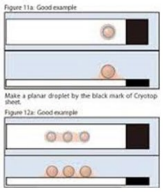
Aspirating Embryos with Minimal Volume



Minimum Drop Size

minimal size that allow us to keep oocytes or embryos without damage due to desiccation
Ambient temperature and relative humidity
2-3 embryos per device





Probability of fracturing = **CR** × µ × *V*

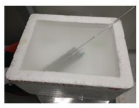
e probability of vitrification is = **CR** × µ × 1/*V*

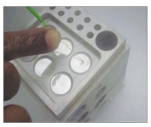
Step 3: Loading on the carrier device and plunging it in liquid nitrogen (LN_2)

Sinking
vs
floating devices

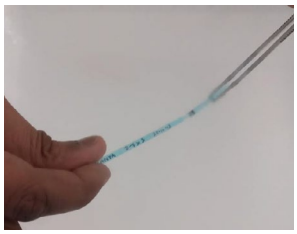
Avoiding Vapor

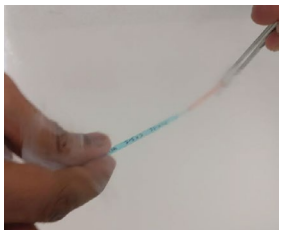
- The vapor coat: prevents a truly high rate of cooling
- Eliminating vapor is important
- Filled to the brim – 0.5 cm
- Cryocane,
- Cap
- LN2 Slush
- Pre-cooled metal surfaces



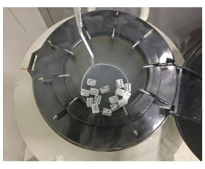


Capping the Device





Proper Placement and Documentation



Regular filling of LN2

Traceability and witnessing

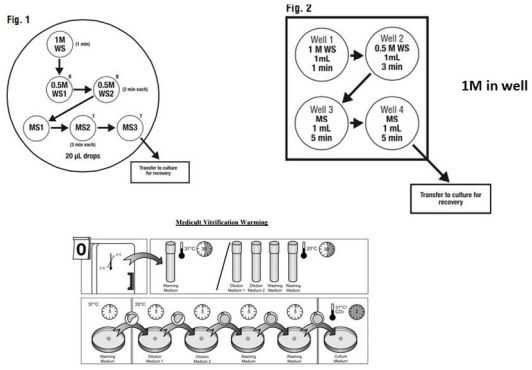
Document everything – esp time of ES and VS

Avoid Multiple patients - separate

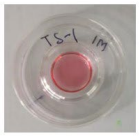
Samples should remain under liquid nitrogen to avoid devitrification

How to set-up Vitrification Warming Media

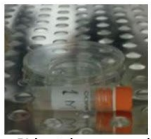
WELL vs DROPS



How to set-up Vitrification Warming Media

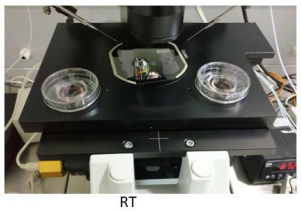


37 °C x 1 hour

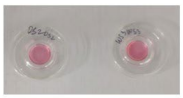


Dishes to be prewarmed before adding media

WARMING RATE



RT

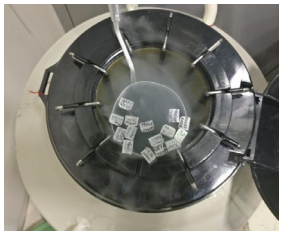


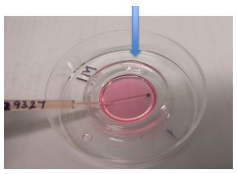
RT

37 °C

Warming

- Seki and Mazur, 2012 demonstrated that the warming rate is dominant over the cooling rate : closed devices
- Warming rates are extremely important:
  -Nucleation of unfrozen freezable water at low temperatures, which may lead to the freezing of this water upon warming, with attendant injury
- keep the samples for 1–3 s in air to avoid fracture damage caused by gas bubbles occurring in the too rapidly immersed samples

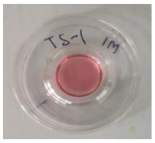




- Spillage
- Media Volume

- Embryos under vision during warming
- Push rather than aspirate if attached
- Embryos float to the top
- Change microscope settings
- Gentle

- **Centre well vs 4 well dishes for warming**



Things to Avoid

- *Safety: cryopreserving multiple patients simultaneously*
- Never place culture dishes or dilutions from different patients on the microscope stage simultaneously.
- During each step in the process one should verify a patient's name and repeat it aloud

- DEVITRIFICATION
  - IMPROPER CAPPING TECHNIQUE
  - IMPROPER HANDLING LEADING TO EXPOSURE/IRREGULAR FILLING OF CYLINDERS
  - WARMING TECHNIQUE

ICE CRYSTALLIZATION
INADEQUATE TIME FOR EQUILIBRATION
MORE THAN TIME TAKEN TO TRAVERSE FROM MELTING POINT TO GLASS TRANSITION TEMPERATURE
EXPOSURE TO VAPOR
CAPPING THE DEVICE UNDER LN2
WARMING RATE < COOLING RATE

Osmotic Injury

- CONCENTRATION OF VITRIFICATION MEDIA MORE THAN WARMING MEDIA WHICH BEING HYPOTONIC RUSHES IN
- TEMPERATURE OF VITRIFICATION MEDIA – not as recommended

Take Home Message

- Freeze inferior embryos first especially at a new set up
- Use device which sinks than floats
- Universal warming media - SF is nothing more than another way of performing vitrification.
- Freeze 2-3 embryos per device (no selective warming)
- Day 2 vs Day 3 vs Day 5 freezing – multifactorial besides operator's preference
- Blastocyst – hatching n post warming incubation

THANK YOU

4. KPIs in clinical practice: what is ideal?

Key Performance Indicator (KPI)

- Performance measurement to identify deviations from the optimum or from established limits, and then being able to act when such deviation exceed certain limit.
- Plays an important role in avoiding adverse consequences and maintaining optimal performance.

Why KPIs ?

- Evaluating the introduction of a technique or process
- Establishing minimum standards for proficiency
- Monitoring ongoing performance
- Benchmarking
- Quality improvement.

KPIS: How do they work?

- Comparing the result with standards
- Standardized methods of measurement
- Specificity of data to be collected and periodicity of measurement

KPIs: Key Features

- Significant
- Reliable
- Routine

LABORATORY KPIs

CLINICAL KPIs

Laboratory KPIs

- Employed for internal quality control in IVF/ICSI programs, using indicators such as oocyte fertilization, cleavage embryo rates, percentage of top quality embryos, etc.
- Can be plotted and compared with established limits for the mean and standard deviation values, so that deviations can be easily recognized as warnings or action points

Advantages

- The KPIs score strategy application could result in an immediate evaluation of the patient's clinical and laboratory performance in the ART cycle
- In addition, internal quality control benchmarks could be evaluated

Association of Clinical Embryologists – Guidelines on Good Practice in Clinical Embryology Laboratories 2012

A6. Evaluation and quality assurance(KPIs)

A6.1. The laboratory should establish quality indicators for systematically monitoring and evaluating the laboratory's performance
evaluated on a regular basis
previous year's results as benchmarks.

A6.2. Intervals between analysis should be based on the laboratory case-load.

A6.3. Laboratories should calculate their own alert conditions for all parameters.

A6.4. Deviation from these bench marks should warrant investigation in line with the clinic's non-conformance policy.

ACE laboratory key performance indicators.*

OVERALL

Based on patients under the age of 40 who had at least three oocytes collected or more.

	KPI	Calculation (×100 for %)*	Suggested benchmarks
1	IVF fertilisation rate	2PN + 3PN/No. inseminated*	>65%
2	IVF abnormal fertilisation rate	≥3PN/No. inseminated*	<5%
3	IVF 1PN rate	1PN/No. inseminated*	<5%
4	ICSI fertilisation rate	2PN/No. injected*	>65%
5	ICSI 1PN rate	1PN/No. injected*	<5%
6	ICSI damage rate	No. degenerate/No. injected*	<10%
7	Failed fertilisation rate	No. of cases with 0 fertilised/No. inseminated*	<5%
8	Low fertilisation rate	No. of cases with < 30% 2PN of Met II*	<10%
9	Cleavage rate	No. cleaved/No. 2PN*	>90%
10	IVF oocyte maturity	No. fert. + Unfertilised Met II/No. oocytes collected*	>80%
11	ICSI oocyte maturity	No. of Met II/No. of oocytes collected* (at time of injection)*	>80%
12	Utilisation rate	No. transferred or frozen/No. 2PN*	>50%
13	Blastocyst formation rate	No. 2PN with progression to D5/D6/Total no. of 2PN*	>50%
14	Frozen embryo survival rate	No. survived/No thawed*	>70%
15	Follicle yield	No of oocytes collected/No follicles punctured*	>80%

*These are suggested benchmarks. Centres should calculate their own benchmarks and trigger points based on their patient population and data.

ACE laboratory key performance indicators.*

FRESH CYCLES

These should be calculated for both D2/3 and D5/6.

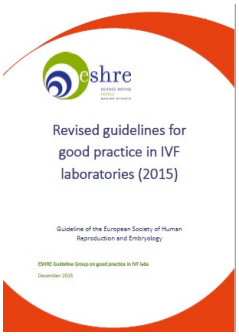
Pregnancy rates (%)	Suggested benchmarks D2/3	Suggested benchmarks D5/6
Positive hCG per oocyte retrieval (OR)	40	45
Positive hCG per embryo transfer (ET)	45	50
Clinical preg. (FH on scan at 7 weeks)/OR	35	40
Clinical preg. (FH on scan at 7 weeks)/ET	40	45
Multiple birth rate per ET	<10%	<5%

FROZEN CYCLES

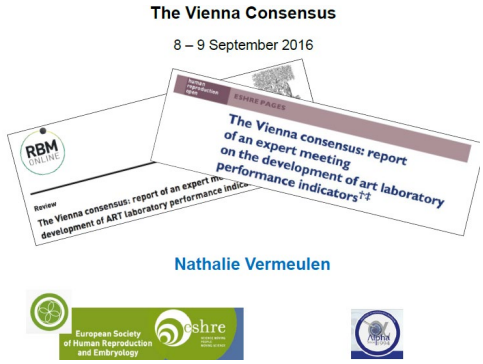
These should be calculated for both D2/3 and D5/6.

Pregnancy rates (%)	Suggested benchmarks D2/3	Suggested benchmarks D5/6
Positive hCG per thaw cycle	35	40
Positive hCG per thaw cycle + ET	40	45
Clinical preg. (FH on scan at 7 weeks)/THAW	30	35
Clinical preg. (FH on scan at 7 weeks)/ET	35	40
Multiple birth rate/ET	<10	<5%

*These are suggested benchmarks. Centres should calculate their own benchmarks and trigger points based on their patient population and data.



- ESHRE revised guidelines for good practice in IVF laboratories (2015)
- KPIs repeatedly mentioned but not covered in detail



Vienna Consensus Meeting

AIM:

- To establish KPIs for use in monitoring "fresh" IVF and ICSI cycles
- To achieve an international consensus regarding a minimum list of IVF laboratory Indicators and KPIs
- Specific definitions for these Indicators
- Recommended values for each KPI

Types of Indicators

- **RIs (reference indicators):** related to the oocytes coming into the laboratory - indirect indicators of the response to ovarian stimulation
- **PIs (performance indicators):** were those for which data should be documented and stored
- **KPIs** were those related to the ART laboratory

Data collection for indicators - monthly basis

Reference population for Indicator Values

- Female patients <40 years old;
- Own fresh oocytes;
- Ejaculated spermatozoa (fresh or frozen);
- No PGD/PGS (PGT)
- Routine IVF and ICSI

RI -Indirect Indicator of Ovarian Stimulation

- Good stimulation: yields a well-expanded cumulus oophorus complex (COC) with higher number of MII oocytes
- Poor stimulation: results in abnormal COC morphology and fertilization and increased rate of aneuploidy
- Oocytes retrieved should be 80–95% of follicles measured in stimulated cycles
- Proportion of MII oocytes at the time of ICSI should be 75–90%

Deviations from the expected range needs investigation of any changes in stimulation protocol

Recommendations for ART modalities

- No KPI for sperm recovery rate because this is heavily dependent on the processing method
- Recommendations for IUI or IVF treatment: post-wash sample showing at least 90% progressive motility
- There was no cut-off value recommendation for ICSI treatment, beyond the spermatozoa ideally being alive

Giant oocytes and oocytes with smooth endoplasmic reticulum cluster should not be injected

Total ICSI failed fertilization rate is not a PI, but should be reported and investigated

Indicators

Table II RIs for identifying performance of the ART laboratory.

RI	Calculation	Benchmark ialue
Proportion of oocytes recovered (stimulated cycles)	$\frac{\text{no. oocytes retrieved}}{\text{no. follicles on day of trigger}} \times 100$	80–95% of follicles measured
Proportion of MII oocytes at ICSI	$\frac{\text{no. MII oocytes at ICSI}}{\text{no. COCs retrieved}} \times 100$	75–90%

MII, metaphase II; RI, reference indicators; COC, cumulus-oocyte complex.

Table III PIs for the ART laboratory.

PI	Calculation	Competency value (%)	Benchmark value (%)
Sperm motility post-preparation (for IVF and IUI)	$\frac{\text{progressively motile sperm}}{\text{all sperm counted}} \times 100$	90	≥95
IVF polyspermy rate	$\frac{\text{no. fertilized oocytes with > 2PN}}{\text{no. COCs inseminated}} \times 100$	<6	
1 PN rate (IVF)	$\frac{\text{no. 1PN oocytes}}{\text{no. COCs inseminated}} \times 100$	<5	
1 PN rate (ICSI)	$\frac{\text{no. 1PN oocytes}}{\text{no. MII oocytes injected}} \times 100$	<3	
Good blastocyst development rate	$\frac{\text{no. good quality blastocysts on Day 5}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$	≥30	≥40

PN, pronucleus; PI, performance indicator; PB, polar body.

KPIs for the ART laboratory.

KPI	Calculation	Competency value (%)	Benchmark value (%)
ICSI damage rate	$\frac{\text{no. damaged or degenerated}}{\text{all oocytes injected}} \times 100$	≤10	≤5
ICSI normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. MII oocytes injected}} \times 100$	≥65	≥80
IVF normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. COCs inseminated}} \times 100$	≥60	≥75
Failed fertilization rate (IVF)	$\frac{\text{no. cycles with no evidence of fertilization}}{\text{no. of stimulated IVF cycles}} \times 100$	<5	
Cleavage rate	$\frac{\text{no. cleaved embryos Day 2}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$	≥95	≥99
Day 2 Embryo development rate	$\frac{\text{no. 4-cell embryos on Day 2}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥50	≥80
Day 3 Embryo development rate	$\frac{\text{no. eight cell embryos on Day 3}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥45	≥70
Blastocyst development rate	$\frac{\text{no. blastocysts Day 5}}{\text{no. normally fertilized oocytes}^a} \times 100$	≥40	≥60
Successful biopsy rate	$\frac{\text{no. biopsies with DNA detected}}{\text{no. biopsies performed}} \times 100$	≥90	≥95
Blastocyst cryosurvival rate	$\frac{\text{no. blastocysts appearing intact}}{\text{no. blastocysts warmed}} \times 100$	≥90	≥99
Implantation rate (cleavage-stage)[b]	$\frac{\text{no. sacs seen on ultrasound}^c}{\text{no. embryos transferred}} \times 100$	≥25	≥35
Implantation rate (blastocyst-stage)[b]	$\frac{\text{no. sacs seen on ultrasound}^c}{\text{no. blastocysts transferred}} \times 100$	≥35	≥60

- The Alpha benchmarks -
Oocyte cryopreservation

	Slow Freezing	Vitrification
Survival	75%	85-95%
Fertilization	No more than 10% (absolute) lower than that of a comparable population of fresh oocytes	
Embryo devel.	The same as for a comparable population of fresh embryos (???)	
Implantation	No more than 10-30% (relative) lower than that of a comparable population of fresh embryos	

- The Alpha benchmarks -
Cleavage stage embryo cryopreservation

	Slow Freezing	Vitrification
Survival (intact)	55%	85%
Survival (>50%)	85%	95%
Implantation	Same as that of a comparable population of fresh embryos	

- The Alpha benchmarks -
Blastocyst stage embryo cryopreservation

	Slow Freezing	Vitrification
Survival	85%	95%
Implantation	Same as that of a comparable population of fresh embryos	

Clinical KPIs

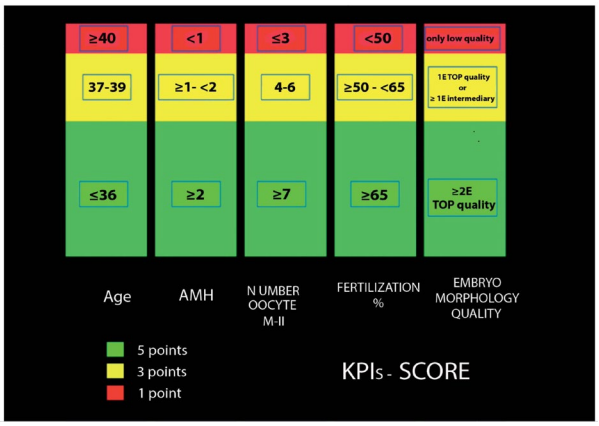
- Age
- BMI
- AMH
- Stimulation protocol
- Number of oocytes obtained

Maximum KPI: 25

- Age <=36
- AMH>=2
- Metaphase ll oocytes >=7
- Fertilisation rate>=65%
- Number of top quality embryos >= 2
- Clinical pregnancy probability is 70%

Maximum KPI: 5

- Age >=40
- AMH <1
- Metaphase 2 oocytes <=3
- Fertilisation rate <50%
- Top quality embryos- none
- Clinical pregnancy probability is 3%



Key performance indicators score (KPIs-score) based on clinical and laboratorial parameters can establish benchmarks for internal quality control in an ART program

José G. Franco Jr[1,2], Claudia G. Petersen[1,2], Ana L. Mauri[1,2], Laura D. Vagnini[2], Adriana Renzi[2], Bruna Petersen[2], M.C. Mattila[1], Vanessa A. Comar[1], Juliana Ricci[1], Felipe Dieamant[1,2], João Batista A. Oliveira[1,2], Ricardo L.R. Baruffi[1,2]

JBRA Assisted Reproduction 2017;**21(2)**:61–6

Clinical KPIs (C-KPIs) – age, AMH and number of oocytes collected
Laboratory KPIs (L-KPIs)– fertilization rate, embryo quality

This paper analyzed if a KPIs–score strategy with clinical and laboratorial parameters could be used to establish benchmarks for internal quality contrc in ART cycles

Key performance indicators score (KPIs-score) based on clinical and laboratorial parameters can establish benchmarks for internal quality control in an ART program

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- This study showed that the values of the total KPIs score have an excellent correlation with the rates of clinical gestation, thus becoming an excellent predictor
- Individualized prognosis for each patient, avoiding a prediction based on global gestational data and all efforts should be directed to increase the total KPIs score in a new attempt if there is no gestation.
- Efforts should be directed to the models of ovarian stimulation, since the age and the AMH values are not susceptible to modifications

Other Factors

- When C-KPI & L-KPIs are maximum but still gestation rate is low then

- Embryo transfer technique
- Endometrial receptivity problem
- Implantation window
- Luteal phase supplementation

Clinic and Laboratory Interaction

Good communication between laboratory and clinic regarding cycle planning and cycle review, ensuring appropriate procedures are ordered, and proper identification of the patient/patient's specimens are crucial for good outcomes in IVF programs

Take Home Message

- The KPIs system was used to detect early warning signals in gamete/embryo cultures
- Each clinic should establish its own key performance indicators and benchmarks
- Develop a systematic, transparent, and consistent approach to data collection and analysis and calculation of KPIs

THANK YOU

Further Reading

1. The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine Reprod. Biomed.Online 2017 25, 146–167.
2. Alpha Scientists In Reproductive Medicine, 2012. The Alpha consensus meeting on cryopreservation key performance indicators and benchmarks: proceedings of an expert meeting. Reprod. Biomed.Online 25, 146–167.
3. Association of Clinical Embryologists – Guidelines on Good Practice in Clinical Embryology Laboratories 2012, Human Fertility, 15:4, 174-189,

5. Why patients don't turn up for repeat IVF?

Introduction

- There has ben significant developments in the field of ART in the last four decades since the birth of the first IVF baby in 1978
- Though the treatment has become simple and effective
- In spite of this, majority of the patients do not come back for a repeat treatment cycle after the failure of the initial attempt
- It is largely due to the fact that ART still remains an expensive, time-consuming and physically & emotionally draining treatment with an uncertain outcome

Why patients don't turn up for repeat IVF

- TREATMENT ASSOCIATED

- Psychological burden
- Physical stress
- Financial burden
- Ovarian hyper-stimulation syndrome (OHSS)
- Ectopic pregnancy

- PATIENT FACTORS

- UNREALISTIC EXPECTTAIONS: efficacy of ART
- Elderly patients feel they are past the reproductive age
- Unable to accept failure
- Higher drop out in elderly patients

Clinic Related

- Inadequate counseling
- Inadequate confidence / faith in the treatment / doctor
- Unfriendly staff
- Inadequate preparation for a long treatment duration requiring
- multiple cycles of ART
- Lower success rates
- High miscarriage rates

Patients Perceptions

- Mahlstedt et al. found that 82% of the patients did not view the decision to enter the IVF program to be a difficult one and that upon entry to the program 56% of patients indicated they would repeat the IVF treatment if the initial procedure was not successful
- Interestingly after the first IVF cycle, only 37% of couples planned to repeat the treatment
- Factors they found that influenced patients' plans not to repeat IVF included emotional strain, medical barriers, and disruption of activities
- They feel they have passed the reproductive age

J Assist Reprod Genet. 1997 Jul; 14(7): 381–384. PMCID: PMC3454774
doi: 10.1007/BF02766144 PMID: 9285321

Factors influencing patients' decision not to repeat IVF

James Goldfarb, Cynthia Austin, Hanna Lisbona, Ricardo Loret de Mola, Barry Peskin, and Sandy Stewart

Abstract

Women who did not pursue a second in vitro fertilization cycle after a failed cycle were surveyed. The major reason for not pursuing a second cycle was financial.

Key words: finance, in vitro fertilization, psychologic stress

for them.

The responses to the open-ended questions were limited, with a maximum of 15 responses to any one question (Table III). The most common response was

Table III. Responses to Open-Ended Questions

Questions	Number
Can you think of any significant positives about IVF experience?	
Professionally competently run	2
Caring supportive staff	2
Staff helpful	1
Staff pleasant	1

GOLDFARB *ET AL.*

Table II. General Attitudes of Couples Regarding Their IVF Cycle ($n = 28$)[a]

Attitude	1	2	3	4	5
Difficult experience physically	3	8	2	12	3
More unpleasant than thought	4	3	3	14	4
Was sure IVF would work	5	11	6	6	0
Team supportive of emotional needs	1	13	5	6	3
Found IVF very difficult emotionally	12	12	2	1	1
Found IVF very difficult financially	14	7	2	4	1
Received adequate guidance emotionally	4	7	6	9	2
Received adequate guidance financially	8	14	3	3	0

[a]1 = strongly agree and 5 = strongly disagree

Physical, Mental and Emotional Stressors in Females

Many patients describe IVF as an emotional roller-coaster

- Female under goes physical pain of taking injections
- Multiple sittings of tVS
- The stress of undergoing ovum pickup
- being informed that very few ova have been retrieved
- Expectation crashes when told not good/ very few embryos have developed
- Finally awaiting result and being told that Bhcg is less than 10 on day 15 after embryo transfer

Financial drain in IVF

- Since IVF is a complex process and needs a series of procedures thus cost rises
- Cost also increases with donor's sperm/eggs, sperm retrival process, ICSI, assisted hatching ,embryo freezing cost etc.
- High cost of hormones, disposables & media
- Limited success rate: many require multiple attempts of fresh and frozen cycles
- Many patients under loan, sell land or jewelry to afford single cycle of IVF

PHYSICALLY TIRING

- Multiple visits
- synthetic hormones, and they make patient feel intense PMS, Mood swings, hot flashes
- temporary weight gain/bloating are common, as are headaches, breast tenderness and nausea
- Some women feel skin irritation at the injection site

TIME CONSUMING

- **Fertility treatment takes time, and may disrupt couples work Schedule and routine at home And the uncertainty of the Outcome just adds more stress**
- **Working class women affected more**

Misinformation and lack of transparency

- **Mostly pts are informed the maximal CPR/LBR, where as it may be different in their individual case due to added pelvic pathology/hormonal milieu**
- **Pts don't want to believe the lower end of success rate so their expectations are always on higher side, so more depression after failure**

Unrealistic expectations: Guaranteed baby/ Male baby

- Many centers advertise/show unrealistic success rates for alluring the couple
- Since a large amount of money is involved they expect 100% result and expect miracles from doctors
- Indians have a great desire for male baby so many times center allure by false promises which later they don't fulfill or deny

Stressed out patients looking for instant results

The prevalence of stress among women was 80%.

- Univariate analysis revealed that predictors of stress were years of marital life
- Duration and type of infertility
- history of gynecological surgery,
- cycles of ovulation induction and intra-uterine inseminations,
- present and past psychiatric morbidity, coping difficulties, gynecological diagnosis, and severity of premenstrual dysphoric disorder

Counselling

- Counseling: type of therapy to provide emotional and psychological support for a person who is undergoing certain difficulties, or experiences in life
- Well trained counselor is a must in IVF setting
- The following situations are usually addressed during IVF counseling:

- implications of treatment
- failed ivf treatment
- succeeding attempt after unsuccessful procedure
- successful ivf treatment and successful birth
- miscarriges
- handling mixed reactions from other sources
- alternative options

Inadequate Counseling

- Counseling has an important role
- COUNSELING not only informs the couple of procedure, side effects and complications but also addresses their questions and apprehensions
- Inadequate time & importance to counseling increases patients stress manifolds
- Individualized counseling after failures has to be emotionally supportive as well as informative about cause of failure and chances of success in future attempts

How to prevent ART drop out

- Better success rates

- proper case selection

- Patient-friendly treatment
- Cost-effective treatment:

- dropout rate is lower with antagonist cycle and mild ovarian stimulation, in contrast to the traditional agonist cycle with standard stimulation dose

- Continuity of care delivered by the same team consisting of the doctor, the nurse and the counselor

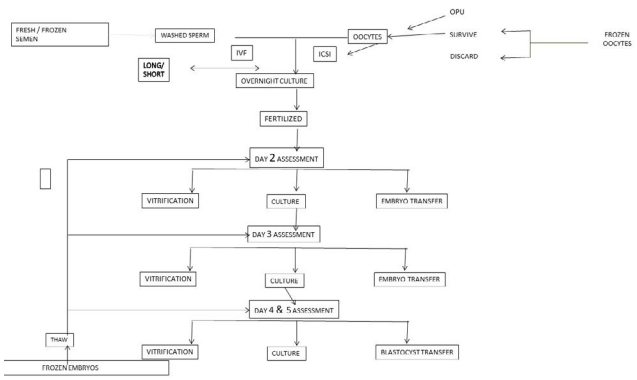
Misinformation and Lack of Transparency

- Availability of 24 X 7 communication and counselling as and when necessary give the couples necessary emotional support and encourages them to come for repeated attempts of treatment
- Discounts on subsequent cycle
- Lower professional fee
- Help from the government and charitable organisations
- Cryopreservation of good quality surplus embryos

THANK YOU

6. Expecting the unexpected in IVF lab?

FUNCTIONING OF AN IVF LAB



You can't improve what you cannot measure!

The Vienna consensus: report of an expert meeting on the development of art laboratory performance indicators[†,‡]

ESHRE Special Interest Group of Embryology[1,*] **and Alpha Scientists in Reproductive Medicine**[2,*]

Table IV KPIs for the ART laboratory.

KPI	Calculation	Competency value (%)	Benchmark value (%)
ICSI damage rate	$\frac{\text{no. damaged or degenerated oocytes}}{\text{all oocytes injected}} \times 100$	≤10	≤5
ICSI normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. MII oocytes injected}} \times 100$	≥65	≥80
IVF normal fertilization rate	$\frac{\text{no. oocytes with 2PN and 2PB}}{\text{no. COCs inseminated}} \times 100$	≥60	≥75
Failed fertilization rate (IVF)	$\frac{\text{no. cycles with no evidence of fertilization}}{\text{no. of stimulated IVF cycles}} \times 100$	<5	
Cleavage rate	$\frac{\text{no. cleaved embryos Day 2}}{\text{no. 2PN/2PB oocytes on Day 1}} \times 100$	≥95	≥99
Day 2 Embryo development rate	$\frac{\text{no. 4-cell embryos on Day 2}}{\text{no. normally fertilized oocytes}^3} \times 100$	≥50	≥80
Day 3 Embryo development rate	$\frac{\text{no. eight cell embryos on Day 3}}{\text{no. normally fertilized oocytes}^3} \times 100$	≥45	≥70
Blastocyst development rate	$\frac{\text{no. blastocysts Day 5}}{\text{no. normally fertilized oocytes}^3} \times 100$	≥40	≥60
Successful biopsy rate	$\frac{\text{no. biopsies with DNA detected}}{\text{no. biopsies performed}} \times 100$	≥90	≥95
Blastocyst cryosurvival rate	$\frac{\text{no. blastocysts appearing intact}}{\text{no. blastocysts warmed}} \times 100$	≥90	≥99
Implantation rate (cleavage-stage)[b]	$\frac{\text{no. sacs seen on ultrasound}^c}{\text{no. embryos transferred}} \times 100$	≥25	≥35
Implantation rate (blastocyst stage)[b]	$\frac{\text{no. sacs seen on ultrasound}^c}{\text{no. blastocysts transferred}} \times 100$	≥35	≥60

[a]Defined as oocytes with 2PN and 2PB on Day 1.
[b]Based on total number of embryos transferred to all patients in the reference group, not just those for whom an implantation occurred.
[c]Definition reached after discussion, as some felt that no. fetal hearts / no. embryos transferred was a more meaningful indicator.
KPI, key performance indicator.

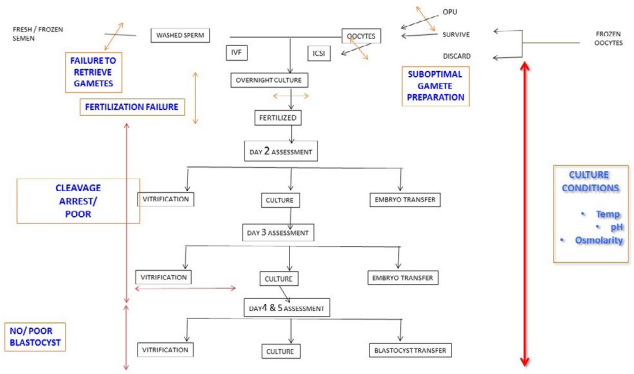
ANY DEVIATION

↓

UNEXPECTED

↓

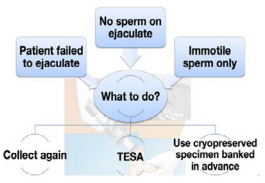
REASON & TROUBLESHOOT



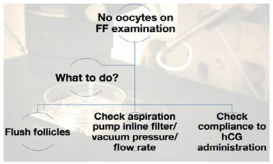
Failure to retrive gametes

SEMEN SAMPLE

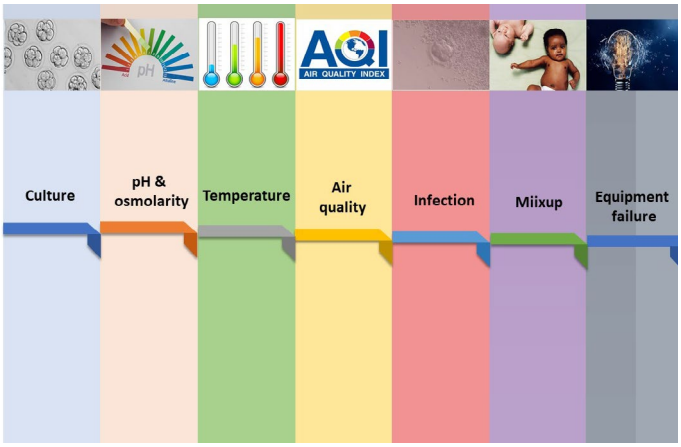
FROZEN SAMPLE
COUNSELLING
TESA
OOCYTE FREEZING

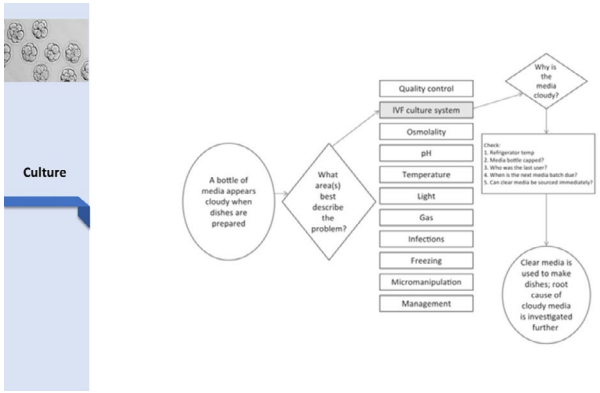


OOCYTES

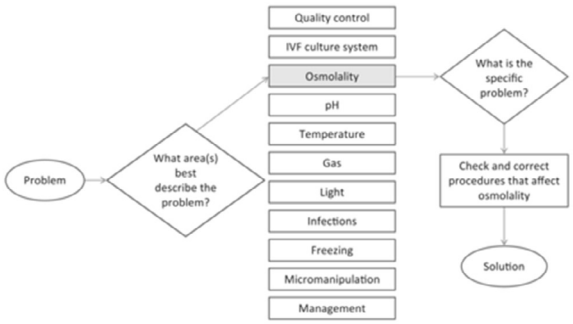


FROZEN OOCYTES IN CASE OF DONOR OPU





You can't improve what you cannot measure!



Osmolarity

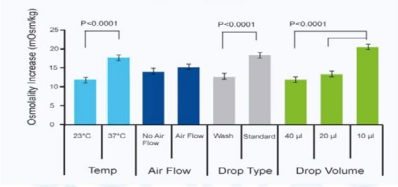
- The optimal osmolarity for pre-implantation embryos is 260 mOsm/L

Company	Medium	Stages[a]	Osmolality range (mean) mosmoles/kg
LifeGlobal® (IVFonline)[b]	global®[c]	All	260–270 (265)
	HTF	Cleavage	280–292 (286)
	Blastocyst	8c to Bl	260–270 (265)
Origio (MediCult)[f]	Universal IVF	Cleavage	277–293 (285)
	ISM1™	Cleavage	272–288 (280)
	ISM2™	8c to Bl	272–288 (280)
	EmbryoAssist™	Cleavage	272–288 (280)
	BlastAssist®	8c to Bl	272–288 (280)
VitroLife®	G-1™ (G5 series™)	Cleavage	256–266 (261)
	G-1™ with HSA (G5 series™)[h]	Cleavage	249–259 (254)
	G-2™ (G5 series™)	8c to Bl	255–265 (260)
	G-2™ with HSA (G5 series™)[h]	8c to Bl	248–258 (253)

Baltz (2012) Methods Mol Biol. 912:61-80. doi: 10.1007/978-1-61779-971-6_5

FACTORS AFFECTING OSMOLALITY DURING DROP PREPARATION:

- VOLUME
- TEMPERATURE
- AIR FLOW
- OIL COVERING

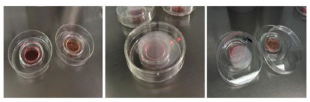


- DISH at RT
- Drop> 20microlitre
- LAF off
- Quick overlay
- WASH DROPS recommended

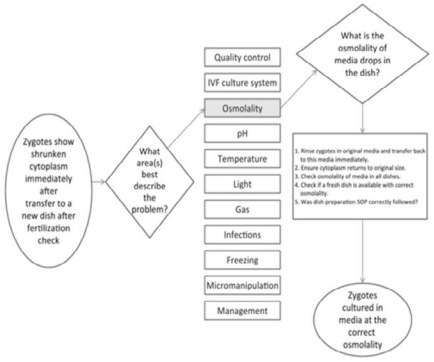
JE Swain et al. 2012 RBM Online 24,142

CHANGES IN OSMOLARITY:

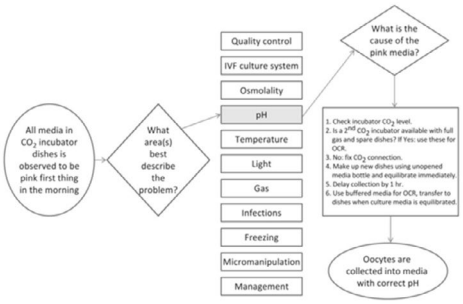
- Embryos change their volume
- **Cytoskeleton Damage**
- **Gene expression**
- **Gene Imprinting**



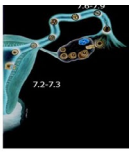
pH & osmolarity



pH & osmolarity

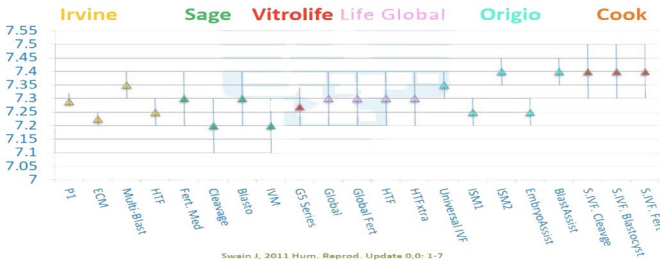


pH



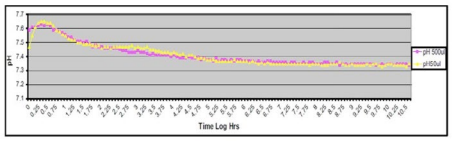
- A precise control over pHi is essential:
  - Enzyme activity (Lane et al., 1999a;1999b)
  - Cell differentiation; growth and proliferation (Ozawa et al., 2006)
  - ***Cell division***; membrane transport; cell-cell communication (Lane et al., 1998)
  - ***Protein and DNA synthesis*** (Squirrell et al., 2001)
  - ***Respiration*** (Lane, 2001)
  - Metabolism, calcium level modulation and cytoskeletal dynamics (Squirrell et al., 2001
- Currently 7.35 ±0.05 in an environment of 5-6% CO2 (Sjöblom, 2004; Quinn, 2004)
- The pH is a logarithmic scale – 0.2 unit decrease = > 60% of H⁺ more
- pH variations < 0.2 affects
  - Gene expression
  - Cell number of the blastocyst
  - Implantation

Cell Stage	pHi (Philips et al. 2000)	pHi Dale et al. (1998)
Germinal vesicle (intact)	7.04 ± 0.07	7.3 ± 0.3
Metaphase I oocyte	7.03 ± 0.04	N.A.
Metaphase II oocyte	6.98 ± 0.02	7.4 ± 0.1
Metaphase II oocyte (aged)	N.A.	7.5 ± 0.2
2 Pronuclei oocyte	N.A.	7.4 ± 0.1
2-8-cell embryo	7.12 ± 0.01	N.A.



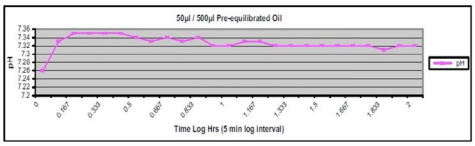
Swain J, 2011 Hum. Reprod. Update 0,0: 1-7

Time log for pH of 50 μL and 500 μL medium under oil:

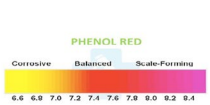


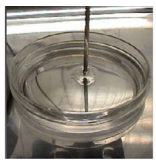
50 µL=8 H 500 µL = 9.58 H

Time log for pH of 50 μL under pre-equilibrated oil:



< 1 H if Oil is pre-equilibtated




Micro pH Probe in 50ul Drop under Oil

- pH: sub optimal just 3 min outside the incubator
- No activity should take longer than 3 min
- A stop watch is an embryologists best friend

TEMPERATURE

CONTROL:

- During follicular aspiration;
- during transport of aspirates;
- During "egg search" and handling of COCs;
- During ICSI

O-234 Wednesday, October 27, 2010 05:00 PM

ASSESSMENT OF EFFECT OF FOLLICULAR FLUID TEMPERATURE AT EGG RETRIEVAL ON BLASTOCYST DEVELOPMENT, IMPLANTATION AND LIVE BIRTH RATES. R. Sherbahn. Advanced Fertility Center of Chicago, Gurnee, IL.

IVF Results By Follicular Fluid Temperatures

	Low Temperature <36.4		Ideal Temperature 36.4 - 36.9		High Temperature >36.9	
Blast Devel Rate (from 2PN)	1408/ 4165 33.8%	P < 0.0001 vs. Ideal	2331/ 5684, 41.0%		139/ 457 30.4%	P < 0.0001 vs. Ideal
Implantation Rate	483/ 1406 34.3%	P=0.0006 vs. Ideal	761/ 1898, 40.1%		39/ 170 22.9%	P < 0.0001 vs. Ideal
Live Birth Rate Per Retrieval	293/ 665 44.1%	P=0.0006 vs. Ideal	484/ 917, 52.8%		29/ 77 37.7%	P = 0.015 vs. Ideal

Vol. 94., No. 4, Supplement, September 2010

Impact of different culture temperature on human embryo development.

Temperature	Meta phase IIs	Fertility rate, %	Day 3 cell no.	Blast rate, %	Usable blast rate, %	Aneuploidy rate, %	Implantation rate, %
36°C	399	86.2	7.0 ± 0.1^a	51.6^a	41.2^a	42.5	67.4
37°C	406	82.0	7.7 ± 0.1^b	60.1^b	48.4^b	46.1	73.3

Note: A sibling embryo split design was used,and incubator type, temperature variation, and pH were accounted for as confounding variables. Data indicate that 37°C is superior to 36°C in terms of embryo development. (Adapted from Hong et al. [94]).
a,b Different superscripts within a column represent a statistically significant difference, $P < .05$.

Swain. Optimizing IVF laboratory conditions. Fertil Steril 2016.

TEMPERATURE

37 ° C

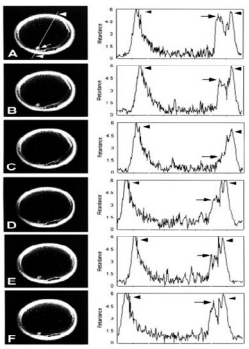
31.9 ° C (1.5 min)

27.1 ° C (5 min)

37 ° C (3min)

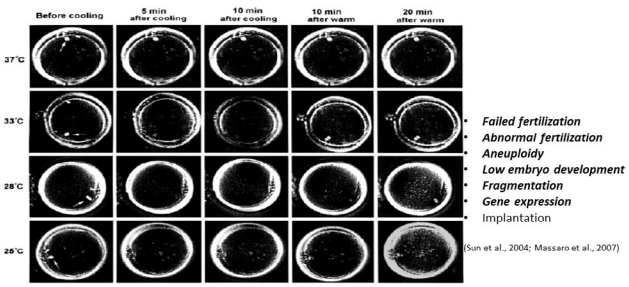
37 ° C (min)

37 ° C (20min)



[Wang et al., 2001]

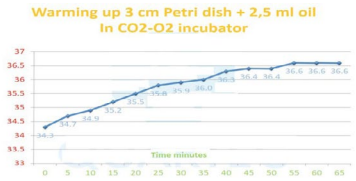
SPINDLE DYNAMICS IN RELATION TO CHANGES IN TEMPERATURE

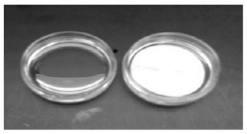


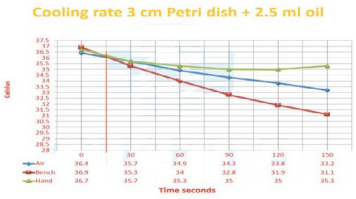
- *Failed fertilization*
- *Abnormal fertilization*
- *Aneuploidy*
- *Low embryo development*
- *Fragmentation*
- *Gene expression*
- Implantation

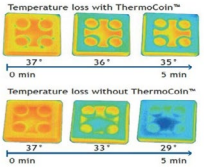
(Sun et al., 2004; Massaro et al., 2007)

Spindle disassembly is temperature- and time-dependent.









Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates

Wei-Hua Wang, Ph.D.,[a] Li Meng, Ph.D.,[b] Richard J. Hackett, M.Sc.,[c] Rudolf Oldenbourg, Ph.D.,[d] and David L. Keefe, M.D.[c,d]

Objective: To examine the effects of different thermodynamic control systems on the temperature stability of human eggs during in vitro manipulation, with the integrity of meiotic spindles imaged using the LC-PolScope (Cambridge Research & Instrumentation, Inc., Woburn, MA).

Design: We performed intracytoplasmic sperm injection (ICSI) and/or imaging of eggs with the temperature regulated by three different systems: thermostated coverslip (system 1), thermostated coverslip combined with objective heater (system 2), and conventional stage warmer (system 3).

Setting: Academic in vitro fertilization clinic.

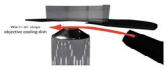
Patient(s): Oocytes were aspirated from stimulated ovaries of patients undergoing oocyte retrieval for ICSI.

Intervention(s): Measurement of temperature regulation in media surrounding eggs during in vitro manipulation and imaging.

Main Outcome Measure(s): Rate of oocytes with spindles, fertilization rates, and clinical pregnancy rates after ICSI.

Result(s): We imaged spindles in more oocytes with system 2 (
could not be imaged for system 3 because of technical limitation
when oocytes were imaged and used for ICSI with system 2 (7
3 (64.0%). Most importantly, a significantly higher clinical preg
manipulated with system 2 (51.7%) than with system 1 (25.0%
found in average ages, number of previous cycles, number of eg

Conclusion(s): Imaging meiotic spindles with the PolScope pro
Rigorous thermal control during ICSI stabilized spindles and inc
rates achieved after ICSI. The presence of birefringent spindles
in vitro conditions. (Fertil Steril® 2002;77:1274–7. ©2002 by A



Patient information and clinical outcome.

	System 1	System 2	System 3
No. of patients	40	29	52
Average patient's age	33.8 ± 4.4	34.1 ± 4.6	34.1 ± 4.4
Average no. of cycles	2.3 ± 1.4	2.8 ± 1.2	2.6 ± 1.8
Day 3 FSH	6.1 ± 1.8	6.3 ± 2.6	6.2 ± 2.5
E_2 level (pre-hCG)	1346.4 ± 608.3	1344.8 ± 552.4	1417.6 ± 763.5
E_2 level (day for hCG)	1780.3 ± 805.1	1809.0 ± 815.6	1926.8 ± 980.8
No. of eggs examined	402	298	433
No. of eggs/patient	8.3	10.0	10.3
Eggs with spindle (%)	61.4[a]	81.2[a]	NA
Fertilization rate (%)	56.7[a]	78.8[a]	64.0[a]
Pregnant rate (%)	25.0[a]	51.7[a]	23.1[a]

TEMPERATURE AND SPERM

Reproductive BioMedicine Online (2014) 28, 436–442
www.sciencedirect.com
www.rbmonline.com
ELSEVIER
ARTICLE

Influence of temperature and sperm preparation on the quality of spermatozoa

Annelies Thijssen [a,b,*], Elke Klerkx [a], Carin Huyser [c], Eugene Bosmans [a], Rudi Campo [a], Willem Ombelet [a,b]

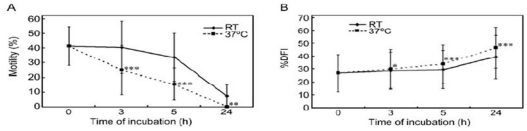
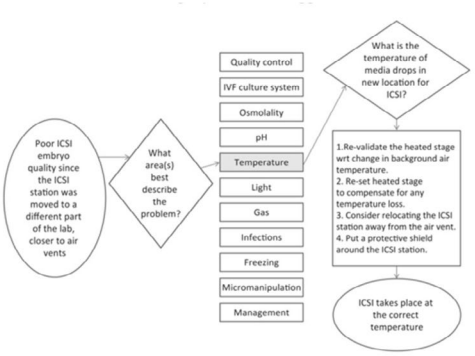


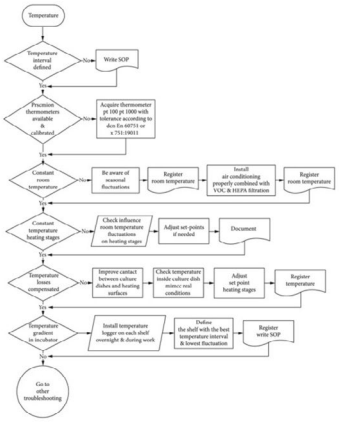
Figure 2. Effect of incubation temperatures and time on the motility and DNA fragmentation index (%DFI) of sperma semen (not frozen and thawed). Sperm motility (A) and DFI (B) as a function of incubation time (abscissa) at room tem diamonds) and 37°C (squares) ($n = 24$) are given on the ordinate. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the val

The proportions of acrosome-reacted, apoptotic and dead spermatozoa were significantly lower in samples incubated for 24 h at RT compared with 35C (P < 0.001, P = 0.01 and P < 0.001, respectively)

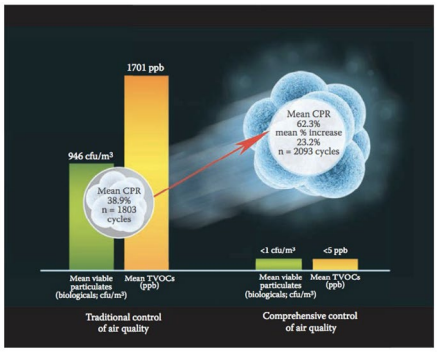
Temperature



Temperature



Air quality



AQI AIR QUALITY INDEX

Air quality

IMPORTANCE OF AIR QUALITY IN IVF
Evidence from Human Studies

Study	Population	Method	Outcome
Mayer et al, 1999	RCT of infertile IVF couples	Embryo culture in incubators with and without VOC filtration	Higher PRs when embryos were cultured in incubators with VOC filters
Racowsky et al, 1999	Infertile IVF couples, observational	IVF and embryo culture in labs and incubators with and without VOC filtration	Reduction in miscarriage in cycles performed in labs and incubators with carbon-activated filters
Boone et al, 1999	Infertile IVF couples, observational	Construction of cleanrooms for IVF activities	Reduction in air particles associated with an increase in the number of high-quality embryos
Worrillow et al, 2002	Infertile IVF couples, observational	IVF in clean rooms with VOC filtration. Outside and inside ambient air monitored for 2 years	Seasonal correlation between temperature/humidity of outside ambient air serving the IVF air system and VOC levels with impact on IR

GAS COMPOSITION

- Medical grade CO2

2. Qualitative and quantitative composition

Medical carbon dioxide specification is:

carbon dioxide purity 99.5% v/v min

The medical carbon dioxide cylinder specification complies with the current European Pharmacopeia monograph (0375).

OXIDATIVE STRESS

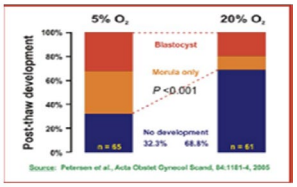
- Reduced embryo development
- Increased fragmentation
- Zona hardening
  - Impaired blastocyst hatching
  - Reduced IR
  - Increased MZ twinning

- REDUCE OS
  - Include antioxidants in media
  - Reduce oxygen tension
  - Reduced exposure to sperm (high ROS generator)

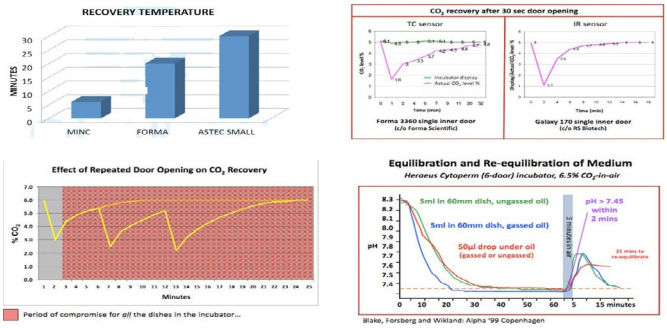
Effects of reduced oxygen concentration in a predominantly blastocyst transfer program.

Endpoint	21% O_2 (%)	5% O_2 (%)
Clinical pregnancy	56/115 (48.7)[a]	74/115 (64.3)[b]
Implantation	95/267 (35.6)[b]	122/247 (49.4)[b]
Live birth	49/115 (42.6)[b]	66/115 (57.4)[b]

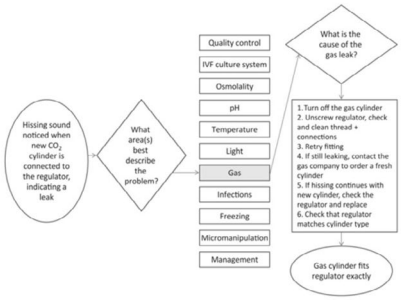
Swain. Optimizing IVF laboratory conditions. Fertil Steril 2016.

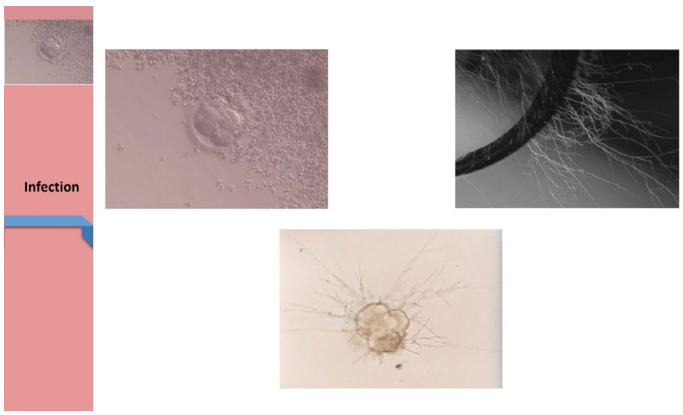


INCUBATORS


RECOVERY TEMPERATURE
MINUTES
MINC
FORMA
ASTEC SMALL
CO_2 recovery after 30 sec door opening
TC sensor
IR sensor
Forma 3360 single inner door
(c/o Forma Scientific)
Galaxy 170 single inner door
(c/o RS Biotech)
Effect of Repeated Door Opening on CO_2 Recovery
% CO_2
Minutes
Period of compromise for all the dishes in the incubator...
Equilibration and Re-equilibration of Medium
Heraeus Cytoperm (6-door) incubator, 6.5% CO_2-in-air
5ml in 60mm dish, ungassed oil)
5ml in 60mm dish, gassed oil)
50µl drop under oil
(gassed or ungassed)
pH > 7.45
within
2 mins
pH
15 minutes
Blake, Forsberg and Wikland: Alpha '99 Copenhagen


AQI
AIR QUALITY INDEX
Air
quality


Hissing sound noticed when new CO_2 cylinder is connected to the regulator, indicating a leak
What area(s) best describe the problem?
Quality control
IVF culture system
Osmolality
pH
Temperature
Light
Gas
Infections
Freezing
Micromanipulation
Management
What is the cause of the gas leak?
1. Turn off the gas cylinder
2. Unscrew regulator, check and clean thread + connections
3. Retry fitting
4. If still leaking, contact the gas company to order a fresh cylinder
5. If hissing continues with new cylinder, check the regulator and replace
6. Check that regulator matches cylinder type
Gas cylinder fits regulator exactly


Infection

Infection

Microbiological Monitoring

Settle plates

Mold and blood-agar Petri dishes

Surface sampling

Swabs and contact plates

Five-finger glove printing

Incubators, workstations, air, floor, wall

Limits Colony Forming Unites (CFU)		
Settle plates (90mm diameter) CFU/4 hours	Contact plates (55mm diameter) CFU/plate	Glove print CFU/ glove
<3	<3	<3

Infection

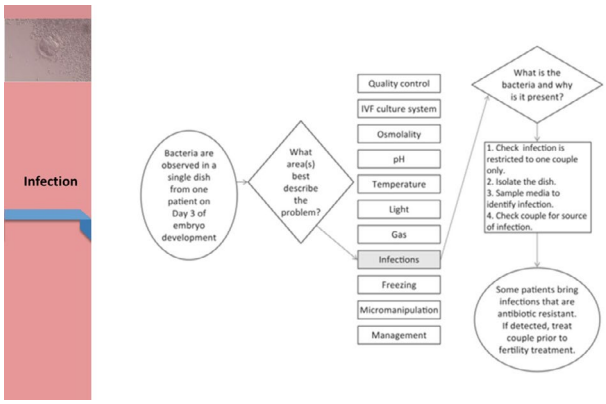
WORK AREA
Is the cell culture hood properly set up?
Is the cell culture hood in an area free from drafts and through traffic?
Is the work surface uncluttered, and does it contain only items required for your experiment?
Did you wipe the work surface with appropriated disinfectant before work?
Are you routinely cleaning and sterilizing your incubators, refrigerators, freezers, and other laboratory equipment?

PERSONAL HYGIENE
Did you wash your hands?
Are you wearing personal protective equipment?
Do you ware rings and juwelry?
If you have long hair, is it tied in the
Are you using a pipette to work with liquids?

Infection

REAGENT AND MEDIA
Have you sterilized any reagents, media, and solutions you have prepared in the laboratory using the appropriate procedure?
Did you wipe the outside of the bottles, flasks, and plates with disinfectant before placing them on your work surface?
Are all your bottles, flasks, and other containers capped when not in use?
Are all your plates stored in sterile re-sealable bags?
Do you use media after the expiration date ?

HANDLING
Are you working slowly and deliberately, mindful of aseptic technique?
Did you wipe the outside of the bottles, flasks, and plates with disinfectant before placing them on your work surface?
Are placing the caps or covers face down on the work area?
Are you using sterile glass pipettes or sterile disposable plastic pipettes to manipulate all liquids?
Are you using a sterile pipette only once to avoid cross contamination?
Are you careful not to touch the pipette tip to anything non-sterile, including the outside of the bottle threads?
Did you mop up any spillage immediately, and wiped the area with disinfectant ?



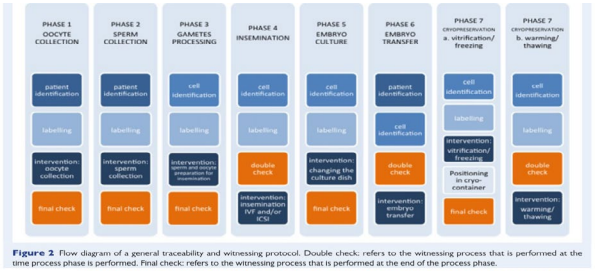


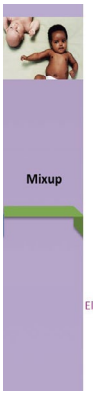
Figure 2 Flow diagram of a general traceability and witnessing protocol. Double check: refers to the witnessing process that is performed at the time process phase is performed. Final check: refers to the witnessing process that is performed at the end of the process phase.

LABELLING
DOCUMENTATION
TIME OUT
WITNESSING

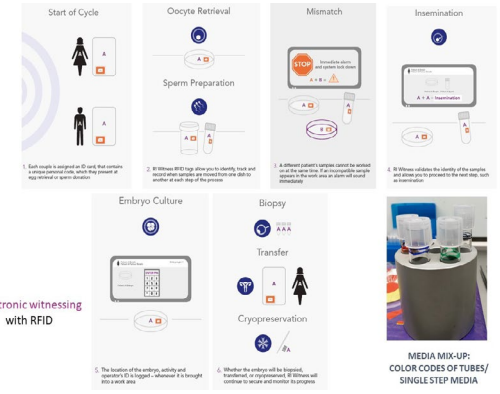
Table 1 Process phases, number of process steps, number of failure modes and relative risk priority numbers before and after implementating the electronic witnessing system.

Process phases	Process steps n (%)	Failure modes n (%)	High/moderate risk modes without EWS (RPN >15)	Highest RPN without EWS	High/moderate risk modes with EWS (RPN >15)	Highest RPN with EWS
1. oocyte retrieval	4 (21.1)	8 (25.0)	2	30	0	10
2. sperm collection	2 (10.5)	5 (15.6)	2	30	0	10
3. gamete processing	2 (10.5)	4 (12.5)	0	10	0	4
4. insemination	3 (15.8)	3 (9.4)	0	10	0	4
5. embryo culture	1 (0.5)	2 (6.3)	0	10	0	4
6. embryo transfer	3 (15.8)	4 (12.5)	1	30	0	10
7. cryopreservation	4 (21.1)	6 (18.8)	1	30	0	10

EWS = electronic RPN = relative risk priority.



Mixup



Electronic witnessing with RFID

MEDIA MIX-UP: COLOR CODES OF TUBES/ SINGLE STEP MEDIA

Equipment failure

Equipment failure at 2nd fertility clinic may have damaged hundreds of embryos

Failure at California fertility clinic could affect hundreds of patients

by Tim Stelloh / Mar.12.2018 / 6:14 AM ET / Updated Mar.12.2018 / 7:06 AM ET

- Operate within tolerable limits
- Periodic maintenance
- Equipment serviced regularly to prolong its life – avoids catastrophe.
- Daily QC monitoring
- Follow manufacturer's guidelines on frequency of QC
- Daily monitoring - first event in the morning as instruments have stabilized overnight
- Temperature - logged into a database
- Back up power

Back up facilities: ***two incubators, and a spare cryostorage vessel, separate oocyte aspiration pump & injector***

Equipment failure

Table 2.5 List of equipment, parameters for QC, and frequency of QC

Equipment	Parameter for QC	Frequency of QC	Comments
Incubator	Temperature	Daily	Annual preventive maintenance
	CO_2	Daily	
	Humidity	Daily	
Heating surfaces	Temperature	Daily	
Heating bath	Temperature	Daily	
	Water level	Daily	
Heating block	Temperature	Daily	
Microscope	Image quality	Daily	Annual preventive maintenance
CASA	Sperm count	Daily	Annual preventive maintenance
	Motility (%)	Daily	
	Motility (velocities)	Daily	
	Morphology (%)	Daily	
Controlled rate freezer	Sufficient refrigerant	Each use	Annual preventive maintenance
	Start temperature	Each use	
	Seeding temperature	Each use	
	Final temperature	Each use	
Storage dewers	Liquid N_2 level	Daily	
Refrigerator	Temperature	Daily	
Freezer	Temperature	Daily	
Heating, ventilation, and air conditioning systems	Room temperature	Daily	Clean filters/humidifiers periodically
	Room humidity	Daily	
QC equipment			
Thermometers	Temperature (accuracy/precision)	Periodically	
pH meters	pH (accuracy/precision)	Each use (daily)	Annual preventive maintenance
Osmometers	Osmolality (accuracy/precision)	Each use (daily)	Annual preventive maintenance
Hygrometers	Humidity (accuracy/precision)	Periodically	
Timers	Time (accuracy/precision)	Periodically	
CO_2 monitor	%CO_2 (accuracy/precision)		Fyrite should be changed every 300 determinations

QC, quality control; CASA, computerized semen analyzer.

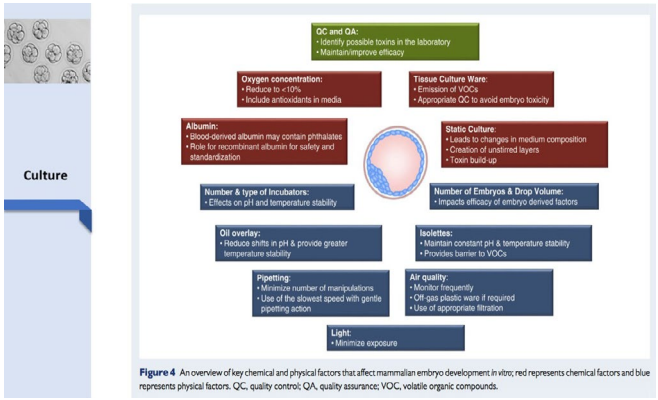


Figure 4 An overview of key chemical and physical factors that affect mammalian embryo development *in vitro*; red represents chemical factors and blue represents physical factors. QC, quality control; QA, quality assurance; VOC, volatile organic compounds.

THANK YOU

7. What lies between an average and successful IVF program

EXCELLENCE IN ART

CLINICAL LAB

CLINICAL SCENARIO 1

A successful IVF clinic started a new lab in a new facility and it reported low results for the initial months .. ….what could go wrong ?

- Location
- Material used in construction? Flooring/adhesive
- Design flaw ? Difficulty in maintenance !!
- Positive pressure ventilation ?
- HEPA filters …inbuilt ? CODA ?
- Gas supplies same as parent ?
- Personnel

These flaws can all contribute to greater circulating levels of volatile organic compounds (VOCs), and other contaminants inside an IVF lab. VOCs can be harmful to the growing embryos and have been found to severely reduce success rates.

Air Quality

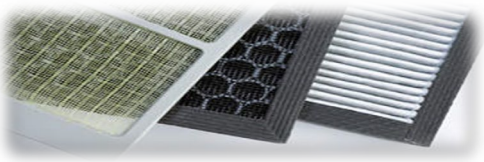
- Air quality/VOCs likely impact embryo development
- Prudent to take preventative measures (Standard of care)
- HVAC VOC filtration
- dedicated, proper intake placement , # exchanges, filters, etc
- Inline gas filters
- Appropriate design & building materials
- Burn-in & off-gassing before use

Burn-In & Validation

- Clean walls, ceilings, floors, cabinets/counters.
- Clean equipment (incubators)

- Increasing temperature of the new area by 10-20 or even higher temperatures
- Increasing the ventilation rate which aid in the removal of VOC
- Lab should be left closed during this time
- AHUs and lighting switched on and left running

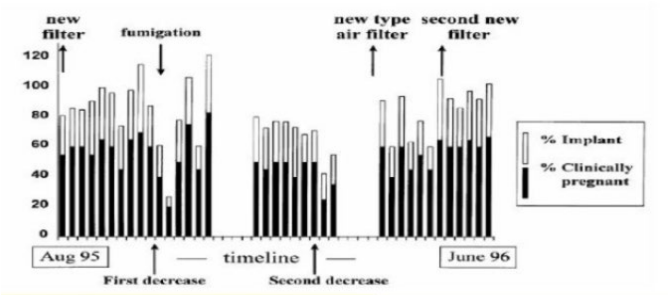
- Area purged with high ventilation repeatedly neutralises the offgassing by paint, adhesives, general building materials
- Run HVAC and incubators for ≥ ~2 weeks
- Validate
- Particle counts, VOCs
- Activated Charcoal in SS buckets
- Document the “commissioning” of the lab



INCUBATORS

- Purchase months in advance of their intended use
- Run decontamination program
- Microbial sampling for aerobic bacteria and fungi
- VOC levels determined
- Inline filters
- Activated charcoal granules

Flaws in AHU designing



Cohen et al. 1997

CLINICAL SCENARIO 2

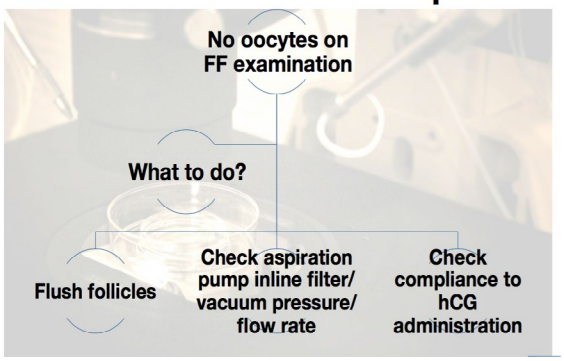
- A 26 yr old pt with regular cycles BMI 24
- Left salpingectomy done in 2014 for ectopic pregnancy HSG showed rt tubal cornual blockage
- Husband semen analysis normal
- TVS shows polycystic ovaries bilaterally
  - AMH 6
  - FSH 3.5
  - LH 10
  - E2 50
  - TSH 2.8
- Declined hysteroscopic tubal canulation and laparoscopy
- Decided to have IVF treatment

- OCP prescribed for 21 days .
- Antagonist protocol with 150 IU of Rec FSH prescribed for 10 days
- Cetrolix as antagonist
- 22 follicles developed E2 2618
- Triptorelin 0.2 mg given as trigger 35 hrs before OPU
- Follicle Sizes:
  - 17-18mm 4
  - 15-16mm 5
  - 12-14 mm 8
  - 8-10mm
- ET 9mm
- Egg retrieval from right ovary attempted
- 4 follicles aspirated: 2x15-16 and 2x 13-14
- No eggs retrieved

- What will you do
  - Continue collection or stop
  - What will you ask
- 3 more follicles aspirated 1x17-18mm 1x15-16, 1x13-14 .
- No eggs retrieved
- What will you do next
  - Abandon
  - Continue and aspirate all the follicles
  - All follicles aspirated and no eggs retrieved

- First step
  - Check date and time of trigger
  - Time interval between trigger and antagonist
  - Was th etrigger supervised
- Second step
  - Consider Flushing the Follicles
  - Check suction pump/ vacuum pressure/ flow rate
-
- Third step
  - Stop collection
  - Give rescue HCG and collect 36 hrs later

Lab perspective determining clinical decision – Example 2



- How will you counsel this patient ?
- Patient wants to have IVF again
- What is your action plan
  - Protocol
  - Dose Of drug
  - Day of collection earlier or later
  - Trigger
- Plan for next Cycle
  - Antagonist cycle
  - Reduce dose of drug to 125
  - Step up if required
  - Dual trigger using GnRh agonist at 40 Hrs with HCG 1500 IU at 34 hrs

J Hum Reprod Sci. 2015 Jul-Sep; 8(3): 170–174. PMCID: PMC460117
doi: 10.4103/0974-1208.165152 PMID: 2653886

Empty follicle syndrome: Successful pregnancy following dual trigger

K. Deepika, Suvarna Rathore, Nupur Garg, and Kamini Rao

CLINICAL SCENARIO 3

An IVF center suddenly has doubled their number of cases per month but reporting lower pregnancy rates… what could be the reason?

- The number of incubators were same , stability of incubators compromised, not withstanding the load of the cases couldn't maintain the co2 and temp properly
- The number of embryologist wasn't increased leading to stressful work conditions and over worked staff, resulting compromised total quality management in the lab
- We need to follow the staffing norm and think how many staff of which categories are required in the ART facility fortarget level of utilization ?
- However, for an ART clinic a strong element of success is dependent on the availability of the right quantity of the right categories of staff

LABORATORY STAFFING NORMS

Table 2.3 Staffing norms for ART facilities in the United States

	Average number of laboratory staff	
Number of IVFs	McCulloh[a]	Boone and Higdon[b]
0	0.47	2.92
100	1.6	4.3
250	3.3	6.4
400	5.0	8.5
1000	11.7	16.9

McCulloh : Staff: 0.47 + Number of IVFs / 88.5
Boone & Higdon: 2.92 + number of procedures x 0.002

CLINICAL SCENARIO 4

- 28 yr old patient operated for grade 4 endometriosis and endometrioma excision done in 2016
- In the OT note Bilateral spillage from tubes mentioned
- Patient has been trying to conceive for 1 yr
- TVS shows recurrent endometrioma 2cms on rt side and 3.5 cms on the left side. Hydrosalpinx not noted
  - AFC 8
  - AMH 1.2
  - FSH 8
  - LH 9.5
  - E2 65

- How would You like to proceed ?
  - Natural cycle folliculometry and TI
  - Ovulation induction folliculometry
  - Stimulated IUI
  - IVF

- If IUI
  - what protocol and how many cycles
  - 2 cycles of IUI have failed pt now wants to go for IVF
- Would you operate on her before IVF
- Which protocol would you prefer

- Options of either stimulated IUI for 3-4 cycles or IVF
- Counsel women with endometrioma regarding the risks of reduced ovarian function after surgery and the possible loss of the ovary.
- The decision to proceed with surgery should be considered carefully if the woman has had previous ovarian surgery
- Use assisted reproductive technologies for infertility associated with endometriosis, esp. if tubal function is compromised or if there is male factor infertility, or other treatments have failed

FOGSI

- In stage I and II endometriosis, treatment with super ovulation and IUI improve fertility compared to expectant management
- Clinicians should take into consideration, age, duration of infertility, ovarian reserve and male factor (16). [Evidence level A]
- Previous ovarian surgery results in longer stimulation, higher FSH requirement, decreased oocyte number but no difference in fertilization, pregnancy outcome in subsequent ART cycles
- Surgical management of endometrioma does not significantly increase IVF pregnancy rate and ovarian response to stimulation compared to no surgery

- COS using GnRh agonists or antagonists is effective in IVF patients with mild to moderate endometriosis and in those with endometrioma who did not undergo surgery [Evidence level A]
- Ultra-long protocol of GnRh agonists for a period of 3 – 6 months before ART improves the clinical pregnancy rates
- In women undergoing IVF, stage III and IV is associated with poor implantation and lower clinical pregnancy rate
- In infertile women with endometrioma smaller than 3 cm cystectomy prior to ART does not improve pregnancy rates
- In women with endometrioma larger than 3 cm, cystectomy is indicated prior to ART when it is associated with pain or inaccessibility of follicles

- Pt was given 2 cycles of GnRH agonist on 21/02/18 and 21/03/18 and then underwent antagonist cycle from 16/04/18 using 450 IU of Rec FSH

- Is an antagonist necessary
- What else could have been done
- Would you like to change the stimulation protocol or continue if so why

- Rec FSH continued
- Slow growth of follicles noted on day 8
- 11mm on day 6 and 12 mm on day 8
- What can we do ? Continue same or anything different?

- Check LH level on day 5-6 of stimulation
- Antagonist required only if LH levels not suppressed
- Or top up with agonist Lupride on daily basis (long protocol)
- Slow growth of follicles on day 8 with rec FSH may indicate need for Rec LH or HMG (Min dose 150 IU)

Hum Reprod. 2008 Feb;23(2):310-5. Epub 2007 Dec 3.

Urinary hMG versus recombinant FSH for controlled ovarian hyperstimulation following an agonist long down-regulation protocol in IVF or ICSI treatment: a systematic review and meta-analysis.

Coomarasamy A[1], Afnan M, Cheema D, van der Veen F, Bossuyt PM, van Wely M.

Author information

Abstract

BACKGROUND: Since the most recent Cochrane review on hMG versus rFSH for controlled ovarian hyperstimulation following a long down-regulation protocol, several new trials have emerged.

METHODS: We conducted a systematic review and meta-analysis of randomized trials comparing the effectiveness of hMG versus rFSH following a long down-regulation protocol in IVF-ICSI cycles, on the primary outcome of live birth per woman randomized, as well as several other secondary outcomes. Searches were conducted in MEDLINE, EMBASE, Science Direct, Cochrane Library and databases of abstracts (last search January 2007).

RESULTS: Seven randomized trials, consisting of a total of 2159 randomized women, were identified. A meta-analysis of these trials showed a significant increase in live birth rate with hMG when compared with rFSH (relative risk, RR = 1.18, 95% CI: 1.02-1.38, P = 0.03). The heterogeneity test was non-significant (P = 0.97), suggesting that there was no statistical inconsistency between the seven studies. The pooled risk difference (RD) for the outcome of live birth rate was 4% (95% CI: 1-7%) for these study populations. There was an increase in clinical pregnancy rates with hMG when compared with rFSH (RR = 1.17, 95% CI 1.03-1.34). No significant differences were noted for gonadotrophin use, spontaneous abortion, multiple pregnancy, cancellation and ovarian hyperstimulation syndrome rates.

CONCLUSIONS: For the populations in the randomized trials, hMG was associated with a pooled 4% increase in live birth rate when compared with rFSH in IVF-ICSI treatment following a long down-regulation protocol.

- On day 14, 8 follicles noted
  - 2x17-18mm
  - 2x 15-16
  - 3x 14-15
  - 1x12
- Rec HCG given as trigger and egg Collection done 35 hrs later

- 7 brown eggs were collected
- 5 M 11
- 4 Fertilised
- Day 3
  - 2x Grade 2 8cell
  - 2x grade 3 6 cell
- To transfer or freeze
- Which day Day 3 or 5

Cochrane Database Syst Rev. 2016 Jun 30;(6):CD002118. doi: 10.1002/14651858.CD002118.pub5.

Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology.

Glujovsky D[1], Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D.

MAIN RESULTS: We included 27 RCTs (4031 couples or women).The live birth rate following fresh transfer was higher in the blastocyst transfer group (odds ratio (OR) 1.48, 95% confidence interval (CI) 1.20 to 1.82; 13 RCTs, 1630 women, I(2) = 45%, low quality evidence) following fresh transfer.This suggests that if 29% of women achieve live birth after fresh cleavage stage transfer, between 32% and 42% would do so after fresh blastocyst stage transfer.There was no evidence of a difference between the groups in rates per couple of cumulative pregnancy following fresh and frozen-thawed transfer after one oocyte retrieval (OR 0.89, 95% CI 0.64 to 1.22; 5 RCTs, 632 women, I(2) = 71%, very low quality evidence).The clinical pregnancy rate was also higher in the blastocyst transfer group, following fresh transfer (OR 1.30, 95% CI 1.14 to 1.47; 27 RCTs, 4031 women, I(2) = 56%, moderate quality evidence). This suggests that if 36% of women achieve clinical pregnancy after fresh cleavage stage transfer, between 39% and 46% would do so after fresh blastocyst stage transfer.There was no evidence of a difference between the groups in rates of multiple pregnancy (OR 1.05, 95% CI 0.83 to 1.33; 19 RCTs, 3019 women, I(2) = 30%, low quality evidence), or miscarriage (OR 1.15, 95% CI 0.88 to 1.50; 18 RCTs, 2917 women, I(2) = 0%, low quality evidence). These data are incomplete as under 70% of studies reported these outcomes.Embryo freezing rates were lower in the blastocyst transfer group (OR 0.48, 95% CI 0.40 to 0.57; 14 RCTs, 2292 women, I(2) = 84%, low quality evidence). This suggests that if 60% of women have embryos frozen after cleavage stage transfer, between 37% and 46% would do so after blastocyst stage transfer. Failure to transfer any embryos was higher in the blastocyst transfer group (OR 2.50, 95% CI 1.76 to 3.55; 17 RCTs, 2577 women, I(2) = 36%, moderate quality evidence). This suggests that if 1% of women have no embryos transferred in (planned) fresh cleavage stage transfer, between 2% and 4% will have no embryos transferred in (planned) fresh blastocyst stage transfer.The evidence was of low quality for most outcomes. The main limitation was serious risk of bias, associated with failure to describe acceptable methods of randomisation, and unclear or high risk of attrition bias.

Brown oocytes: Implications for assisted reproductive technology

Fertilization, embryo development, and successful pregnancy can be achieved after transfer of embryos derived from brown oocytes at the same rate as embryos from morphologically normal oocytes. Therefore, brown oocytes are probably normal and this morphological criterion does not seem to be indicative of any adverse outcome in IVF. (Fertil Steril® 2006;86:1522–5. ©2006 by American Society for Reproductive Medicine.)

- Navid Esfandiari, D.V.M., Ph.D., H.C.L.D.
- Hasan Burjaq,

Cellular Physiology and Biochemistry
Cell Physiol Biochem 2016;39:677-684
DOI: 10.1159/000445658
Published online: July 22, 2016
Accepted: June 15, 2016
Karger Open Access
677
Original Paper

High Serum FSH is Associated with Brown Oocyte Formation and a Lower Pregnacy Rate in Human IVF Parctice

Hongyi Xu* Kai Deng*,b Qingbing Luo* Juan Chen* Xin Zhang* Xiaoyan Wang*,b Honglu Diao* Changjun Zhang*
*Reproductive Medicine Center, Renmin Hospital, Hubei University of Medicine, Shiyan, China; bWellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

- High serum FSH levels during the IVF-ET cycle were correlated with the formation of brown oocytes and affected embryo development, leading to a decrease in the pregnancy rate
- In contrast, no significant correlation was observed between brown oocytes formation and patients' conditions like age, BMI, or duration and causes of infertility
- Therefore, limiting the FSH dose during controlled ovarian hyperstimulation is important for the production of high-quality oocytes

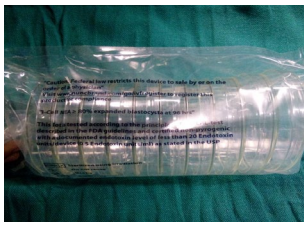
CLINICAL SCENARIO 5

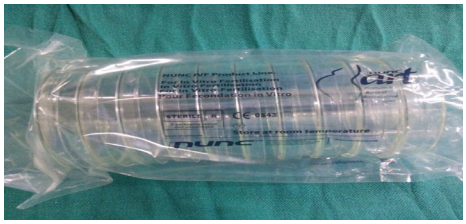
ART centre in Karad near Mumbai established in 2001 .
Carries out nearly 200 cycles per year in batches. Has permanent embryologist.
Fertilization rate: 90 percent
Cleavage rate: 92 percent

The embryologist moves to UK for higher education. The centre hires a fly by night embryologist who does cases in batches and carries his own disposables and media. The results suddenly fall and centre earns a bad name

Question 1. What probably went wrong at this laboratory ? Do you think the disposables and media were the culprit

Question 2 . What is the importance of high quality IVF grade plastic ?





Gametes and embryos are never exposed to a substance that will deleteriously impact their environment

Exposure can occur during gamete acquisition, via culture in media, or via plastic ware with which the medium has come in contact, or via any airborne volatile or nonvolatile agent that may affect the culture material either directly or indirectly

Great variability exists between different manufacturers of certain plastic ware as well as between different lots of the same item

Culture of human gametes and embryos is performed in a plastic ware

Purchase plastic ware products that have already tested non-toxic using a mouse embryo assay (MEA)

Table 2.6 Contact materials and availability of prior mouse embryo testing

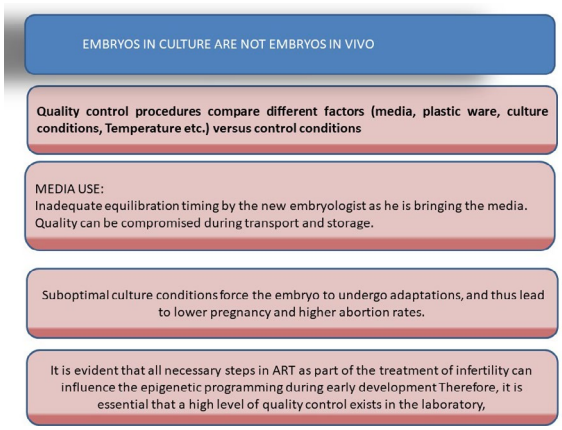
Item	Available with prior mouse embryo assay?
Culture medium	Yes
Culture dishes	Yes
Embryo transfer catheters	Yes
Pipettes, serological	No, requires testing
Pipettes, micropipettes for moving embryos	Yes
Pipettes, Pasteur	Yes
Microtools for ICSI, AZH, and holding	Yes
Centrifuge tubes	No, requires testing
Culture tubes	No, requires testing
Pipette tips	Yes
Gases for maintenance of CO_2 levels	No, requires testing
Filters for gases or medium	No, requires testing

ICSI, intracytoplasmic sperm injection; AZH, assisted zona

PLASTICWARE AND VOCs

Table V. Compounds released from cell tissue culture grade petri dishes

Material	>50 ng/sample	≤50 ng/sample	
Styrene	920.00	*n*-Pentane	50
Toluene	180.00	3-Methylpentane	50
Acetone	150.00	Nonanal	50
2-Butanone	130.00	Butanal	40
Acetaldehyde	100	3-Pentanone	40
n-Butane	100	n-Hexane	30
Benzaldehyde	100	Butene isomer	30
Hexanal	70	Benzene	23
Ethylbenzene	64.00	*n*-Octane	20
2-Hexanone	58.00	*n*-Nonane	20
		Decanal	20
		Cumene	10
		Propylbenzene	10
		Octanal	10
		m- & *p*-Xylenes	7.5
		o-Xylene	5.80

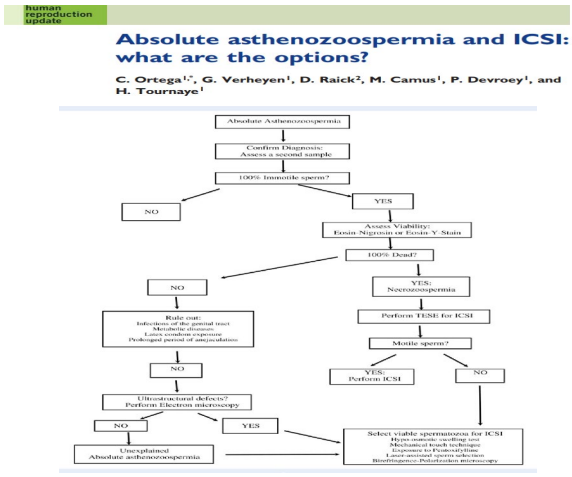


CLINICAL SCENARIO 6

- 28 yr old patient proceeding for IVF .
- Husbands semen analysis
  - Count 4mill/ml Motility
  - rapid progressive 4%
  - Sluggish progressive 20 %
  - Normal forms 4 %
- Preparing for ICSI
- Husband working In UK coming home for 3 to 4 days
- Semen banking not done
- Not agreeable to have donor semen backup
- No erectile problems identified

- Pt stimulated with 225 IU Of rec FSH using antagonist protocol
  - 12 follicles stimulated
  - ET 8mm
- On day of egg retrieval
  - Semen count 1mill/ml
  - Rapid progressive 0 %
  - Sluggish progressive 4 %
  - Normal forms nil %
- What is to be done ?

- 2nd sample
- HOS test
- Sperm Mobil Media (pentoxyphylline / theophylline)
- Laser assisted immotile sperm selection (LAISS)
- TESE



- Kovacic et al., (2006) reported the largest series (47 cycles) using testicular sperm, with a fertilization rate of 66% and a pregnancy rate of 38.3%.
- Kahraman et al. (1997) reported the first pregnancy and delivery of a healthy child after ICSI treatment with immotile testicular spermatozoa in a patient with absolute asthenozoospermia.

Fertil Steril. 1996 Aug;66(2):331-4.

The use of testicular sperm for intracytoplasmic sperm injection in patients with necrozoospermia.

Tournaye H[1], Liu J, Nagy Z, Verheyen G, Van Steirteghem A, Devroey P.

Testicular sperm were recovered in all treatment cycles and fertilization occurred in six of seven cycles. Overall normal fertilization and transfer rates were 67% and 71%, respectively. One live birth was obtained after five ETs.

CLINICAL SCENARIO 7

An old centre with huge workload notice that their results are plummeting. An experienced embryologist advices them to hold audits to see where they are heading?

- What should be the frequency of audits?
- What parameters should be analysed?
- Internal / External audits

INTERNAL AUDITS

- Continous Monitoring of Laboratory Performance in an Established IVF Unit
- Performance of the IVF laboratory can be monitored by auditing
  - Fertilization rates
  - Ceavage rates
  - Embryo quality
  - Pregnancy rate
  - Implantation rate, and
  - Multiple birth rates
- Frequency should be depending on the center's activity.
- However, when interpreting these statistics, consideration must be given to the activity
- of the unit and the types of patients treated, etc .

- Furthermore, pregnancy rate alone should not be used as a QC measure to audit the performance of the laboratory
- This is not least because it is affected by other variables, but also because a drop in pregnancy rate identifies a problem encountered at the time of treatment usually weeks before
- A good QC program detects problems before they affect the pregnancy rate
- Moreover, using pregnancy rate as a QC measure does not identify the source of the problem
- Record Keeping and Documentation
- Fundamental to the success of an QA program is accurate documentation

Table 2.5 List of equipment, parameters for QC, and frequency of QC

Equipment	Parameter for QC	Frequency of QC	Comments
Incubator	Temperature	Daily	Annual preventive maintenance
	CO_2	Daily	
	Humidity	Daily	
Heating surfaces	Temperature	Daily	
Heating bath	Temperature	Daily	
	Water level	Daily	
Heating block	Temperature	Daily	
Microscope	Image quality	Daily	Annual preventive maintenance
CASA	Sperm count	Daily	Annual preventive maintenance
	Motility (%)	Daily	
	Motility (velocities)	Daily	
	Morphology (%)	Daily	
Controlled rate freezer	Sufficient refrigerant	Each use	Annual preventive maintenance
	Start temperature	Each use	
	Seeding temperature	Each use	
	Final temperature	Each use	
Storage dewers	Liquid N_2 level	Daily	
Refrigerator	Temperature	Daily	
Freezer	Temperature	Daily	
Heating, ventilation, and air conditioning systems	Room temperature	Daily	Clean filters/humidifiers periodically
	Room humidity	Daily	
QC equipment			
Thermometers	Temperature (accuracy/precision)	Periodically	
pH meters	pH (accuracy/precision)	Each use (daily)	Annual preventive maintenance
Osmometers	Osmolality (accuracy/precision)	Each use (daily)	Annual preventive maintenance
Hygrometers	Humidity (accuracy/precision)	Periodically	
Timers	Time (accuracy/precision)	Periodically	
CO_2 monitor	%CO_2 (accuracy/precision)		Tyrite should be changed every 300 determinations

QC, quality control; CASA, computerized semen analyzer.

DIALOGUE AND INTERACTION BETWEEN THE CLINICIAN AND THE EMBRYOLOGIST

External audits

The assessment of internal controls, processes, and documentation is oriented more to a certification process than a standard audit.

This is all the more reason to ensure that the organization is fully prepared for the auditing experience, and that the compliance process works well and is successful.

CLINICAL SCENARIO 8

- 37 year old pt with BMI 36 recently married is seen in your clinic wanting to conceive quickly

- AMH 0.6
- FSH 12
- LH 5
- E2 86
- TSH normal prolactin normal
- AFC 4

- Husband's semen analysis

- Count 8 mill
- rapid progressive 40 %
- normal forms 4 %

- Proceeding for IVF
- How would you prepare her to optimise response ?

- Weight loss
- DHEA for 3 months
- For Husband
- Check for varicocele and any anatomical problems
- Lifestyle modification
- Antioxidants

- TVS Day 2 one Follicle of 11mm and rest of the three follicles 8-9mm
- How will you pretreat this pt before IVF treatment
- To deal with follicular asynchrony

- OCP
- Estradiol pretreatment in luteal phase

J Obstet Gynaecol India. 2016 Aug; 66(4): 295–299.
Published online 2016 Apr 29. doi: 10.1007/s13224-016-0877-z
Short Commentary
PMCID: PMC4912497
PMID: 27382226

Precycle Estradiol in Synchronization and Scheduling of Antagonist Cycles

Shilpa Saple, Mukesh Agrawal, and Simi Kawar

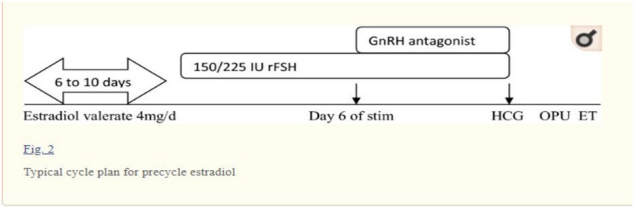


Fig. 2
Typical cycle plan for precycle estradiol

Fertil Steril. 2008 May;89(5):1118-22. Epub 2007 Jul 20.

A luteal estradiol protocol for expected poor-responders improves embryo number and quality.

Frattarelli JL, Hill MJ, McWilliams GD, Miller KA, Bergh PA, Scott RT Jr.

- What protocol would you like to use

- Microflare
- Antagonist

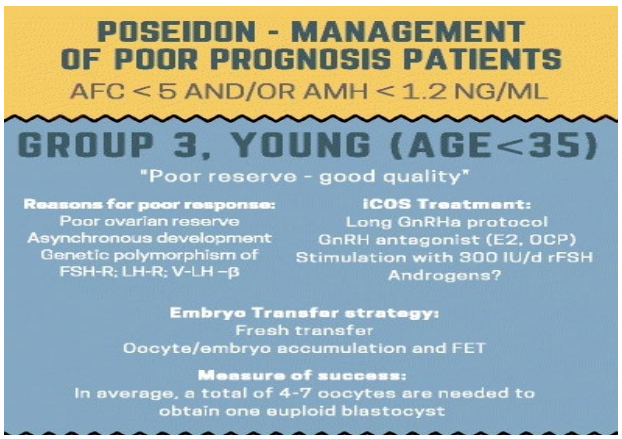
- HMG or rec FSH
- Pt was stimulated using 300 IU HMG
- 4 follicles developed and 3 eggs retrieved on day 12
- Follicle size

- 18mm
- 16mm
- 15mm
- 14mm

- ET 6.5mm
- 3 eggs obtained
- IVF done
- 2 eggs being fertilised by multiple sperms (polyspermy)
- What are the implications
- How do we modify cycle for next attempt ?

What are the implications of polyspermy ?
How do we modify cycle for next attempt ?

- Rec FSH or HMG
- Which dose
- Stimulation protocol
- Day of trigger



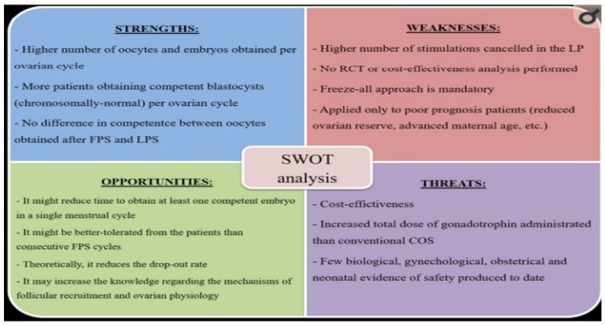
Stimulation

Consider dual stimulation for poor ovarian response patients to maximise egg collection

Front Endocrinol (Lausanne). 2018; 9: 317.
Published online 2018 Jun 14. doi: 10.3389/fendo.2018.00317
PMCID: PMC6010525
PMID: 29963011

Double Stimulation in the Same Ovarian Cycle (DuoStim) to Maximize the Number of Oocytes Retrieved From Poor Prognosis Patients: A Multicenter Experience and SWOT Analysis

Alberto Vaiarelli,[1,*] Danilo Cimadomo,[1] Elisabetta Trabucco,[2] Roberta Vallefuoco,[2] Laura Buffo,[3] Ludovica Dusi,[3] Fabrizio Fiorini,[4] Nicoletta Bernocchi,[4] Francesco Maria Bulletti,[5] Laura Rienzi,[1,2,3,4] and Filippo Maria Ubaldi[1,2,3,4]



Dose Of Gonadotrophins

- Current evidence supports a maximum daily dose of 300 IU of rFSH in the expected POR patient as higher doses do not increase neither the clinical pregnancy rate nor the live birth rate

Baker VL, Brown MB, Luke B, Smith GW, Ireland JJ. Gonadotropin dose is negatively correlated with live birth rate: analysis of more than 650,000 assisted reproductive technology cycles. Fertil Steril. 2015;104:1145–1152.e1-5. doi: 10.1016/j.fertnstert.2015.07.1151

Hum Reprod. 2017 Jul; 32(7): 1537–1538.
Published online 2017 May 25. doi: 10.1093/humrep/dex208
PMCID: PMC5946864
PMID: 28541398

Efficacy and safety of follitropin alfa/lutropin alfa in ART: a randomized controlled trial in poor ovarian responders

P. Humaidan,[1,2] W. Chin,[3] D. Rogoff,[4] T. D'Hooghe,[5] S. Longobardi,[5] J. Hubbard,[4] and J. Schertz[4], on behalf of the ESPART Study Investigators

- In patients aged <35 years (*n* = 118) a greater mean number of oocytes retrieved was observed with r-hFSH/r-hLH (3.5) compared with r-hFSH (3.3)
- In patients aged ≥35 years (*n* = 821), a lower mean number of oocytes retrieved was observed with r-hFSH/r-hLH (3.3) compared with r-hFSH .
- Lower incidence of total pregnancy outcome failure in patients receiving r-hFSH/r-hLH compared with those receiving r-hFSH alone

Polyspermy

- Polyspermy occurs due to poor egg quality and possible postmaturity
- In older patients better to trigger at 16mm follicle in such cases

Day of Retrieval

J Ovarian Res. 2018; 11: 23.
Published online 2018 Mar 16. doi: 10.1186/s13048-018-0398-8
PMCID: PMC5857093
PMID: 29548330

With low ovarian reserve, Highly Individualized Egg Retrieval (HIER) improves IVF results by avoiding premature luteinization

Yan-Guang Wu,[1] David H. Barad,[1,2] Vitaly A. Kushnir,[1,3] Qi Wang,[1] Lin Zhang,[1] Sarah K. Darmon,[1] David F. Albertini,[1,4] and Norbert Gleicher[1,2,4,5]

- Follicles of POA/oPOI patients biologically as well as clinically, indeed, behaved very similarly to previously reported older women above age 43 years
- More mature (81.5 ± 4.5 vs. 55.8 ± 8.3%, $P < 0.05$) and fewer atretic oocytes (7.9 ± 3.0% vs.28.2 ± 7.2%, $P < 0.05$) were obtained with ER t 16mmm folliclws than SR, while immature oocyte numbers were similar
- These observation suggest PL of follicles with SR

Stimulation

- If endometrial development poor consider freezing embryos . Inform about post thaw survival of embryos.
- If progesterone levels low on day of HCG consider fresh transfer if endometrial thickness optimal

CLINICAL SCENARIO 9

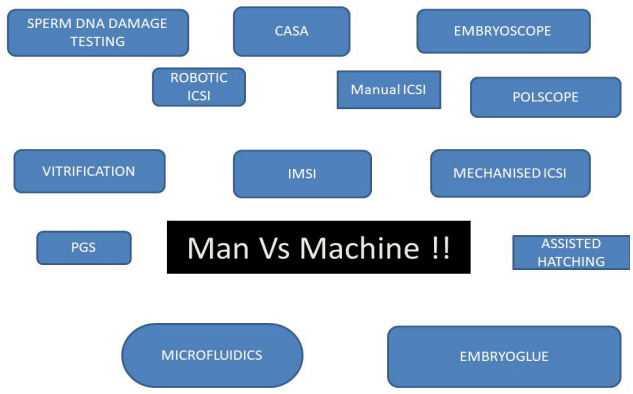
In a change of scenario , if you were an infertility patient, how would you select an IVF centre, what would be your criteria of a good clinic ?

- It takes time and patience to select a successful IVF center.
- When you screen potential IVF sites, ask questions! Research the clinics in your area.
- Compare the various sites based on different qualities such as:

- Are the physicians and embryologists qualified enough?
- What specialties are they certified in?
- Do the clinics use accredited labs?
- What types of amenities and services are offered to patients?

- IVF labs should maintain the best quality standards and should adopt standard operation protocols confirming to the practices followed globally to ensure the best of results and get their labs accredited.
- Get your IVF clinic registered with the National Registry for IVF clinics and ART banks managed by the Indian Council of Medical Research (ICMR)

- The auditing covers not just the Laboratory and Medical management areas but also patient safety, disaster management, Patient relations, grievance handling, counseling, administrative and HR protocols.
- The certification proves that that the clinic was already 99% prepared and the audit help us improve and achieve 100% benchmark ".



NEVER BASE YOUR DECISION ON WHAT GADGETS AN IVF CENTRE HAS

CONCLUSIONS

- There is no single "best" way to do anything in the IVF lab.
- Make your choices according to careful analysis of

- Ability to control the operational characteristics (equipment)
- Efficiency and simplicity of the procedural steps
- Ability to minimize mistakes: reduce process and operator errors
- Ability to optimize the requisite control variables
- Ability to minimize the impact of adverse factors

- Define Indicators and select / set Benchmarks, then monitor your system processes and outcomes

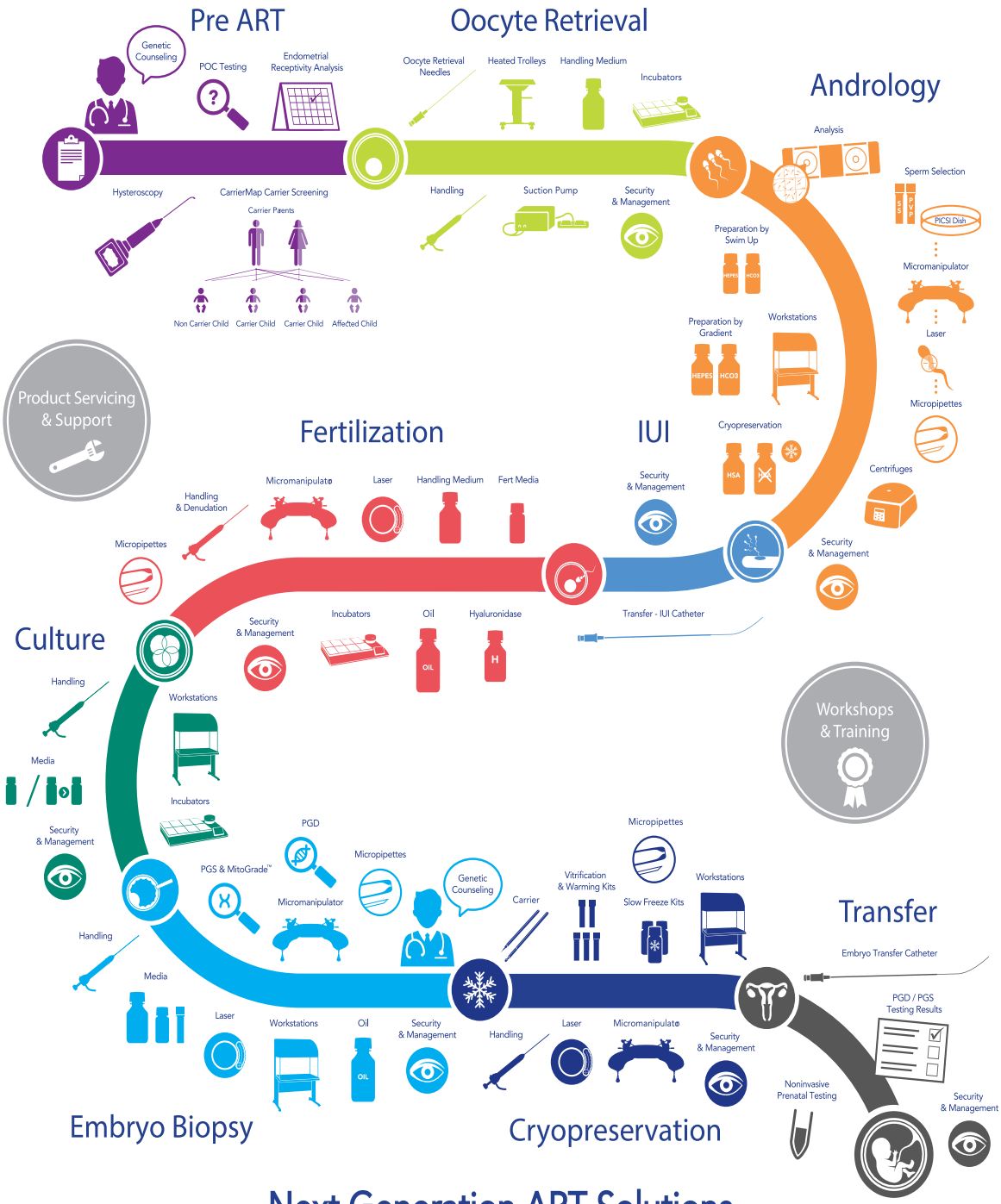
Stimulation

THANK YOU

Indian Fertility Society &
Origio India Initiative

CooperSurgical Fertility Companies

CooperGenomics™
a CooperSurgical company



Next Generation ART Solutions

Origio India Pvt Ltd

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