ВРТ / ИКСИ в позднем репродуктивном возрасте

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Advanced Maternal Age

- **Definition**

Chronological : Age above 35yrs

Biological

Ovarian reserve markers
- FSH
- AMH
- AFC

Response to stimulation

All parameters should be included in the definition

Introduction

- Worldwide tendency to postpone pregnancy until later reproductive life :

  Building up a career
  Late marriages & increased divorce
  Lack of social incentives to support parenthood
  Misleading idea that ART can overcome fertility decline with aging

- Patients of AMA comprise 10-20 % of IVF population

- ICSI cycles after 40 yrs : Europe 25%
  Egypt 10%

- Oocyte pool decreases from 1-2 millions at birth to 300,000 - 500,000 at puberty to few hundred eggs at menopause

  Faddy et al. 1992

- 300 - 400 oocytes will be ovulated during reproductive years

- Decline of fertility starts 10 - 13 yrs before menopause & is an expression of accelerated ovarian ageing

  Helen S. et al. 2003 HR

How Does Biology Work ?

Aging is associated with fertility decay due to a decline in both ovarian reserve and oocyte / embryo competence mainly as a result of:

Spindle & meiotic errors
Reduced mitochondrial activity
Increased aneuploidy

Pellestor et al. Hum Genet 2003, 2005

- **Oocyte aneuploidy increases with age**

< 35 yrs	10%	40 yrs	30%
43 yrs	40%	> 45 yrs	90%

Capalbo et al 2017, Franasiak JM et al. FS 2014

This explains the sharp increase in chromosomal defects after 35yrs

from 2.5 - 3 / 1000 births to 37

BJOG International 2010

Infertility Work-up in AMA

- Recommended after 6 months of seeking pregnancy SOGC 2011
- Exclude age related factors : fibroids, endometriosis , polyps
- Multi –marker approach to evaluate ovarian reserve

Broekmans et al. 2006

- TSH, PRL, Tubal patency , HS
- Hypertension, DM
- History of previous radiation / chemotherapy
- Details about previous IVF trials : poor response or cancellation

Ovarian Reserve Markers

- Correlate with egg quantity / not quality
  Predict response to stimulation
  Poor predictors for pregnancy
  Help in determining FSH dose & stimulation protocol

Fauser B et al.2007

FSH : D_3 FSH (> 14 IU /L) : First sign of ovarian aging , usually occurs between 35 & 40 yrs

AFC : FSH sensitive / correlates with primordial follicles number

AMH : Produced by GCs of antral & pre-antral follicles

Ovarian **Reserve** Tests & Age

	24 - 33y	34 - 38y	39 y
AMH ng/ml	2.1	1.6	1.1
AFC	11	10	7
FSH IU/L	6.9	7.4	7.9

Imog et al. 2011

Poor Responders

ESHRE Criteria Bologna: at least 2 criteria

Ferraretti et al. HR 2011

1-Age > 40yrs presenting with other risk factors for PR

2-Previous POR ≤ 3 oocytes with standard dose stimulation

3- Abnormal ovarian reserve (AFC <5-7 fol. or AMH < 0.5-1.1 ng/ml)

Or : Any age + 1 cycle ≤3 eggs retrieved + abnormal reserve tests

POSEIDON Groups of Poor Response

Patient- Oriented Strategies Encompassing IndividualizeD Oocyte Number

New measure : Number of oocytes needed to retrieve to obtain at least one euploid embryo

Changed definition of PR to have homogenous population

4 Groups based on:

Age and expected embryo euploidy rate

< 35 yrs 60% , 40 - 42 yrs 30% > 42 yrs 15 %

Ovarian reserve biomarkers (AFC, AMH)

Previous response & oocyte quality

Thor Haahr et al . 2015

POSEIDON Groups

- I < 35 yrs , AFC ≥ 5 , AMH ≥ 1.2 ng /ml , < 9 retrieved eggs
  young pt . with unexpected poor/ suboptimal response

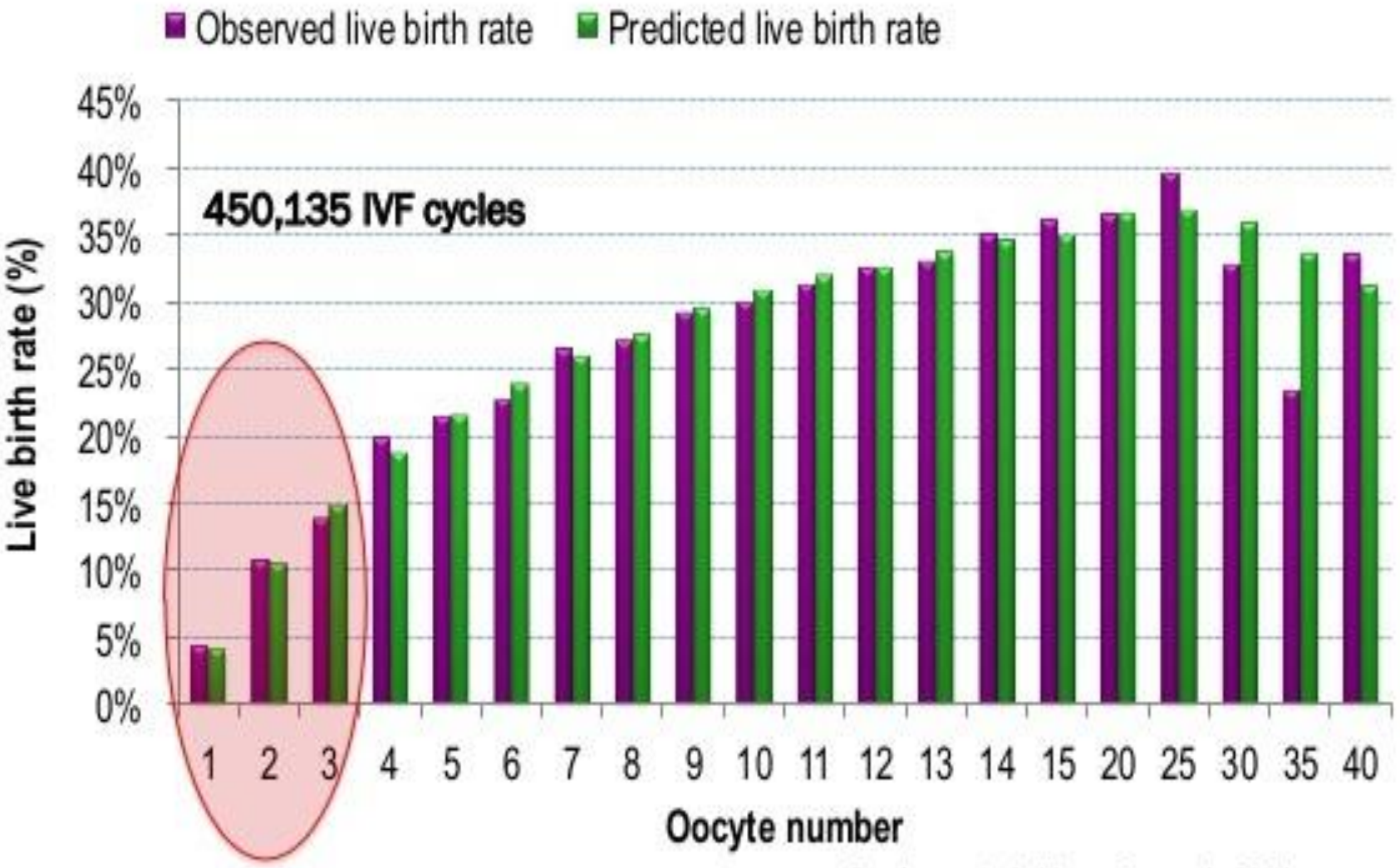
- II > 35 yrs, AFC ≥ 5 , AMH ≥ 1.2 ng /ml , < 9 retrieved eggs
  older pts. with unexpected suboptimal response

- III < 35 yrs , AFC < 5, AMH < 1.2 ng /ml , < 5 retrieved eggs
  young with poor reserve

- IV > 35 yrs ,AFC <5, AMH < 1.2 ng /ml , < 5 retrieved eggs
  older pts. with poor reserve

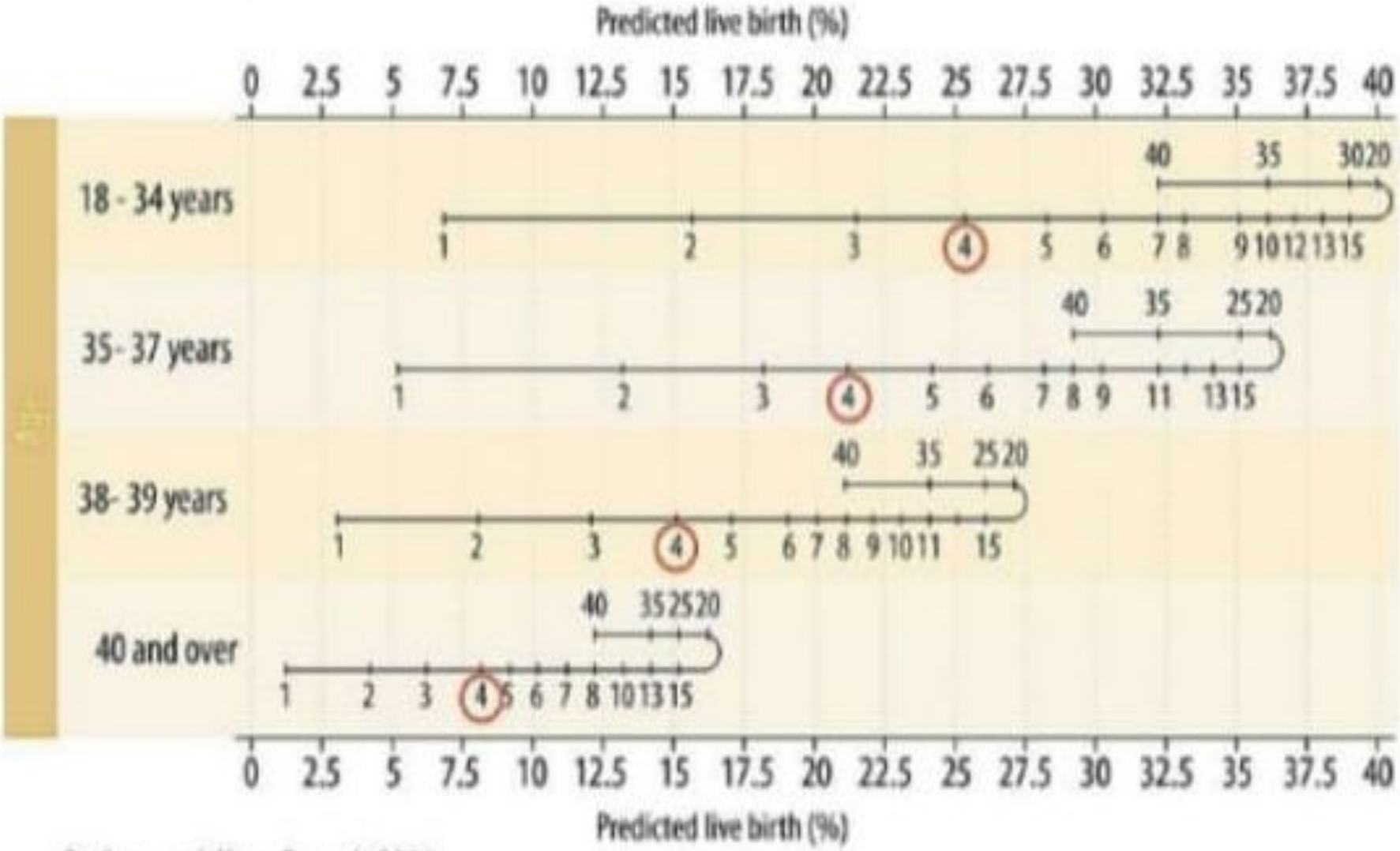
This changes the prognosis of women with the same number as well as different oocyte yields

Number of Oocytes and LBR



Sunkara et al. *Hum. Reprod.*, 2011

LBR by No. Oocytes and Age



Sunkara et al. Hum. Reprod., 2011

Treatment Strategies in Practice

Current strategies

- Maximizing ovarian response
  - Natural or mild stimulation IVF protocol
  - Personalized COS
- Freeze - all embryos
- Enhancement of embryo selection via BC – stage PGT-A & frozen single ET
- Endometrial scratching
- Oocyte cryopreservation / medical & social freezing
- Egg donation

- **Future solutions :** Mitochondrial transfer
  - Chromosome therapy
  - In vitro generation of gametes
  - In vitro activation

Ubaldi F J. Frontiers in Gin Endocrinology 2019

Maximizing Ovarian Response

- Keep in mind :

Gns can not generate follicles ex-novo

Max. threshold dose of Gns 300 IU FSH /d ± 75-150 IU LH/d

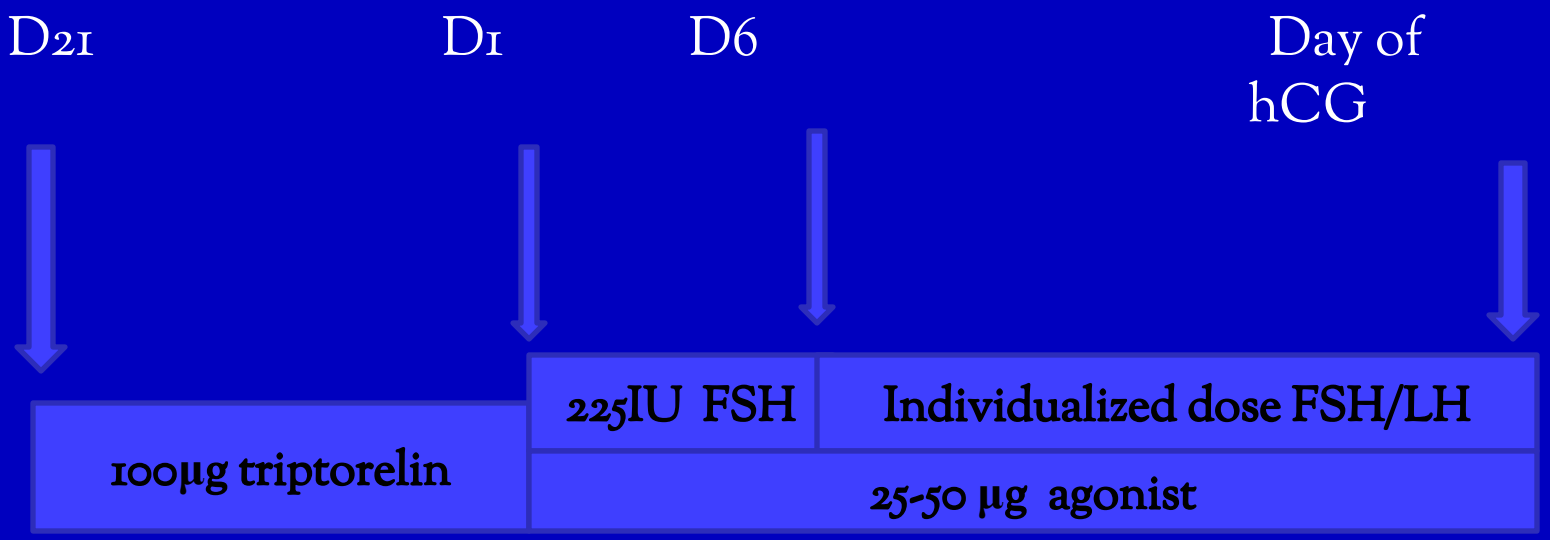
Still OHSS can occur

- Antagonist / Agonist protocols are equally effective in PRs

RCOG Guidelines 2019

- hCG trigger at 16mm fol. Size: less premature luteinization , doubles CPR above 43y from 7.7% to 15.5% Wu et al. 2015
- D2 transfers give better IVF outcome

Mini-dose Long Agonist Protocol



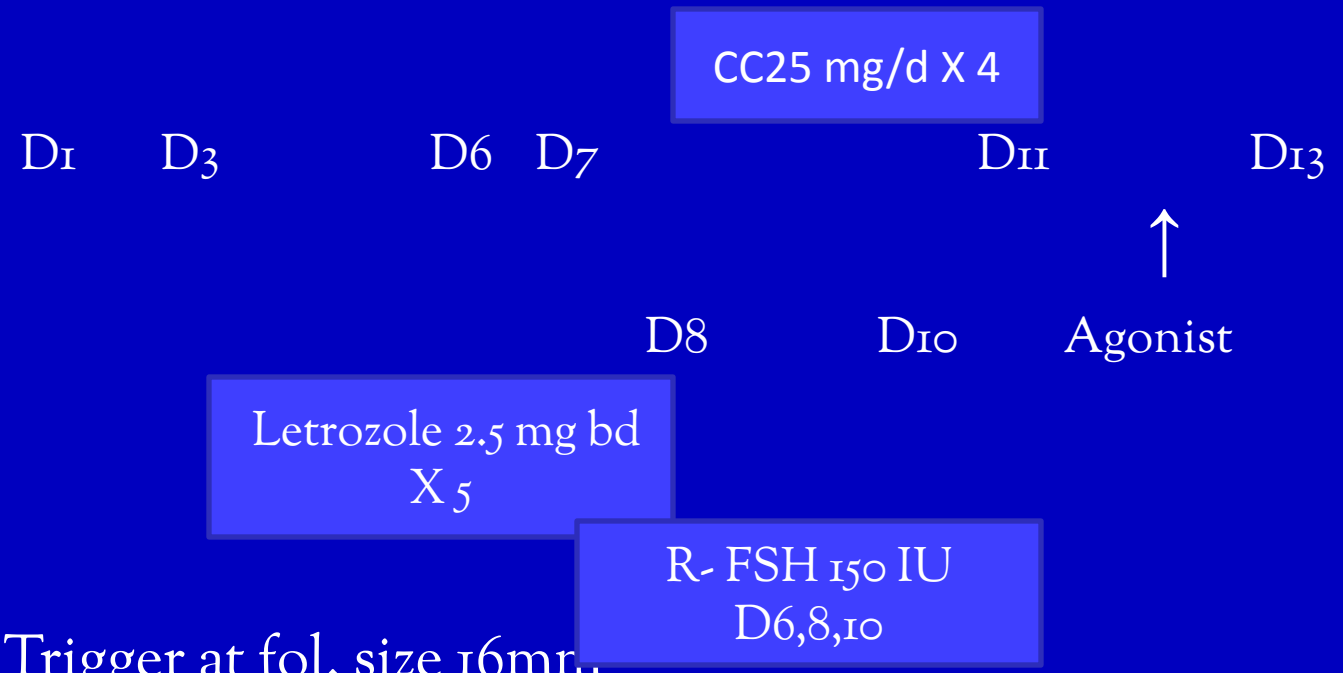
Limited oocytes available for fertilization
Longer time –to-pregnancy
Higher cancellation rate

Progestin

Down regulation

Wessman et al . Fertil Steril 2003

Minimal Stimulation



Trigger at fol. size 16mm
Ibuprufen 600mg on day of agonist
If LH rise : early OCP
Vitrification : oocytes /embryos
BC freezing
FET : natural / HRT cycle

Modified Dr J. Zhang

Combined r- FSH & r-LH 2:1

Role of LH in folliculogenesis & implantation

- Stimulates the pathway $P_4 \rightarrow$ Androgens $\rightarrow$ Estrogens
- Enhances ovarian sensitivity to FSH leading to final maturation & successful luteinization by hCG
- Increases IR & CPR

Patermor 2007 / Reprod Biomed Online

LH polymorphism (V-βLH) shorter half life
Causes ovulatory dysfunction , POF, ↑PRL , Infertility & Endometriosis

Mafra et al. 2010 / Obstetr Gynecol Reprod Biol.

Greater needs for r-FSH > 2500IU Alviggi et al 2009

RCOG Guidelines 2019 : Gns dose > 300 IU is not recommended for predicted PRs

Preferred regimen in expected poor responders
(AMH≤0.82 and/or history of POR)

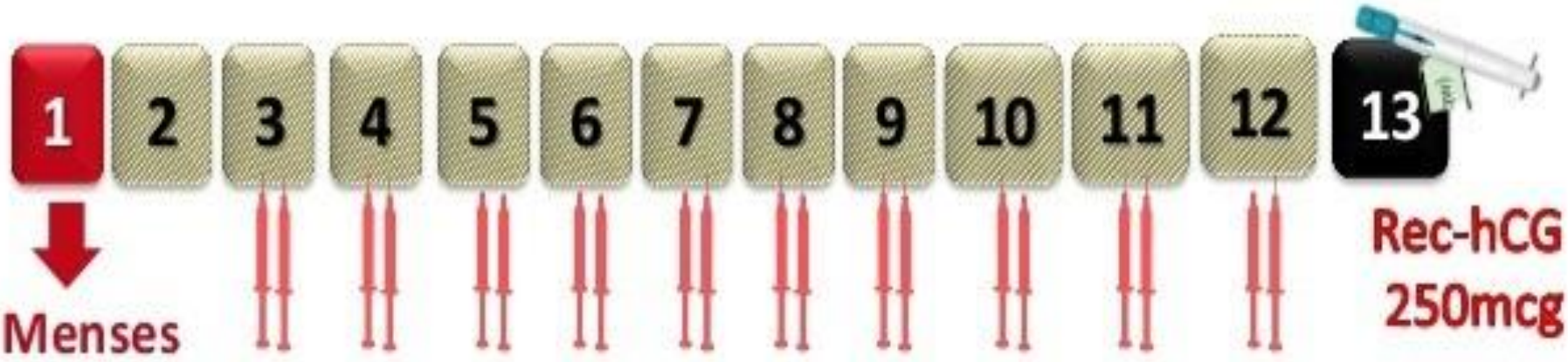
Rec-hFSH + rec-hLH (2:1 ratio) from Sd1

Gonadotropin dose per day 450 IU:

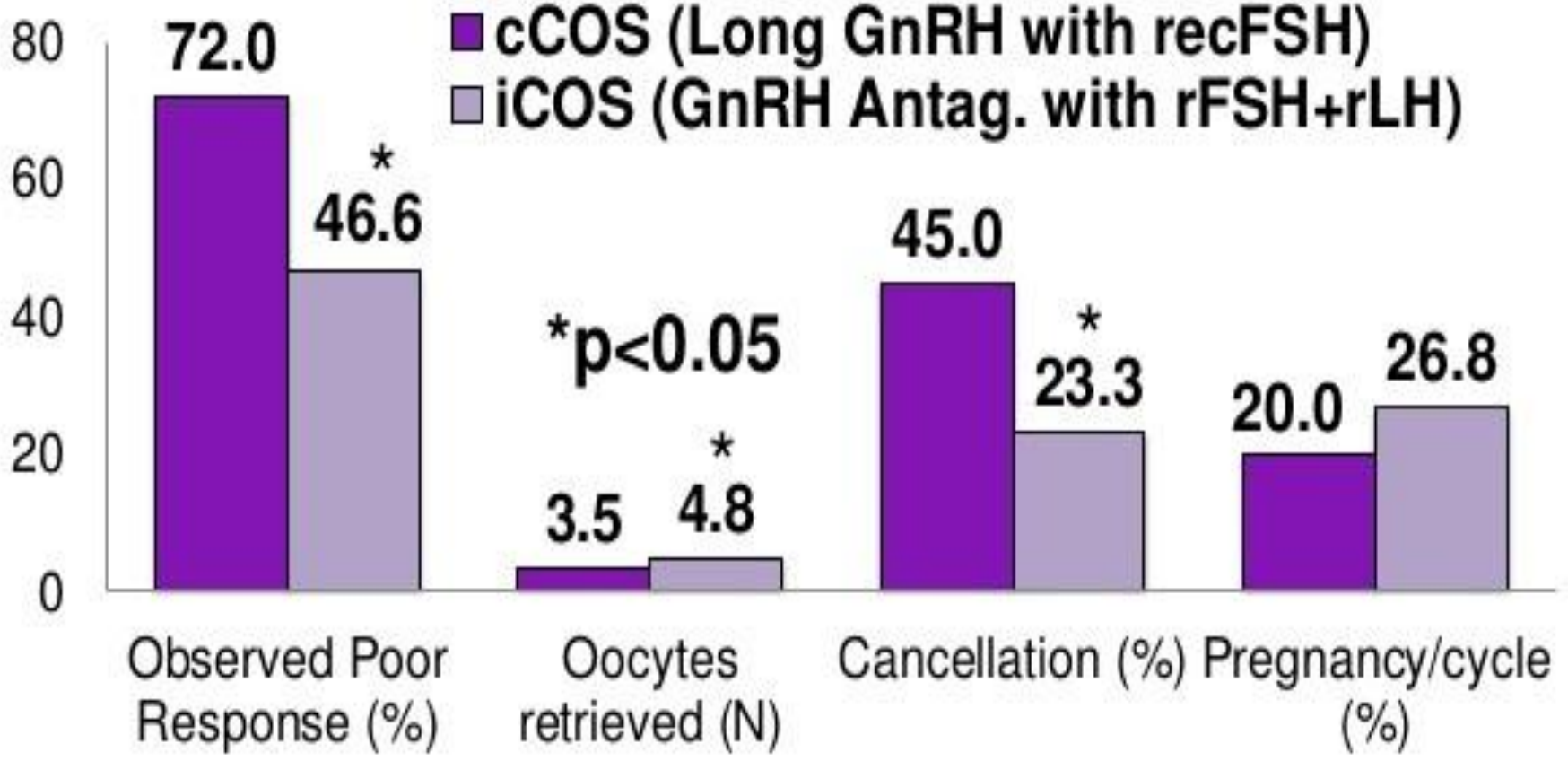
- *rec-hFSH 300 IU + rec-hLH 150 IU)*

GnRH antagonist (flexible): mean 13mm

LH trigger with rec-hCG (mean 17-18 mm



Individualized vs. Conventional COS in Expected Poor Responders (N=118)



Expected poor response: AMH<0.82 ng/dL; Observed poor response <5 oocytes retrieved;

Duostim Protocol de Ziegler 2015

- 2 GnRH- antagonist protocols (300 IU FSH /d)
- COS 1 : start D6 post OCPs COS2 : start right after OPU1
- Triggering : GnRH-a in COS 1&2
- **Results** : Similar number of eggs & BC in COS1 & 2
  Twice as many oocytes & BC in a 4-week time frame
- No statistically significant difference in number of retrieved MII oocytes 5.7 ±3.3 vs 5.1 ± 3.4 or
- Biopsied euploid blastocysts / stimulated cycle 44.8% vs 46.9% in LP stimulation vs FP. F. M Ubaldi 2016
- Only cost effective when it increases CLBR by 20 %
  (now hardly reaches 18 %)

Double Ovulation Trigger

GnRH-a with a reduced or standard dose of hCG 40 hrs and 34hrs prior to OPU respectively

Kasum et al 2016

Indications : 1- EFS

K. Deepika et al. 2015 / Journal of Human Reprod Sciences

R. Beck-Fruchter et al. 2012 HR

2- Poor responders

Significant increase in number of retrieved eggs , M II eggs, FR, IR , PR & LBR

Oliveira et al 2016 J BRA Assist Reprod

Natural & Modified Natural Cycle

Natural : Less cost & side effects with more natural hormonal environment but, fewer oocytes/ embryos and less success rate

LBR In PRs ≤ 35y 2.5% 36-39 y 2.4% ≥ 40y 1 % Polyzos et al. 2012

Modified NC

250 µg Cetrorelix is started concomitantly with 150IU r-FSH when the leading follicle 13-14mm till hCG injection

Cancellation Rate: 12/45= 26.7% vs 3/45= **6.7%** in antagonist arm
CPR / cycle / ET similar
LBR / ET similar
Total FSH dose less

Kadoch et al .
Segawa et al. Fertil Steril 2009

RCOG Guidelines 2019: Modified NC is probably not trecommended over conventional stimulation for expected PRs

Freeze - all Policy

- Rationale : Improves endometrial receptivity
  - ↓ OHSS & MPR
  - ↑ Cumulative PR and cost effectiveness in high responders
- Indications : OHSS prevention
  - Increased P_4 on day of hCG (>2.5 ng /ml) Xu et al. 2012
  - Dual stimulation in poor responders

Outcome : FAP v Fresh D_3 ET n= 530

LBR 46.34% v 35.9 % OPR 39.7% v 31.1 %

Roque et al 2016 In AMA No RCTs . 4 small retrospective studies showing no benefit

Freeze - all Policy For Poor Responders

n= 83,000 1st cycle
Cumulative PR

Acharya et al 2018

	Fresh ET	Frozen ET
	32 %	15 %
LBR	25 %	10 %

Advantages of FAP: Decrease preterm birth & LBW

Pelkonen et al. HR 2010

Disadvantages : Evidence of benefit is limited to high responders
Cumulative outcome is not different in poor responders
Increases time to pregnancy
Questionable cost effectiveness in PRs
Higher risk of LGA (RR1.54) & PET (RR1.29)

Maheshwari et al HR Update 2018

- **Oocyte cryopreservation :** eggs, ovarian tissue , IVM oocytes

Postpone parenthood: Pre-cancer Rx

Social issues (after 37 yrs : Doyle et al 2019)

It is a reproductive insurance , reducing the need for egg donation

- **Egg donation :**

Especially after many failed euploid ETs

> 35 % PR in females in their 50s US data

Associated immunological problems impairing placentation

Preimplnatation Genetic Testing of Aneuploidies PGT-A

Aim : Avoid ET of aneuploid embryos & Increase PR /ET especially after SET to prevent multiple gestation , miscarriage & vital chromosomal syndromes

Ubaldi FM et al. HR 2015 , Dahdouh & Chen MA 2015

Types of PGT-A :

Comprehensive chromosome testing	CCT
Comparative genome hybridization array	CGH -a
Single nucleotide polymorphisms - array	SNP-a
Next generation sequencing	NGS

Types of PGT-A :

Cleavage stage biopsy D3

PBs biopsy D 0-1

Tropho-ectoderm biopsy D 5-7 (Mosaicism 28-90%)

Embryo Genetic Screening

- **MA & SR Mastenbroek et al 2013 HR Update**

9 RCTs

FISH & D_3 biopsy

PGS significantly lowered LBR for women of AMA 13-23 % vs 26 % without PGS

PGS in good prognosis women & in RIF gave similar outcomes

Technical drawbacks & chromosomal mosaicism underlie this inefficacy of PGS

New PGS approaches should be evaluated carefully

Rubio et al 2017 D2 PGT-A in women 38-41y : Sign. higher CLBR using PGT-A 52.9% vs 24.2 % / first ET

36 % vs 21.9% / patient

Dramatically less miscarriage rate 2.7 % vs 39 % in controls

Polar body testing CCT did not increase LBR in 205 women 36-40y 24% with or without testing Verpoest et al . HR 2018

Endometrial Scratching

Mechanisms :

Induce endometrial decidualization — Timeya T. et al J. Reprod & Infertility 2014

Provokes secretion of cytokines & GFs — Liand Hao 2009

Recruits stem cells to the endometrium — Taylor 2004 , Du & Taylor 2007

How? Biopsy (Pipelle sampler) , scratch , hysteroscopy ± versa point

Raziel et al 2007, Narvekar et al 2010

Biopsy / Scratch are superior to hysteroscopy

2 SR : Potdar et al 2012, El-Toukhy et al. 2013

Endometrial Scratching

Timing : one week before the treatment cycle

Barash et al 2003 , Raziel et al. 2007, Zhou et al 2008

Immediately before starting ovarian stimulation

In follicular phase of index cycle (no benefit !)

Karimzad et al . 2010. Zhou et al 2008

Before triggering ovulation

Not on OPU day

Cochrane SR 2015, Nastri et al 2012

Can improve IVF outcome

Botdar et al 2012 SR & MA

Doubles LBR 48.9 % vs 22.5%

Barash A. 2003 FS , Zhou L 2008

Doubles PR & LBR **66.7% vs 30.3 %**

Timeya T. et al J. Reprod & Infertility 2014

Pipelle study : Same LBR 26.1 %

Sarah Lensen et al 2016

MA & SR 2019 / HR Open n= 2537 : It remains unclear whether we should do scratching prior to IVF

N E Hoogenhuijze et al. 2019

Awaiting results from SCRaTCH and Endometrial Scratch Study

Mitochondrial DNA Transfer

Oocyte mitochondria: Functionally immature

6000 in germ cells to 300,000-400,000 in MII

Decrease after fertilization & with embryo development

Chappel S. 2013

Primarily inherited from the mother

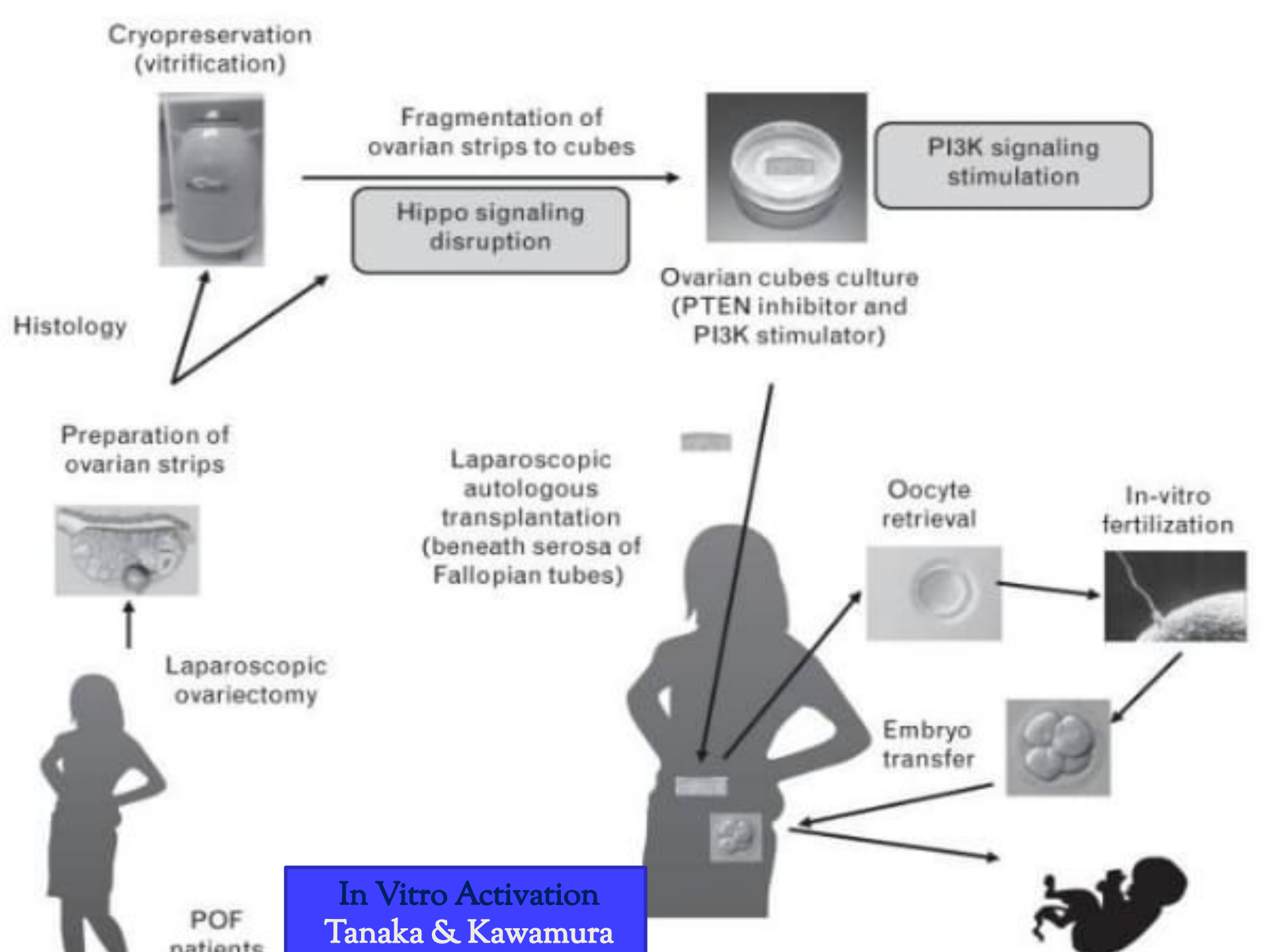
mtDNA susceptible to mutations (has no histones , introns , repair enzymes)

Leese 2012, Bentov et al 2011

Leading to energetic stress in the oocyte or embryo or

Myopathy, encephalopathy, lactic acidosis , stroke later in life

Moont S. et al. 2013


Cryopreservation
(vitrification)
Fragmentation of
ovarian strips to cubes
Hippo signaling
disruption
PI3K signaling
stimulation
Histology
Ovarian cubes culture
(PTEN inhibitor and
PI3K stimulator)
Preparation of
ovarian strips
Laparoscopic
autologous
transplantation
(beneath serosa of
Fallopian tubes)
Oocyte
retrieval
In-vitro
fertilization
Laparoscopic
ovariectomy
Embryo
transfer
POF
patients
In Vitro Activation
Tanaka & Kawamura

- **AUGMENT** 3 centers

- Autologous germline mitochondrial energy transfer from ovarian cortex
  (less prone to progressive mutations as mtDNA from somatic cells)

  Dori C. Woods et al. 20

Steps : Isolation of egg precursor cells by magnet – assisted cell sorting
Extraction of mt.
Injection of mt. into autologous oocytes

- CPR/ cycle 22% CPR/ ET 32 % n = 60

  Fakih MH et al. 2015

❖ AUGMENT: Might be beneficial in ageing oocytes & RIF via in vitro differentiation (IVD) of oogonial stem cells .

Silvestris et al HR 2018

AUGMENT

- E. Labarta et al 2019 FS : Triple blind RCT in PRs .

  mean age 36+ ys n= 250 control

  n= 253 AUGMENT arm

Conclusion : Mitochondrial injection does not benefit
Developmental capacity of treated oocytes or
Euploidy status of embryos nor
PR

So AUGMENT should not be considered a novel way of ovarian rejuvenation in poor prognosis pts with bad embryos

Adjuvant Therapy

GH Stimulates FSH receptor expression on granulosa cells
Increases intra-ovarian IGF-1

4-18 IU sc /d from day of stimulation (7. 5 IU /d from D6)

7/9 studies : **No change or significant improvement**

J.Dor et al HR . Eftekhar et al Archives of OBGYN 2013

↑ collected eggs 5.9 vs 3.7 ↑ PR 25.7vs 11.4 % (P=NS) Chung-Hoon et al

Recently :Duffy et al. ↑ CPR MA 2013
Kolibianakis et al. ↑ PR MA 2009
Kyrou et al. ↑ LBR MA2009

Sustained release GH 20 mg D2+ midluteal + late luteal doses in PRs >39y
= Sign. more mature oocytes Choe SA et al. 2018

No clear evidence of benefit in PRs Yue- Ming Xu et al 2019

Adjuvant Therapy Cont...

- Androgens : DHEA / Testosterone
  Augment FSH receptor expression
  Promote GCs development
  Increase pre-antral & antral follicles

Cochrane MA 2015 : 1496 PRs
No evidence of benefit on CPR
Safety ?

Nagels HE et al. 2015 , Sunkara et al. 2011

Anti -estrogen, Aromatase inhibitors :

Better embryo quality, less eggs — Baart et al 2007

↑Endometrial receptivity — Devroey 2004

Letrozole ---- better endometrium & folliculo- genesis
Can be used in mild protocol but probably not recommended by RCOG guidelines 2019

100 mg CC or 5mg Letrozole +150-225IU r-FSH Vs conventional protocol

Comparable results , ↑PR > 37yrs , Less cost

Yoo et al 2011 Clin Exp. Reprod Med

Aromatase inhibitors cab be used with high dose FSH

Schoolcraft et al .2008, G Velasco et a2005

CC alone or with Gns or Gns alone are equally recommended in

Potential Future Approaches to Treat AMA Inferility

- Minimally / non-invasive embryo biopsy : investigate leftover IVF products (proteomics, metaboloites, nucleic acids)

Spindle chromosomal complex transfer
Chromosome therapy
Isolation of oogonial stem cells OSCs
Induced (somatic) pluripotent cells
In vitro generation of new gametes : Ovarian cortex
Bone marrow stem cells

Silvisters et al. HR 2018, Kuwamora et al 2019

Age and IVF Outcome

Age	20-30yrs	31-35	36-40	41.....
FR	67%	68%	70%	67%
G1,2 Es	54%	52%	53%	55%
PR	58%	55%	46%	26%
CPR	51%	46%	38%	18%
Miscarriage R	8%	12%	19%	35%

S. Sunkara ,T. El Toukhy 2017

Cycle Cancellation & LBR ≥ 40 yrs

	Initiated cycles	Cancellation Rate	LBR %
Klipstein et al .2005	2750	19.9%	9.7
Tsafrir et al. 2007	1217	16.6 %	4.7
Serour et al. 2010	2386	16%	6.7
ESHRE 2010			8.6

Outcome of IVF in AMA

Number of eggs needed to find one euploid embryo

35-37 yrs	5 eggs
38-40 yrs	7
41-42 yrs	10
> 42 yrs	20

Vaiarelli et al . 2018

- Implantation potential on euploid BC is independent of maternal age 45-50 %

Cimadomo D. et al. 2018

Implantation Rate as a function of maternal age

25-29 y	18.2 %	30-34 y	16.1%
35-39 y	15.3%	40-44 y	6.1 %

ASRM Practice Committee 2006

IVF Outcome in AMA

- LBR after IVF Canadian ART data

  < 35 yrs 37.4% 35-39 yrs 26.5 % > 40yrs 11.4 %

  J. Gunby FS 2011

- LBR /cycle 6.7 % (range 10% - 0.5%)

  40-42 y 7.4%

  > 43 y 1.1%

  Serour et al. 2014

HEFA 2014 LBR 38-39y 19.2 /cycle 40-42y 12.7%

43-44y 5.1% ≥ 45 yrs 1.5 %

When to stop IVF in AMA ? at ≥ 45 yrs .

Mehmet at al . 2013

Still reasonable LBR up to 44 yrs

Most pregnancies occur within the first 3 cycles

After 45 stop ART procedures using patients' own oocytes

Pregnancy in AMA

Increased risk of spontaneous miscarriage

17 %- 28% 25-39 yrs

34% -52 % ≥40 yrs

Canadian Data

53% ≥ 45yrs (10% at 25-29yrs)

Norwegian data n=421,201 BMJ 2019

- Increased risk of maternal & obstetrical complications :

Maternal death Ectopic Hypertension & PET

Gestational diabetes Prematurity IUGR

Fetal and neonatal death Operative delivery

Smajdor et al. 2008

Pregnancy in AMA

- Increased risk of aneuploidy & other chromosomal abnormalities

  Down's risk 1 : 204 at 35 y

  1 : 65 at 40 y

  1 : 2 at 45y

  Hook et al. Obst Gynecol

Some authors link pregnancy after 35 yrs , usage of fertility drugs & androgens with an increased risk of developing ovarian cancer.

Cochrane review 2019: The risk is slightly higher in nulliparous and for borderline tumors

Age , infertility itself , parity , genetics & BMI should be taken into consideration

So ,What is the Magic Formula ?

- None
- Fertility preservation (oocyte / ovarian tissue freezing)
- Careful assessment of oocyte reserve
- Optimization of ovarian stimulation through individualizing treatment plan: iCOS , oocyte /embryo banking via duoStim protocol and enhanced embryo selection for SET by PGT-A
- Realistic expectations based on proper counseling and the best available practice
- Egg donation

Conclusions

- ATR outcome is adversely affected by AMA
- Launching social campaigns & educating young generations are important to promote awareness of age impact upon fertility
- Properly counsel females regarding risks of pregnancy especially above 40 yrs
- Ovarian stimulation must be tailored individually
- Androgen adjuvant therapy and day 2 ET increase CPR in PRs
- In virto activation and mitochondrial transfer are developing areas in ART
- Egg donation is the last efficient alternative

Have you Guessed

What FONA stands for ?

Friend of Natalia Artymuk

If you have a friend that's true.
Count your blessings for this gift .
For , she / he will stay with you.
When the rest have gone adrift .

Quoted

THANK YOU

For

Listening

Abbreviations
As they appear in the slides 1, 2,3 etc..

- AMA : advanced maternal age
- TSH :thyroid stimulating hormone
- PRL: prolactin
- HS: hydrosalpinx
- DM : Diabetes mellitus
- AFC: antral follicle count
- AMH: anti- mullerian hormone
- GCs : granullosa cells
- POR : poor ovarian response
- COS : controlled ovarian stimulation
- BC : blastocyst
- PGT-A preimplantation genetic testing for aneuploidies
- ET: embryo transfer
- Gns: gonadotropins
- OHSS :ovarian hyperstimulation syndrome
- hCG : human chorionic gonadortopin
- CPR cumulative pregnancy rate
- CC: clomiphene citrate
- OCP: oral contraceptive pills
- FET: frozen embryo transfer
- HRT: hormone replacement therapy
- IR: implantation rate
- POF: premature ovarian failure
- PRs : poor responders
- OPU : ovum pickup
- MII : metaphase II
- LBR: live birth rate ,
- CLBR : cumulative live birth rate
- EFS: empty follicle syndrome
- FR: fertilization rate
- NC : natural cycle
- MPR: multiple pregnancy rate
- OPR: omgoing pregnancy rate
- FAP : freeze –all policy
- LBW
- : Low birth weight
- LGA: large for gestational age
- PET: pre-eclampsia
- RR;Relative risk
- IVM: in vitro maturation
- Rx: treatment PBs ; polar bodies
- MA 7 SR : meta-analysis & systemic review
- PGS: preimplantation genetic screening
- RIF: repeated implantation failure
- CCT: Comprehensive chromosome testing
- GFs ; growth factors
- mt DNA: mitochondrial DNA
- IGF-1 : Insulin growth factor
- DHEA: dehydroepiandrosterone
- ART: artificial reproductive techniques
- IUGR: intra-uterine growth restriction
- i COS : individualized controlled ovarian stimulation