CRYOPRESERVED-THAWED HUMAN EMBRYO TRANSFER : SPONTANEOUS NATURAL CYCLE IS SUPERIOR TO HUMAN CHORIONIC GONADOTROPIN–INDUCED NATURAL CYCLE

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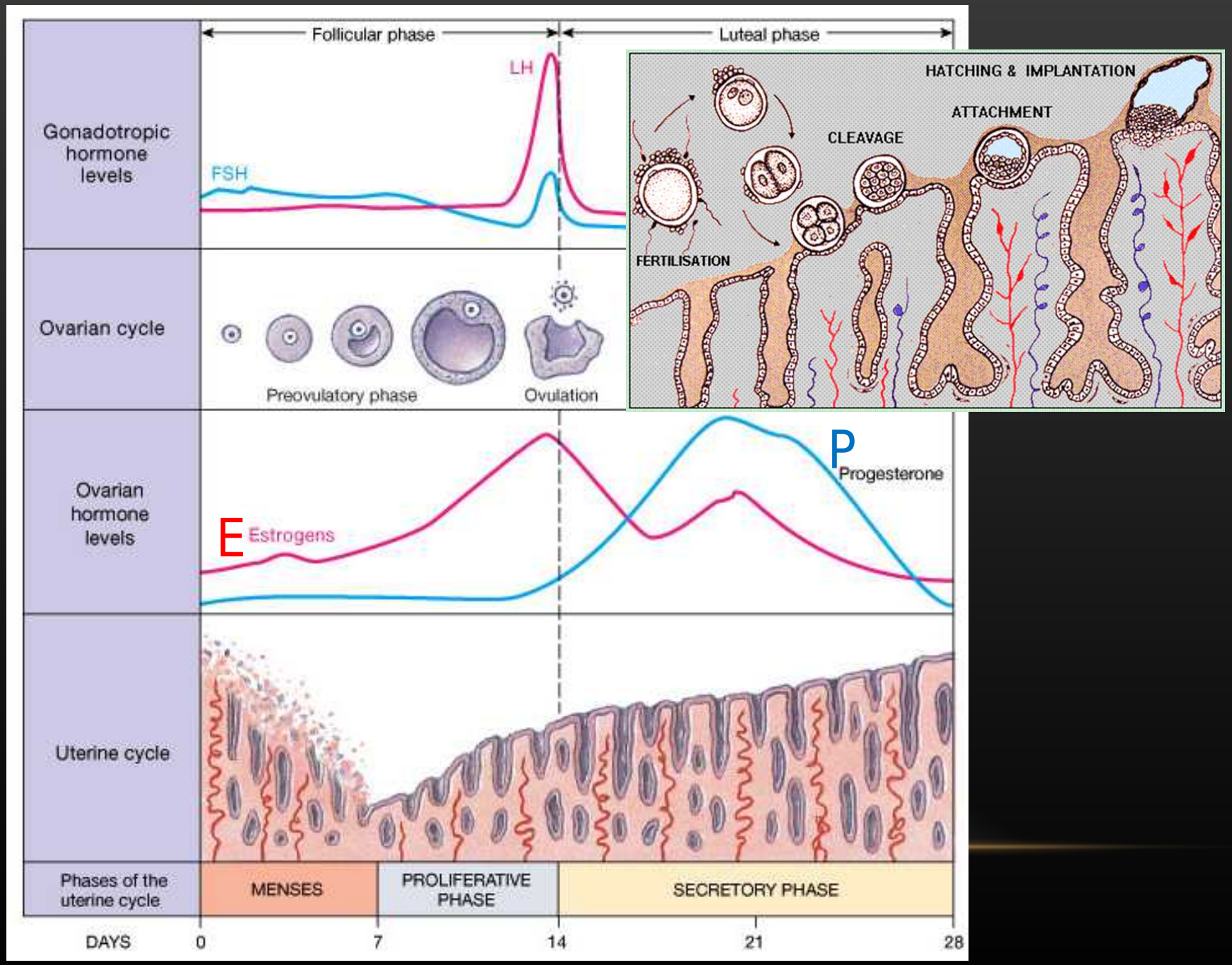
Follicular phase
Luteal phase
Gonadotropic hormone levels
LH
FSH
Ovarian cycle
Preovulatory phase
Ovulation
Ovarian hormone levels
E Estrogens
P Progesterone
Uterine cycle
Phases of the uterine cycle
MENSES
PROLIFERATIVE PHASE
SECRETORY PHASE
DAYS
0
7
14
21
28
HATCHING & IMPLANTATION
ATTACHMENT
CLEAVAGE
FERTILISATION

INTRODUCTION

- Cryopreservation of surplus embryos
  - ↑ cumulative chance of conception per oocyte retrieval
  - 1st successful pregnancy – 1983
- *Preparation of the endometrium*
  - Gonadotropins-GnRH agonists
  - Clomiphene citrate (selective estrogen receptor modulator)
  - Exogenous administered estrogens & progestatives

- Natural cycle
- Endometrial preparation (exogenous E & P, with or without the addition of a GnRH agonist)
- *No conclusions could be drawn on the superiority of any one regimen in terms of ongoing pregnancy rate, (lack of randomized trials),*
  Cochrane review, Ghobara & Vandekerckhove

HCG

- Induce ovulation / Terminate the follicular phase / ET independent of the natural endogenous LH surge
- Well-proved biologic base / Clinical predictability / Ease of planning

- Terminated by hCG vs. spontaneous LH rise → lower probability of ongoing pregnancy in IUI cycles

- Kosmas et al.

- Impact on endometrial receptivity (EM hCG receptors)

FOR FROZEN-THAWED ET

- Hypothesis of the current trial
  - ➔Natural LH surge cycle would be superior to a hCG-induced unstimulated cycle
- Present study:
  - ➢Natural cycle (without EM preparation)
  - ➢Difference in the ongoing pregnancy rate between
  - ➢spontaneous LH-P rise (Study group)⇔hCG for final oocyte maturation & ovulation (Control group)

MATERIALS AND METHODS

- Prospective randomized trial
- Tertiary referral University Hospital

PATIENTS

- 168 patients, outpatient clinic
- October 1, 2007 ~ November 30, 2008
- Inclusion criteria:
  - Maternal age $\leqq$ 36 years
  - Regular menstrual cycle (25–34 days)
  - Previous conventional IVF or intracytoplasmic sperm injection (ICSI) with embryo cryopreservation on day 3,
  - Normal intrauterine cavity
  - Consent: frozen-thawed ET in an unstimulated cycle.

- Exclusion criteria
  - The use of testicular sperm for ICSI
  - Early (day 3) follicular phase FSH levels ≦ 12 IU/L
  - American Fertility Society grades ≧ III for endometriosis
  - BMI ≧ 29 kg/m2
- Patients could participate in the study only once

PROCEDURES

- Day 2: TVS + serum hormone (FSH, LH, E2, P)

Control group:

- Endometrium $\geqq$ 7 mm, Follicle $\geqq$ 17 mm

  ➔ 5000 IU urinary hCG (final oocyte maturation)
- Monitor serum P & E2 (confirm ovulation)
- Excluded if premature ovulation confirmed (serum)

Natural cycle

- Day 8: Check LH

  → Confirm LH rise → monitor LH, E2, P the day after

- LH surge: ↑ 180% > the latest serum value available in that patient and continued to rise

- Ovulation: following D1: ↓ E2 + ↑ P >1.5 nmol/L

- No luteal support
- All ultrasound performed by a single physician
- LH, FSH, hCG, E2, P levels - automated Elecsys immunoanalyzer *(Roche Diagnostics, Mannheim, Germany)*
- Intra-assay and interassay coefficients of variation:

➔LH: <3% & < 4%, FSH: <3% & <6%, hCG: <5% & <7%, E2: <5% & <10%, P: <3% & <5%

TIMING OF THE FROZEN-THAWED ET

- Embryo Frozen: day 3 (preimplantation development) in a previous treatment attempt
- Frozen-thawed ET planned
  - Study group, 5 days after the LH rise
  - Control group, 5 days after the hCG administration

IVF-ICSI TREATMENT, EMBRYO CULTURE/SELECTION

- IVF-ICSI Treatment: as described by Van Landuyt et al.
- Oocytes / embryos - cultured in sequential culture media
- Embryo selection:
  - At least 6 blastomeres with $\leqq$20% fragmentation
  - With >20% but <50% fragmentation, if had reached the 8-cell stage on day 3

SELECTION OF FROZEN-THAWED EMBRYOS FOR TRANSFER

- Cleavage-stage embryos → thawing
  → cultured overnight in sequential media
  → Evaluate further cleavage next morning

- Max. 2 embryos could be replaced per frozen-thawed ET
  → Only 1 straw containing up to 2 embryos was thawed
  → At least 1 embryo survived with all cells intact → If not → 2nd straw was thawed

STATISTICAL TESTS

- Estimate the sample size

  => No published data were available on frozen-thawed ET (Natural ⇔ Semi-natural cycles “hCG”)

- Difference of 15% in ongoing pregnancy was acceptable (cryo-thawed regimens ⇔ complete natural cycle)

  ✓Change the policy of frozen-thawed ET

  ✓Anticipating the higher number of visits (hormonal assessment)

- Group sequential method:
- 120 / group
  - → Statistical power: 80%
  - → Absolute difference of 15% (ongoing pregnancy rate between the groups)
  - → α level: 0.05 (2-sided z-test)
- 1st interim analysis: P ≦ 0.029 (between 2 groups)

RESULTS

- 168 patients (assigned randomly) → 1st interim

No undergo transfer	Bad embryo quality	Failed folliculo-genesis up to D21 of the cycle
Study group (23/84)	17	6
Control group (21/84)	11	10

first interim analysis

  - 61x spontaneous LH group
  - 63x hCG group

	Spontaneous LH (n = 61)		hCG group (n = 63)	*P* value
Female age (y)	32.9 ± 3.1		32.9 ± 3	NS
Body mass index (kg/m^2)	23.8 ± 2.9		23.1 ± 2.9	NS
Cycle length (d)	28.7 ± 1.5		28.8 ± 1.7	NS
Indication for treatment (% of patients)				NS
Andrologic	70		73	
Tubal	10		15.9	
Idiopathic	20		11.1	
No. of trials	2.2 ± 1.9		2.2 ± 1.8	
Procedure (% of patients)				NS
IVF	8.2		14.3	
ICSI	91.8		85.7	
Type of previous stimulation (% of patients)				NS
Long GnRH agonist–urinary gonadotropins	26.2		27	
Long GnRH agonist–recombinant FSH	1.6		3.2	
Short GnRH agonist–urinary gonadotropins	1.6		7.9	
GnRH antagonist–recombinant FSH	62.3		50.8	
GnRH antagonist–urinary gonadotropins	8.2		11.1	
No. of visits	4.1 ± 1.4	>	2.6 ± 1.1	.001
No. of days until LH-hCG	14.3 ± 2.3	>	13.4 ± 2.6	.02
Embryos transferred	1.6 ± 0.5		1.4 ± 0.5	NS
Quality score of transferred embryos	1.3 ± 0.5	>	1.1 ± 0.3	.02

HORMONAL PROFILE OF PATIENTS

	Spontaneous LH (n = 61)	hCG group (n = 63)	*P* value
Day 3			
FSH (IU/L)	7.3 ± 1.7	7.4 ± 4.9	NS
LH (IU/L)	5.1 ± 1.9	4.7 ± 1.3	NS
E_2 (pg/mL)	41.7 ± 18.6	39 ± 14.4	NS
P (ng/mL)	0.6 ± 0.3	0.7 ± 0.4	NS
Day of hCG administration/LH rise			
FSH (IU/L)	9.9 ± 5	7 ± 4.1	NS
LH (IU/L)	34.6 ± 18.3	17.5 ± 16.7	.001
E_2 (pg/mL)	275.4 ± 121.3	252.7 ± 91.3	NS
P (ng/mL)	0.93 ± 0.45	0.91 ± 1.6	NS

- Control group:

➔ ↑ LH (without P ↑): 36.5% (23/63)

➔ Previously LH rise with hCG

➔ Only 4.3% (1/23) became pregnant

TREATMENT OUTCOME

	Spontaneous LH (n=61)	hCG group (n=63)	Difference, % (95% CI)	P value
Ongoing pregnancy rate-ET	31.1 (19)	14.3 (9)	16.9 (2.1-30.9)	0.025
Miscarriage rate-ET	0 (0)	3.2 (2)	3.2 (10.9~3.2)	NS
Biochemical rate-ET	3.3 (2)	3.2 (2)	0.1 (7.8~8.3	NS

DISCUSSION

- Natural cycle over a natural cycle controlled by hCG
  - Significantly higher ongoing pregnancy rate
  - Lower embryo quality (1.3 ± 0.5 vs. 1.1 ± 0.3, P=.02)
  - Number of visits (4.1 ± 1.4 vs. 2.6 ± 1.1, P=.001)
  - Number of days until hCG administration–LH peak (14.3 ± 2.3 days vs. 13.4 ± 2.6 days, P=.02)

ENDOMETRIAL EFFECTS FROM HCG

- Normal cycles + hCG + frozen-thawed ET of cleavage-stage embryos => PR: 19%, live-birth rate: 15%

Center for Reproductive Medicine of the Free University of Brussel

- Implantation & Ongoing pregnancy

⇔ Receptive endometrium & Functionally normal embryo

- Hypothesis: hCG administration → Endometrial effect → *significantly lower pregnancy rate in*

LH & HCG ACTION

- Same receptor of EM expresses throughout the cycle
- Alternative splicing

⇒Full-length-R only present proliferative ~ mid-luteal phase

⇒Only the extracellular domain is expressed

⇒Deletion of exon 10

→Not affect hCG action / Impaired LH action

LH & HCG ACTION

- LH vs. hCG
  - Much shorter half-life / An intrinsic molecular difference
  - No dose-finding study for an optimal hCG concentration to replace the endogenous LH surge
- Natural cycle
  - Endometrium at the end of the follicular phase
    - ↑ endogenous LH
    - No hCG before apposition of the embryo

HCG GROUP IN THIS STUDY

- (EM thickness ≧ 7 mm, follicle diameter ≧ 17 mm) → hCG administration
- LH rise without concomitant P rise
- 36% of LH rise with PR 4.3%
- hCG & LH : the same receptor / simultaneous presence → affect pregnancy adversely

HCG

During normal menstrual cycle

- Expressed & produced in human secretory endometrium
- Glandular endometrial production ↑ toward the late secretory phase (not the beginning)

⇒Implicate implantation & maintenance of the pregnancy

- Exerts control over the vascular endothelial growth factor

VASCULAR ENDOTHELIAL GROWTH FACTOR

- Key regulator of neoangiogenesis & vascular function
- ↑ toward the end of the secretory phase
- hCG → ↑ Intrauterine VEGF

⇒Suggest involvement in the initiation of *neoangiogenesis (essential for the formation of a functional placenta)*

- Small (N = 112) retrospective study → serial monitoring → ovulation with hCG triggering for timing frozen-thawed ET in natural cycles

  Weissman et al.

  ⇒Number of monitoring visits: control < study group

  ⇒Contrary: no adverse effect on cycle outcome

- Future studies should focus on
  - Natural cycles vs. hCG-induced ‘‘unstimulated’’ cycles

EMERGING EVIDENCE

- Administration of hCG in the late follicular phase

⇒Induces a cascade of events in the endometrium

⇒Started several days later in the presence of endogenous LH rise

⇒Negative impact on ongoing pregnancy rate

- Natural cycle superior to a hCG controlled cycle for planning the frozen-thawed ET (*a Prospective randomized trial)*

THANK YOU FOR YOUR LISTENING