

Growth hormone supplementation improves implantation and pregnancy productivity rates for poor- prognosis patients

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Introduction

IVF - Prognosis

Ovarian responsiveness

Quality of oocytes

recovered



Number of

good -

quality embryos

- Programmes

• Ovarian stimulation => *focus on* capturing 2nd follicles

- Early antral stage in the early follicular phase

- FSH sensitive → be selected

- Growth in the cyclic recruitment process



A R T



↑ endogenous FSH

- Oral agents

- Clomiphene citrate / Aromatase inhibitors

- Exogenous FSH (concentrated purified form)

- Urinary-derived / Recombinant technology

- follicle recruitment & generation of

- pregnancies (esp. with GnRHa to prevent early luteinization)

⇒ Wide variability to its responsiveness

→ Excessive response with risk of OHSS

→ Relative degrees of ovarian resistance

(recovery of few oocytes)



Number of oocytes

- Important given the age-related phenomenon
- Oocytes recovered → maturational integrity (only a proportion) → generating good-quality embryos → subsequent successful implantations → ensuing pregnancies
- e.g. 20% of oocytes in younger women (<35 years) < 10% of oocytes for older women (>40 years)



Ovarian stimulation regimen

- Menstrual cycle dynamics (since 1970s)
 - ⇒ Inevitable progression into IVF
 - ⇒ **FSH** → *key hormone*
 - For follicle recruitment / maturation
 - Focused: oocyte numbers rather than quality
- Ovarian endocrinology & paracrinology (last 20 yrs)
 - Molecular biology
 - **Folliculo-genesis / Cycle dynamics / Age-**



F S H - o n l y r e g i m e n s

- P o o r - r e s p o n d e r : h i g h l i g h t t h e c o m p l e x i t y o f f o l l i c l e r e c r u i t m e n t a n d v i a b i l i t y a n d t h e p r o b l e m o f a u n i v e r s a l s t i m u l a t i o n r e g i m e n
- F o l l i c u l o - g e n e s i s i n v o l v e s e v e n t s p r i o r t o , a n d o t h e r t h a n , F S H d e p e n d e n c e , w i t h t h e r o l e o f **g r o w t h f a c t o r s** a n o b v i o u s c o n s i d e r a t i o n

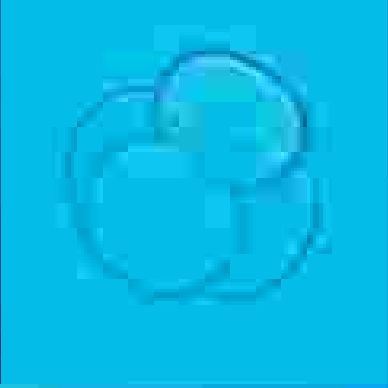


Stimulants other than FSH

- **LH** may improve **embryo quality** and **implantation** (Andersen et al., 2006)
- **Dehydroepiandrosterone (DHEA)** for **follicle recruitment** (Barad and Gleicher, 2005, 2006) in low-responder patients
- **GH** is another adjunctive therapy reported to provide benefit to complex cases (outside routine clinical application)



- Essential role (in various animal species) of growth factors on ovarian function, particularly the **Amplification of FSH action** -*Adashi et al., 1991*
- Facilitate ovulation induction by menotrophins (in the human setting) -*Jacobs, 1972, 1992*
- In poor-prognosis patients
 - ⇒ Pregnancies were difficult to achieve despite gonadotrophin dosages ↑
 - ⇒ *If GH have a role in improving the outcome?*



MATERIALS AND METHODS



Experimental design

Sequential crossover design:

- Sequence (repeated measures design) of different treatments (including at least 2)
- Each patient serves as his or her own control
- “Order effects” / “Carry-over” effects
- Characteristics:
 - Some weaknesses as a research model
 - Highly practicable in a private clinical setting
 - Comparative assessments are believed to be valid



Study period and participants

- PIVET Medical Centre
- 1 January 2002 ~ 31 December 2006
- Patients identified as 'poor-prognosis cases'
 - (i) Oocytes, Metaphase II < 3, even maximal FSH stimulation: 450 IU/day (Max 600 IU/day)
 - (ii) Embryos, majority (>50%): **Marked fragmentation**; PIVET's long-standing embryo-grading system: **1.5**
 - (iii) Repetitive fresh/frozen embryo transfers → no conceive → Lab: **diminished egg or embryo quality**



(Patients' characteristics)

- Majority had undertaken several IVF attempts before GH was offered
- It was only in the latter part of the study period that GH was offered pre-emptively on the 1st or 2nd attempt, generally on the basis of advanced age or limited cycle opportunities.
- Received GH at least 1 cycle during the 5-year period
- Limited the “order effect”

(Number of pre-cycles undertaken in outside clinics was not always clear → the attempt may



Electively using GH

- Offered GH based upon experience from previous cycles or history (either from PIVET or outside clinics)
 - Made their own decision to commence the next cycle with or without GH
 - Under PIVET requirement:
 - Max x6 inj. / 6-months
 - Waited at least 6 months before utilizing it again
 - Min. x2 months' rest between Tx cycles
- ★ Some Tx cycles without GH may well



Analysis

- Compares the outcome (including pregnancy) between $GH+ \Leftrightarrow GH- \Leftrightarrow GHu$



Patient consent

- Patient information sheet
- Growth Hormone
 - Potential side effects (Report headache, visual problems, nausea, vomiting or joint-swelling)
 - Information from current reported studies
 - Safety aspects along with biosynthetic preparation
 - Costing
 - Potential association low GH \leftrightarrow hypothyroidism
 - Potential precipitation of diabetes (Esp. with



Growth hormone protocol

- 2004–2006, GH started & maintained ↑ during the IVF cycle
 - 2002–2003 represented prior GH exposure (26% of transfers)
 - 2005–2006 represented concurrent exposure (57% of transfers)
 - 2004 may be viewed as a transition
 - ↑ usage of GH supplementation over time



Growth hormone protocol

- Saizen 10 IU (Serono, Australia) under 2 schedules

1st 4 years (2000–2004):

- Enhance the development 2nd oocytes (initial recruitment phase) → available for FSH capture (early follicular phase) of the treatment cycle
- Days 7, 14 and 21 (pre-cycle) → Days 2 (Tx-cycle)

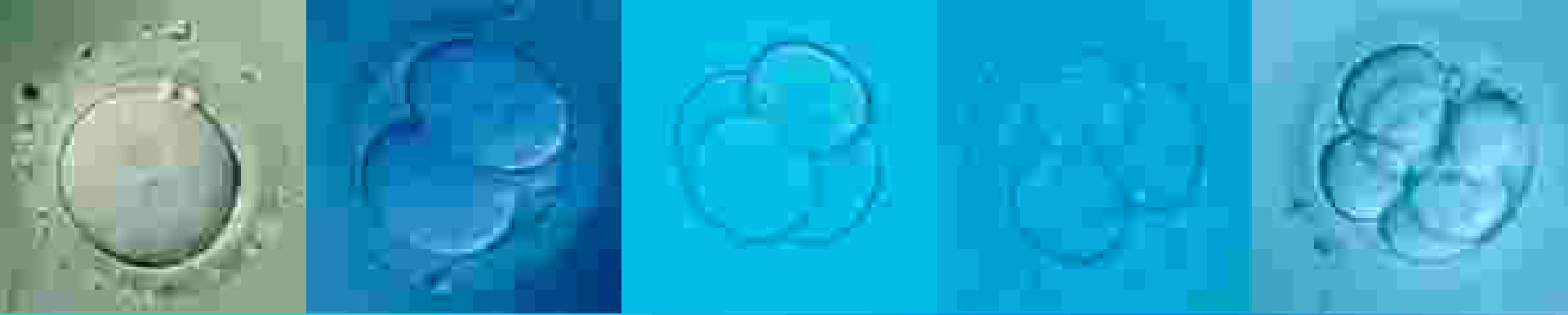
Majority in 2005 ~ 2006 (*Revised blend*):

- Day 21 (pre-cycle) → Days 2, 6, 8, 10 (Tx-cycle) → (if still progressing) Day 12 (Tx-cycle)



Clinical management

- In a flexible manner, different **stimulation regimens**
 - long down-regulation protocol (LDR)
 - Flare-stimulation regimen (FSR)
 - Antagonist protocol (AP - ↑ in recent years)
- Young (<35 years): LDR → FSR → AP
- Older (>35 years): FSR → AP
 - *PIVET: no differences in the pregnancy rates or likelihood of a live-born baby*
- All treated with **recombinant FSH**
 - for 1st cycles, based on age, day-2 FSH and antral follicle count graded (high-GrA ~ low-



- Monitor during Follicular phase:
 - Oestradiol, progesterone & LH:
Basal day -2 → from day 7 on alternate days
 - TVS: follicle dimensions
- **Ovulation** with 10,000 IU HCG
 - Leading follicles reached 18 mm
 - Serum oestradiol 800–1000 pmol/follicle 14 mm
- Post trigger 35 h → Transvaginal **oocyte recovery** (*under IV sedation, Each follicle was*



Luteal phase

- PIVET's long-standing protocol of HCG support => 1000 units on *days 4, 7, 10, 13* (*day 0*: oocyte retrieval)
- Mid-luteal hormone check:
 - Oestradiol & progesterone
 - if given additional support hormones (oestradiol, progesterone, combined oestradiol/progesterone pessary, compounded PIVET products)
- 12 oocytes were recovered



Embryo culture

Sage Biopharma culture media (Gytech, Melbourne, Australia)

*with 5 mg/ml human serum albumin (Gytech)
or occasionally patient's serum (10%)*

- 4–5 h post collection → Oocytes were cultured
- IVF (100,000 motile spermatozoa/ml)
- ICSI (denuded with hyaluronidase)

*Single embryo incubations in 10 μ l drops under oil (Gytech)
in 60 mm Falcon dishes (BD, Australia)
in MINC benchtop incubators (Cook)*

*under an atmosphere of charcoal filtered (5% CO₂ / 5% O₂ /
90% N₂ medical-grade gas)*



Embryo assessment

Grade:

• **Day 3** (4-point system, 1.5 → discarded)

- Gr4 = 8+ cells no fragmentation / early compaction evident
- Gr3 = 7-9 cells, no fragmentation and no compaction
- Gr2 = slow cleavage and/or >20% fragmentation
- Gr1 = arrested or significantly fragmented embryos

• **Day 5** (Gardner's scoring system, 1999) for blastocysts



Embryo Transfer

- *Days 2, 3 or 5*
- 1 or 2 (categorized as poor prognosis) embryos
 - In 10–20 ul of culture media
 - Cook double-catheter system (K-JITS-2005; Cook)
 - Under transvesical ultrasound → uterus
- Deposited just short of the fundus
 - A clear flash identified in the fundal region
 - Negative check on the transfer catheter
- Fertilization rates > expectation (5 embryos growing)

→ blastocyst culture & Day 5 ET, electively



- **Assisted hatching** (According to RTC approval)
 - 3 pre-transfers failed to generate a pregnancy
 - Patient >38 years with baseline FSH \uparrow
- **Cryopreservation**
 - Residual embryos of suitable quality
 - On the day of transfer
 - Slow freezing, propanediol method (Testart, 1986), 10% patient serum or human serum albumin



Main data comparison

- $GH+ \leftrightarrow GH-$, relevant against the GHu
- **Clinical pregnancy (CP) rate** *Per ET procedure*,
 - Fresh ET or post-thaw frozen ET (FET).
- **Productivity rate**
 - Cumulative pregnancy rate *per oocyte retrieval*
 - \triangle total ET, \triangle FET pregnancies (relevant group)
- **Implantation rates**
 - Identifiable gestational sacs in clinical pregnancies
 - Proportion of total number of ET



Main data comparison

- Utilization rate

- Single oocyte retrieval procedure → 2 pronucleate embryos → ‘usable’ embryos (the proportion)

- Denotes the number of embryos transferred + suitable for cryopreservation

- Statistical analysis

- Chi-squared analysis with Pearson’s correction factor

- t-test for comparison between means



RESULTS

Table 1 Basic demographics for patients receiving growth hormone co-treatment.

Parameter	Value
No. of patients:	159
Mean age \pm SD (years)	37.5 \pm 4.1
Type of infertility (%)	
Tubal	21
Endometriosis	32
Unexplained	21
Male	42
Additional factors, e.g. fibroid/adhesions	34
Single cause	49
Multiple causes	51

< 35 years: 101
 35 ~ 40 years:
 216
 > 40 years: 78

PCO or
ovulatory disorders

Average cycles

Before offer GH:

3.05

Per referral case :

1.96

Table 2 Summary of fresh and frozen treatment cycles with or without growth hormone (GH) co-treatment

Parameter	GH-	GH-	GH+	Total
Cycles started	232	256	1686	2174
Oocyte retrievals	221	241	1572	2034
Fresh embryo transfers	193	202	1311	1706
Clinical pregnancies	49	19	499	567
Clinical pregnancy rate per fresh embryo transfer (%)	25 ^{a,c}	9 ^e	38	33
Thawing cycles	84	148	1528	1760
Clinical pregnancies	17	14	494	525
Clinical pregnancy rate per thawing cycle (%)	20 ^{b,d}	9 ^e	32	30
Total pregnancies	66	33	993	1092
Clinical pregnancy rate/oocyte retrieval	30 ^{a,c}	14 ^e	64	54

Similar Cancellation &

Egg collection to transfer rates

Chi-Squared
P < 0.01

79 ET

Table 3 Relationship between clinical pregnancies per transfer, patient age and growth hormone (GH) co-treatment

Influence of age

Treatment	<35 years		35-40 years		>40 years		Total	
	n	Pregnant (%)	n	Pregnant (%)	n	Pregnant (%)	n	Pregnant (%)
GH+	41	38 ^{94%}	103	71 ^{68%}	49	24 ^{49%}	193	25 ^{13%}
GH-	60	9 ^{15%}	113	11 ^{10%}	29	3 ^{10%}	202	9 ^{4%}
GHU	653	46	522	34	131	16	1311	38
Total	754	43	743	29	207	16	1706	33

Significant high (P < 0.001)

26% (GH+ in <35 years)

55% (GH+ in 35-40 years)

20% (GH+ in >40 years)

24% (GH+ in >40 years)

10.6% (GH+ in Total)

x4 (GH+ in <35 years vs GH+ in Total)

x2 (GH+ in 35-40 years vs GH+ in Total)

x8 (GH+ in >40 years vs GH+ in Total)

x2.5 (GH+ in >40 years vs GH+ in Total)

Utilization rate and implantation

	GH+	GH-	GHu	Total
>40 years				
Oocyte retrievals	54	36	167	257
Oocytes/retrieval	7.2	7.9	6.9	7.1
Fertilization rate (%)	57.0	55.0	59.0	58.40
Utilization rate (%)	67.0	77.0	26.0	43.0
Implantation rate (%)	13.0 [≠]	1.3 [≠]	11.0	10.0
Mean FSH ± SD (IU/l)	6.6 ± 3.0	6.5 ± 2.4	6.4 ± 3.7	6.5 ± 3.2
Total				
Oocyte retrievals	221	241	1572	2034
Oocytes/retrieval	8.0	8.9	10.7	10.3
Fertilization rate (%)	55.0	53.0	61.0	60.0
Utilization rate (%)	67.10	72.0	78.0	75.0
Implantation rate (%)	15.2 ^{≠c}	5.1 [≠]	27.40	22.0
Mean FSH ± SD (IU/l)	7.4 ± 2.7	7.4 ± 2.9	5.4 ± 2.4	6.5 ± 2.7

Non significant

Similar number of average ET

P < 0.05

15.2^{≠c} > 5.1[≠]

P < 0.01

Poor prognosis group

Utilization rate and implantation

	GH+	GH-	GHu	Total
<35 years				
Oocyte retrievals	41	71	804	96
Oocytes/retrieval	10.7	9.8	12.2	11.7
Fertilization rate (%)	58.3	58.0	61.9	61.4
Utilization rate (%)	64.4	64.7	84.7	82.2
Implantation rate (%)	20.6 ^{a,b}	4.7 ^a	34.3	29.9
Mean FSH ± SD (IU/L)	7.4 ± 1.6	7.3 ± 1.9	5.3 ± 2.5	5.9 ± 2.4
<i>Poor prognosis group</i> P < 0.01				
35–40 years				
Oocyte retrievals	126	134	601	861
Oocytes/retrieval	9.3	8.4	9.8	9.5
Fertilization rate (%)	52.7	48.4	61.7	58.0
Utilization rate (%)	68.8	81.0	76.2	75.0
Implantation rate (%)	12.8 ^a	7.2 ^a	22.0	18.9
Mean FSH ± SD (IU/L)	8.0 ± 3.1	7.8 ± 3.8	5.6 ± 1.9	6.6 ± 2.9

P < 0.05

Non significant

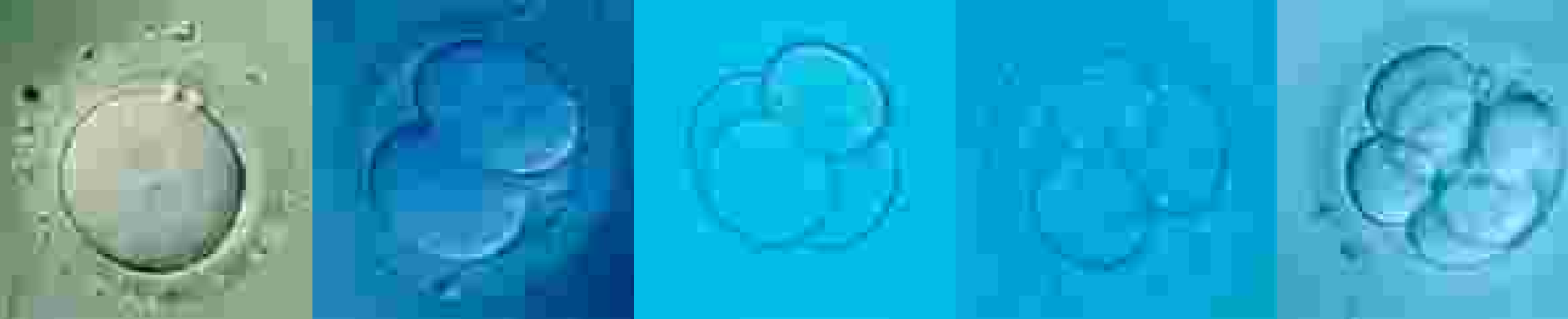


Table 5 Relationships between clinical pregnancies per transfer, method of ovarian stimulation and growth hormone (GH) co-treatment.

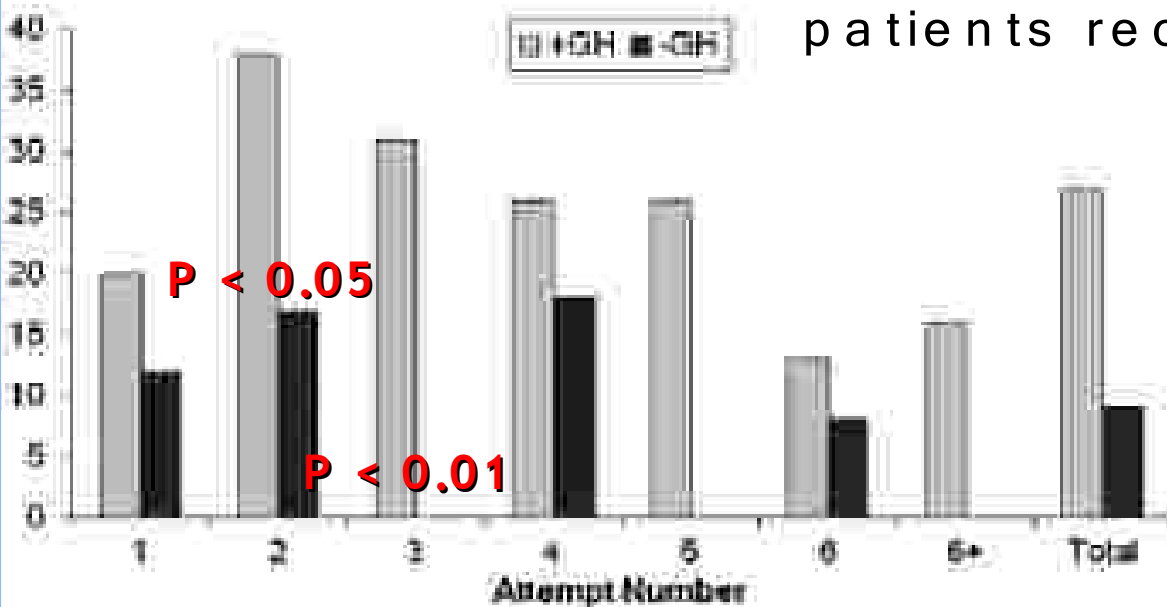
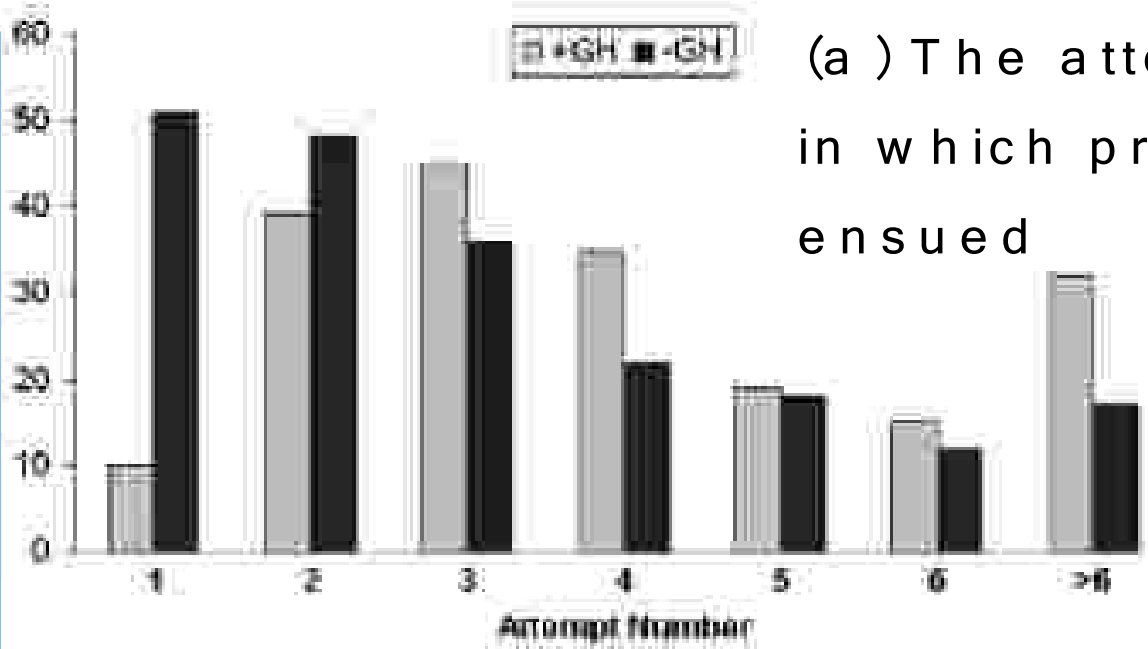
Group	Antagonist		Flare stimulation		Long down-regulation	
	n	Pregnant (%)	n	Pregnant (%)	n	Pregnant (%)
GH+	138	30 ^a	38	21 ^b	17	17 ^c
GH-	67	15	109	7	26	4
GHu	251	39	686	39	374	38

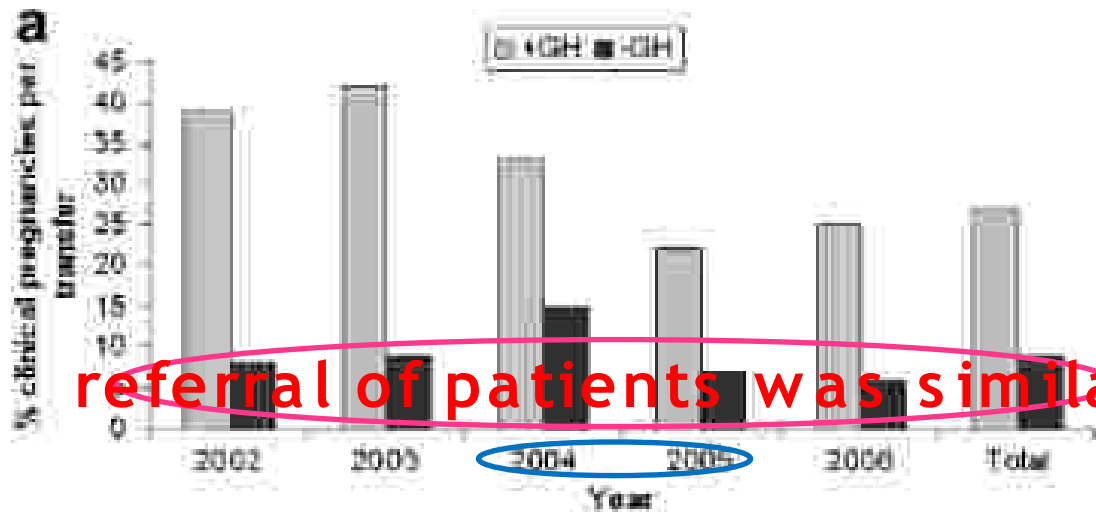
No significant difference

P < 0.05

Poor responders were ↑ managed by an AP protocol

clinical pregnancies per transfer number transfers





referral of patients was similar

No significant

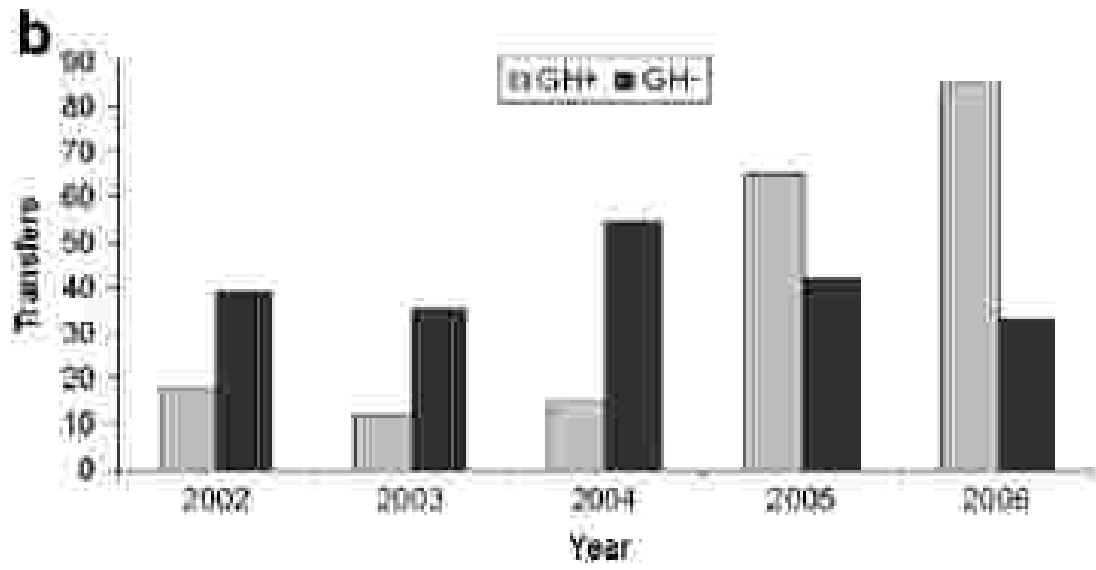


Figure 2 Clinical pregnancy rates (a) and number of transfers (b) in poor prognosis cases with or without GH supplement during individual years of the study period.



Side effects

- No case of diabetes or hypothyroidism emerged
- 2 patients described joint swellings of the hands
 - After a single GH inj. / After a series of 4x inj.
 - Resolved spontaneously over 1 or 2 weeks after GH ceased
- No cases of clinically significant OHSS
 - 2 patients had 12 oocytes recovered
 - Monitored during the luteal phase

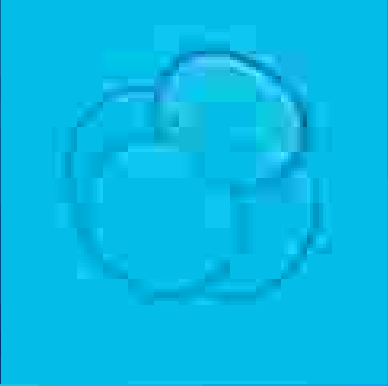
Pregnancy outcome

Parameter	GH+	GH-	GHu
Cycles started	232	256	1686
Cancelled cycles	11	15	114
Oocyte retrievals	221	241	1572
Nil oocytes retrieved	4	4	28
Nil fertilization	17	17	80
Deferred transfer	4	5	175
Fresh transfer cycles with embryos frozen	95	103	879
Cycles with freezing (%)	49	50	56
Fresh transfers	193	202	1311
Frozen transfers	73	145	1528
Total transfers	266	347	2839
Total biochemical pregnancies	7	4	33
Total clinical pregnancies	66 90.4%	33 89.2%	993
Failed pregnancies	23	16	228

Pregnancy outcome

Parameter	GH+	GH-	GHu
Live births from fresh embryo transfer	33	11	387
Live births from frozen embryo transfer	10	6	378
Total live births	43 65.2%	17 51.5%	765
Multiple pregnancies	2	1	70
Total babies delivered	45 P < 0.05	18	836
Miscarriage rate/positive HCG (%)	30/95 (41)	20/37 (54)	261/1026 (25)
Miscarriage rate/clinical pregnancies (%)	23/66 (35)	16/33 (48)	228/993 (23)
Babies/oocyte retrieval (%)	20 ^a	7	53
Mean birthweight ± SD (g)	3111 ± 738	3271.3 ± 446	3043 ± 732 ^b
Mean gestation ± SD (weeks)	38.0 ± 3.2	38.9 ± 0.91	37.6 ^c
Males:females	20:25	7:11	431:405

lower implantation potential



DISCUSSION



Previous Study with GH

- small, limited randomized studies involving GH in ovulation induction and IVF
- ➔ GH: augmentation role to FSH in follicle development via ↑ insulin-like growth factor (IGF)-I activity
- GH may improve pregnancy rate in poor-responder patients though with insufficient info. to confirm
- Oocyte recovery: a variable outcome measure



- Poor-responder groups: may under normally on high dose FSH regimens
 - Max. doses GH may override the role of facilitate the actions of FSH at normal serum concentrations
- ➔ Future studies may need to elucidate the **interaction between FSH dose and GH exposure**



Requirement of GH in follicle development

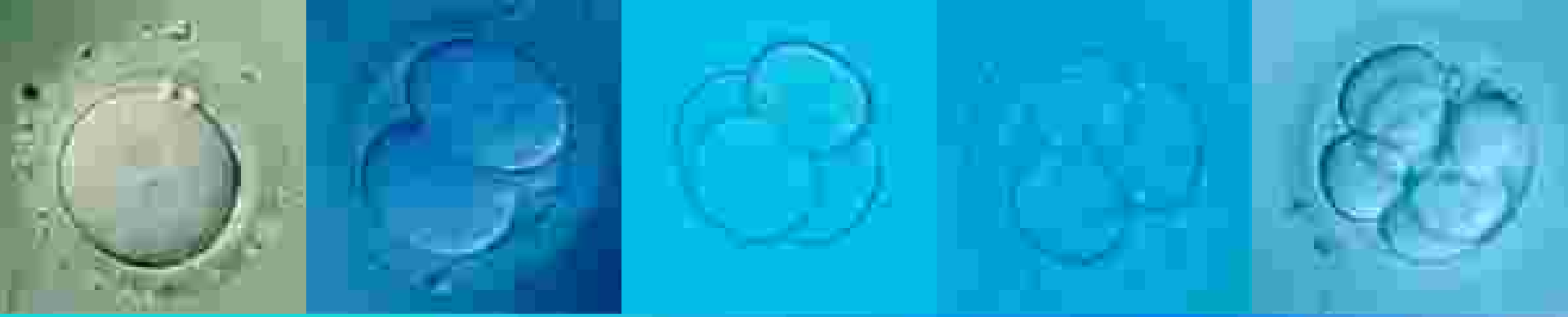
- GH replacement therapy

- Agonadal women → natural conceptions without the addition of any other stimulatory means

- ↑ IGF-1 serum and follicle concentrations in normal ovulating women (promote FSH activity by IGF-1)

Early studies focused on: recruitment increasing & oocyte numbers in poor-responder patients

(Whether they are already receiving high dose gonadotrophin stimulation is unclear)



- GH → more oocytes recovered (only in patients with low IGF-I binding protein-3)
- DHEA → ↑ oocyte recovery rates and embryo quality → augment the ovarian action of FSH in poor responders
- DHEA and GH may differ in some modes of action
- combining both DHEA and GH may warrant specific investigation



- GH receptors: identified on cumulus cells & oocyte
 - GH mRNA: expression in the oocyte
 - GH promoted embryo development possibly by improving cytoplasmic aspects of the oocyte during maturation
- FSH effect on oocyte maturation: cAMP dependent
 - GH & FSH acted via separate pathways
- ↑ [GH] in the follicular fluid: associated with improved oocyte maturity, fertilization, embryo



This report

- The 1st to span such a long time period
- Whole of-cohort analysis : views the total productivity from an egg collection (both fresh & frozen transfers)
- 5-year period, reduced prognosis due to poor embryo numbers and/or quality and to a lesser extent response to FSH.
- Report on the outcome of children conceived from a treatment cycle involving GH during IVF



GH protocol



- The beneficial effect of GH was apparent over all attempt numbers and over time
- Both pre- or peri-treatment cycle administration provided a benefit (2002-2003 / 2004-2005)
- There have been no reports comparing the timing of GH exposure, yet there are two proposed modes of action:
 - A supportive effect on FSH stimulation on ***follicle recruitment and development***
 - A role in ***oocyte maturation and maturity***



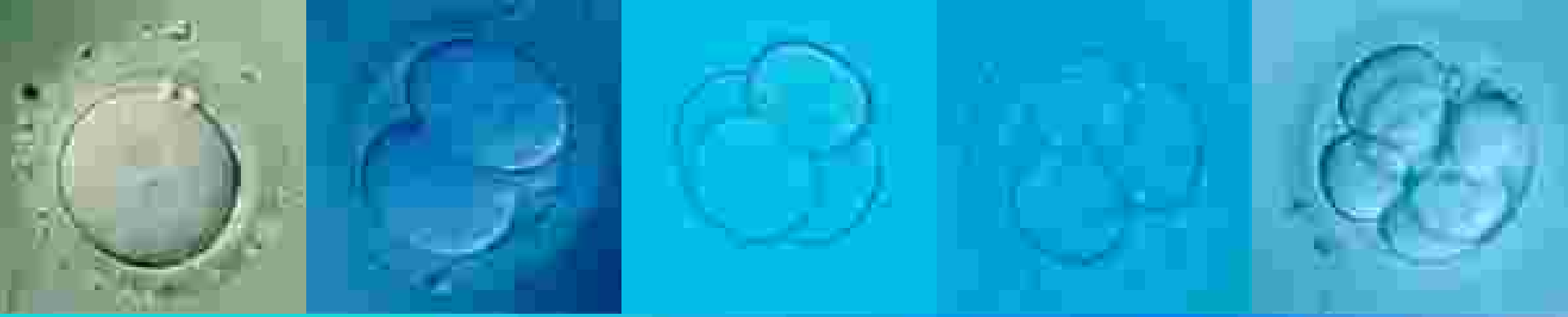
Implantation rate

- GH+ cycles: \uparrow to levels only marginally less than the GH- group, esp. so for > 40 years
- In the thaw cycles, pregnancy rate: GH+ $>$ GH-
- ➔ Better-quality embryos arise following GH co-treatment in poor-prognosis cases
- ➔ GH has an active role in human embryo quality ➔ reflect a role in oocyte maturation rather than oocyte numbers

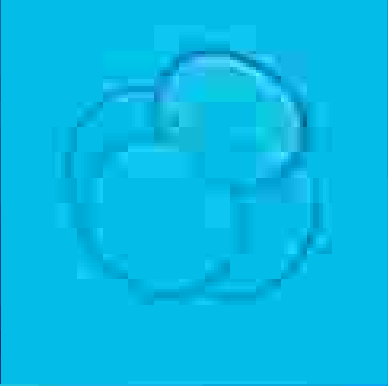


Productivity rate

- Cumulative number of pregnancies
- The best measure of GH effectiveness
- Significantly higher after GH augmentation, although this was (still < Ghu group due to poor-prognosis)



- Local action of cytokines on the permeability of the follicle wall to serum concentrations \rightarrow Differing concentration of GH in follicular fluid
- Little is known about the relative concentrations of GH in serum after co-treatment and whether the concentrations are raised significantly near the time of ovulation induction to influence oocyte maturation.



- The phase of GH administration may therefore produce different outcomes, depending on whether GH was prescribed before treatment, and therefore acts on follicle selection and response to FSH, or later in treatment where the effects may be more to do with oocyte competency.



- G H deficiency, oocyte ageing
 - G H improves clinical outcomes in both younger women (<35 years) and older women
- ➔ women over 40 years have lower ovarian reserve and decreased oocyte quality
- ➔ poor responders under 35 years of age represent women with either premature reduction of ovarian reserve or elevated FSH threshold



m i s c a r r i a g e r a t e

- s i m i l a r i n t h i s s t u d y
- D H E A m a y r e d u c e t h e m i s c a r r i a g e r a t e s i n p o o r r e s p o n d e r s (G l e i c h e r e t a l . , 2 0 0 8) a g a i n s u g g e s t i n g D H E A a n d G H m a y n o t a c t i n t h e s a m e m a n n e r .

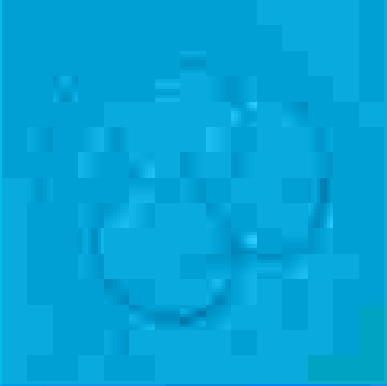
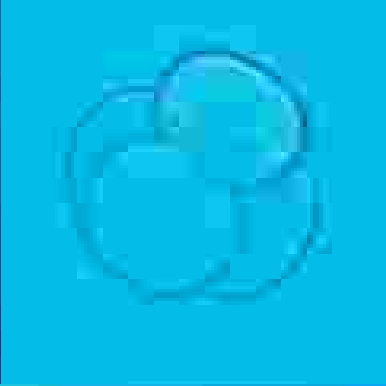


In summary

- 5-year, comprehensive study of IVF/CSI Tx, large group of poor prognosis patients
- *Implantation rates* & resultant *pregnancy rates* along with the *numbers of healthy babies delivered* are significantly higher when GH co-treatment
 - ➔ Confirm a role for GH treatment during an IVF stimulation cycle and the benefits apply to women categorized as poor prognosis
 - ➔ Apparent across time periods, in all age groups, regardless of previous attempts and independent of stimulation protocols



- ➔ Both benefit in fresh & subsequent frozen ET cycles
- ➔ There appeared to be no adverse effects in the children born after GH exposure
- ➔ Suggest improved oocyte quality (rather than numbers of oocytes and embryos)
- Further research is required to ascertain
 - ➔ If the effects observed are due to improved ***follicle health? Peri-ovulatory environment?***



THANK YOU FOR YOUR ATTENTION

ENDING