Intensified ovarian stimulation in a GnRH antagonist protocol with agonist triggering:

A prospective, clinical feasibility study

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Introduction

- Aim of *ovarian stimulation* for IVF: induce **multi**follicular growth → retrieval of multiple oocytes for extracorporal fertilization
- ↑ discomfort & risk of adverse events
- Threat of **severe OHSS**, in young patients → limited the feasibility of maximizing the *oocyte yield / single treatment cycle*

GnRH antagonist ovarian stimulation protocol + **agonist triggering**

- ➔Intensified ovarian stimulation
- ➔Without ↑ the likelihood of severe OHSS

- GnRH agonist (instead of hCG) bolus → Triggering of final oocyte maturation
  - **Pituitary** remains responsive
  - ⇔ ↓ risk of moderate-to-severe OHSS

Cryopreserved all fertilized oocytes by **vitrification** for a later transfer

- GnRH agonist triggering *in OHSS risk patients as a* safe & efficacious option

  (Griesinger et al., 2007, 2010)

- Circumvents the impaired luteal phase after agonist triggering

  (Babayof et al., 2006; Humaidan et al., 2009; Nevo et al., 2003)

- Eliminates the risk of late-onset OHSS

Highly efficacious cryopreservation technique

- Had potential to allow ***temporally splitting*** ovarian stimulation and embryo transfer

- Without a significant loss of treatment efficacy

This prospective clinical study …

- Exploring the feasibility of such ***ovarian stimulation approach***
  - Creating a maximally large number of **fertilized** oocytes from a **single** ovarian stimulation cycle
  - For later transfer in **repetitive** vitrified–warmed cycles
- No previous experience → non-randomized study → derive a first estimate of the tolerability, safety and efficacy

Questions:

(i) Is an **intensified ovarian stimulation protocol with agonist triggering safe** in terms of OHSS occurrence?

(ii) Is this approach **acceptable** to the patient in terms of discomfort?

(iii) What is the **cumulative live birth rate** from **multiple vitrified–warmed embryo replacement** cycles per single oocyte retrieval?

Materials and methods

Patient population – *Inclusion criteria*

(i) ≤ 36 y/o, indicated for IVF or ICSI *(intracytoplasmic sperm injection)*

(ii) No expected or previous poor response (≥ 3 oocytes at retrieval)

(iii) Both ovaries present

(iv) No endometriosis American Fertility Society grade III–IV

(v) neither uterine nor ovarian abnormality on transvaginal sonography

(vi) informed consent

Study protocol

- D2 or 3 of MC(spontaneous or induced)
- ↓ Progesterone, oestradiol, LH (to confirm reference range values)

→ 225–375 IU r-FSH or human menopausal gonadotrophin (HMG) or combination → **once daily** s.c.

→(Chosen dose)Aim: inducing ≥20 follicles

→Expected normo-responders ≤36 y/o → 150 IU daily (*non-intensified stimulation*)

After 5–6 days FSH/HMG

- Start **GnRH antagonist** 0.25 mg daily s.c. → until the day of **triggering final oocyte maturation**
- D7 or 8 of stimulation → TVS, serum oestradiol, progesterone and LH → leading follicle 17–18 mm

oocyte retrieval

- 0.2 mg GnRH agonist (triptorelin) bolus (single s.c. injection)
- Approximately 36 h later→ **IVF or ICSI**

Endometrial transformation

- After agonist triggering
- → **M**edroxy**p**rogesterone **a**cetate (MPA) 10mg 10–14 days oral daily
- Oestradiol < 4000 pg/ml during stimulation
- → Low-molecular weight heparin *(dalteparin 5000 IU/day)* self-administered daily sc
- → Continued until menstruation

Vitrification

- 20 h after IVF or ICSI
- Oocytes at the 2 pronuclear (2PN) stage → vitrified

(Kuwayama et al., 2005)

Cryopreserved embryo transfer

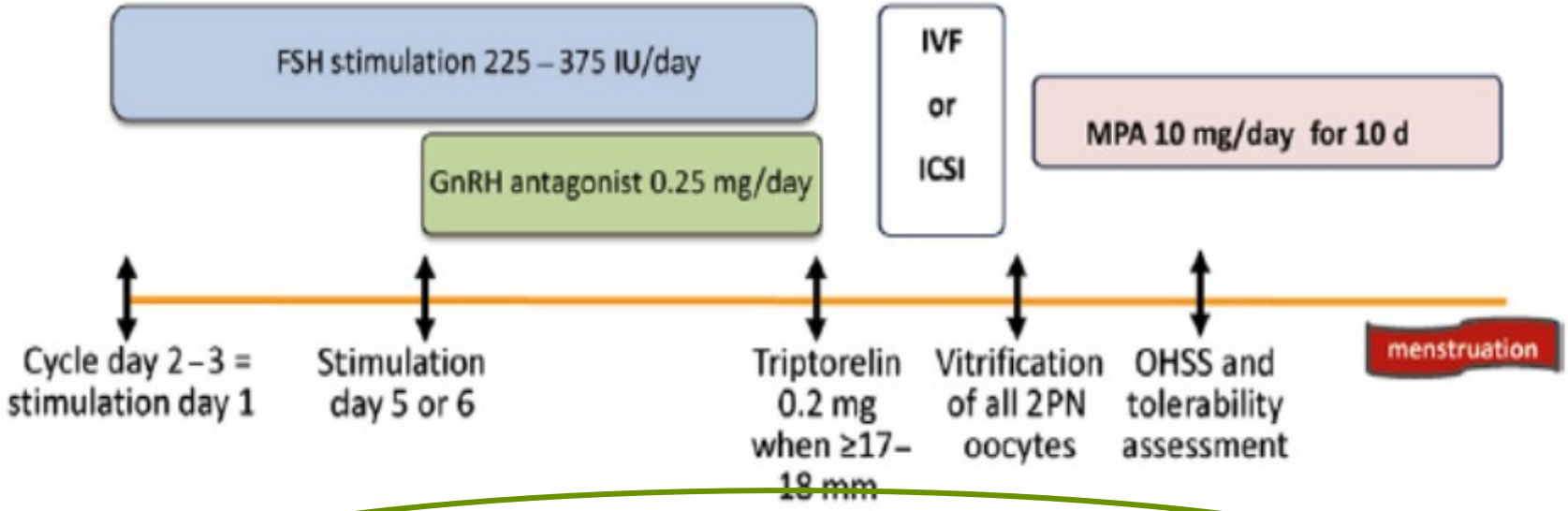
- After spontaneous or induced menses *(Bals-Pratsch et al., 1999)*

➔ Preparation of the endometrium:

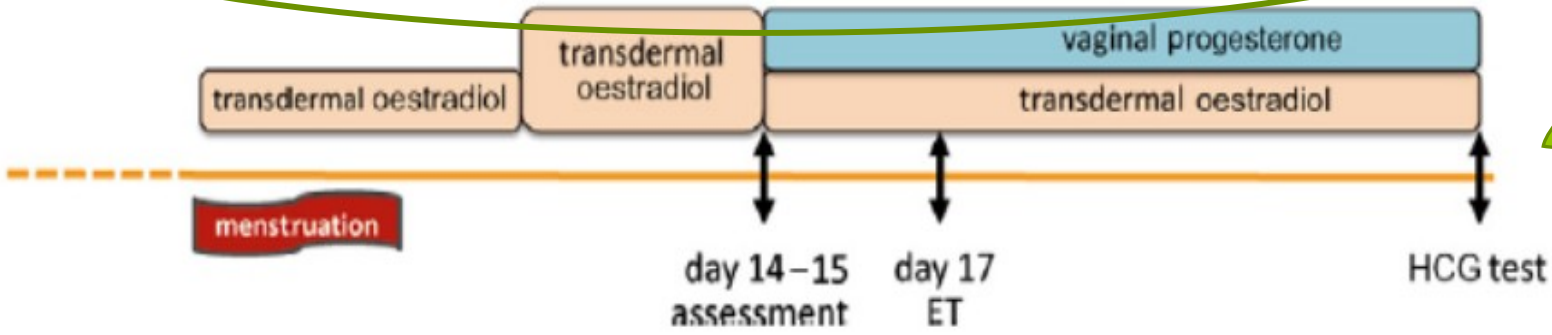
- 14 days x Transdermal oestradiol patches *(Estraderm TTS 100, Novartis Pharma)* or Oral oestradiol *(Progynova, Bayer Vital)*
- Since day 15 Add vaginal progesterone *(**Crinone 8%,** Merck Serono or Utrogest, Kade/Besins)*

Embryo transfer

- Day 3 of progesterone administration
- Day 2 of **preimplantation development**
  - 2PN oocytes → viable after thawing (maximally 3) → further culture → transferred to the uterus at the embryo stage (no selection of embryos according to morphology)



Supplementation of early pregnancy: IM progesterone + transdermal oestradiol → GA 8–10 weeks



Safety & tolerability assessment

- D3 or 4 after oocyte retrieval
- Signs & symptoms of OHSS *(Golan et al., 1989)* & treatment tolerability
- TVS → Ovarian volume & the presence of free abdominal fluid (ascites)
- WBC, CRP, Hct, oestradiol, LH, progesterone

→ In case of Abdominal distension/pain, nausea, vomiting, diarrhoea or headache during later luteal phase → advise to visit

Tolerability assessment questionnaire

- ‘Do you experience today or did you experience within the previous 5 days’:

(i) Abdominal distension

(ii) Lower abdominal pain

(iii) Nausea/vomiting

(iv) Headache

- Scale: 1 (perfect wellbeing) ~ 5 (maximum discomfort)

Outcome parameters

- Primary efficacy outcome: Cumulative live birth rate per patient undergoing oocyte retrieval

- Live birth rate per embryo transfer
- Time-to-conception (duration in weeks agonist administration → + PPT → live birth)
- Number of Biochemical pregnancies
- Number of Clinical pregnancies (+ fetal sac)
- Number of Clinical abortions (+fetal sac → no progression to live birth)

- Fertilization rate
  - ➔Number of **2PN oocytes** / number of MII oocytes injected or cumulus–oocyte–complexes inseminated per patient
- Proportion of **2PN oocytes** cryopreserved per cumulus–oocyte–complex retrieved
- Proportion of **2PN oocytes** cryopreserved per MII oocyte retrieved (ICSI cases)
- Survival rate after cryopreservation
  - ➔Number of vital embryos/number of *thawed* 2PN oocytes

Sample size and statistics

- 30 patients
- Mean ± SD, median ± interquartile range or proportions with 95% CI
- Linear relationship between two variables → **Pearson's correlation coefficient** (Pearson's r)
- Number of oocytes retrieved ⇔ number of oocytes available for freezing (& later transfer) → **regression modelling**

Results

Demographic parameters

Parameter	Study population (n = 30)	
	Statistic	Range
Age (years)	30.0 ± 3.5	25–37
Weight (kg)	64.6 ± 12.0	52–110
Body mass index (kg/m^2)	22.9 ± 4.3	17.4–39.0
Duration of infertility (months)	39.5 ± 22.6	12–96
Cycle length (days)	33.7 ± 12.8	25–90
Cycle rank	2.0 ± 1.3	1–6
No. of previous pregnancies	3/30 (10)	NA
No. of previous live births	2/30 (6.7)	NA

- 19 x regular cycle, 11x irregular cycle
- 13x1st IVF Tx attempt, 11x2nd, 3x3rd , 3x more

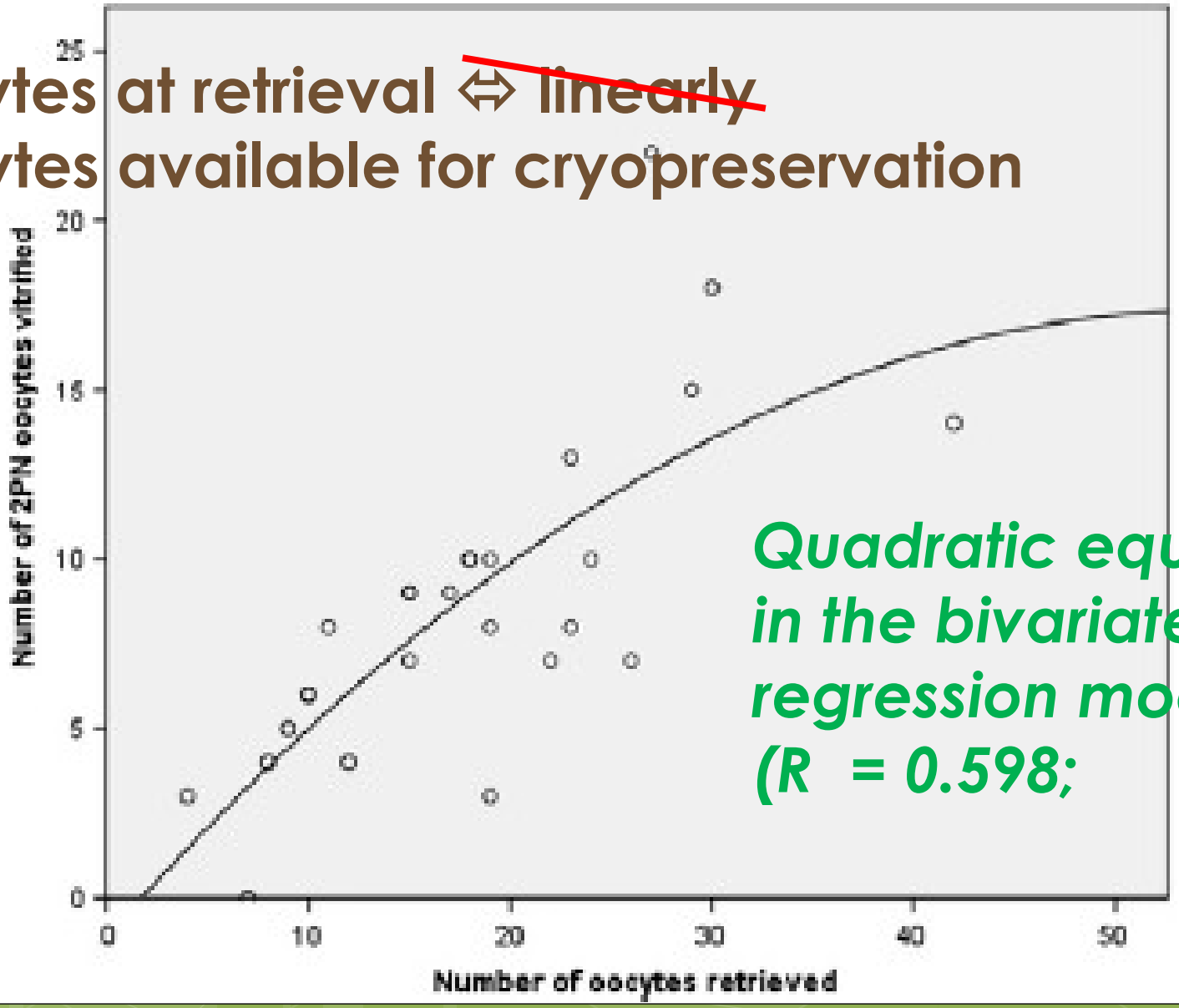
Parameter	Study population (n = 30)	
	Statistic	Range
Stimulation (days)	9.9 ± 2.0	6–17
Total FSH (IU)	2564.9 ± 722.4	1350–3750
FSH/day (IU)	273.3 ± 65.5	150–375
On day of final oocyte maturation		
Oestradiol (pg/ml)	3619.9 ± 2123.0	478–9799
Progesterone (ng/ml)	1.7 ± 0.9	0.6–4.6
No. of follicles >10 mm	18.3 ± 8.2	9–40
No. of COC	17.0 ± 8.5	4–42
No. of MII oocytes (n = 29)	13.4 ± 6.6	3–26
Fertilization rate (%)	65.4 ± 20.5	19–100
No. of 2PN vitrified[a]	8.4 ± 4.5	3–22
2PN oocyte vitrified per COC (%)[a]	50.9 ± 15.2	15.8–100
2PN oocyte vitrified per MII oocyte (%)[a]	65.0 ± 20.0	18.8–100.0
Survival rate (%)[b]	96.3 ± 10.8	50–100
No. of embryos transferred[c]	2.1 ± 0.3	2–3
Modified cumulative embryo score[c]	24.3 ± 8.3	6–42
Luteal-phase haematocrit (%)[d]	37.4 ± 3.8	28–43

Stimulation characteristics

- 3x150 IU (hyperresponse) → 7, 18, 23 oocytes retrieved
- 1x fertilization failure → 29x at least one 2PN oocyte vitrified (8.4 2PN)
- Mean number of oocytes at retrieval: 17
- Number of oocytes at retrieval ⇔ ovarian volume on days 3–4 after OR: significantly correlated (Pearson's r 0.50, P = 0.02).

↑ oocytes at retrieval ⇔ ~~linearly~~

↑ oocytes available for cryopreservation

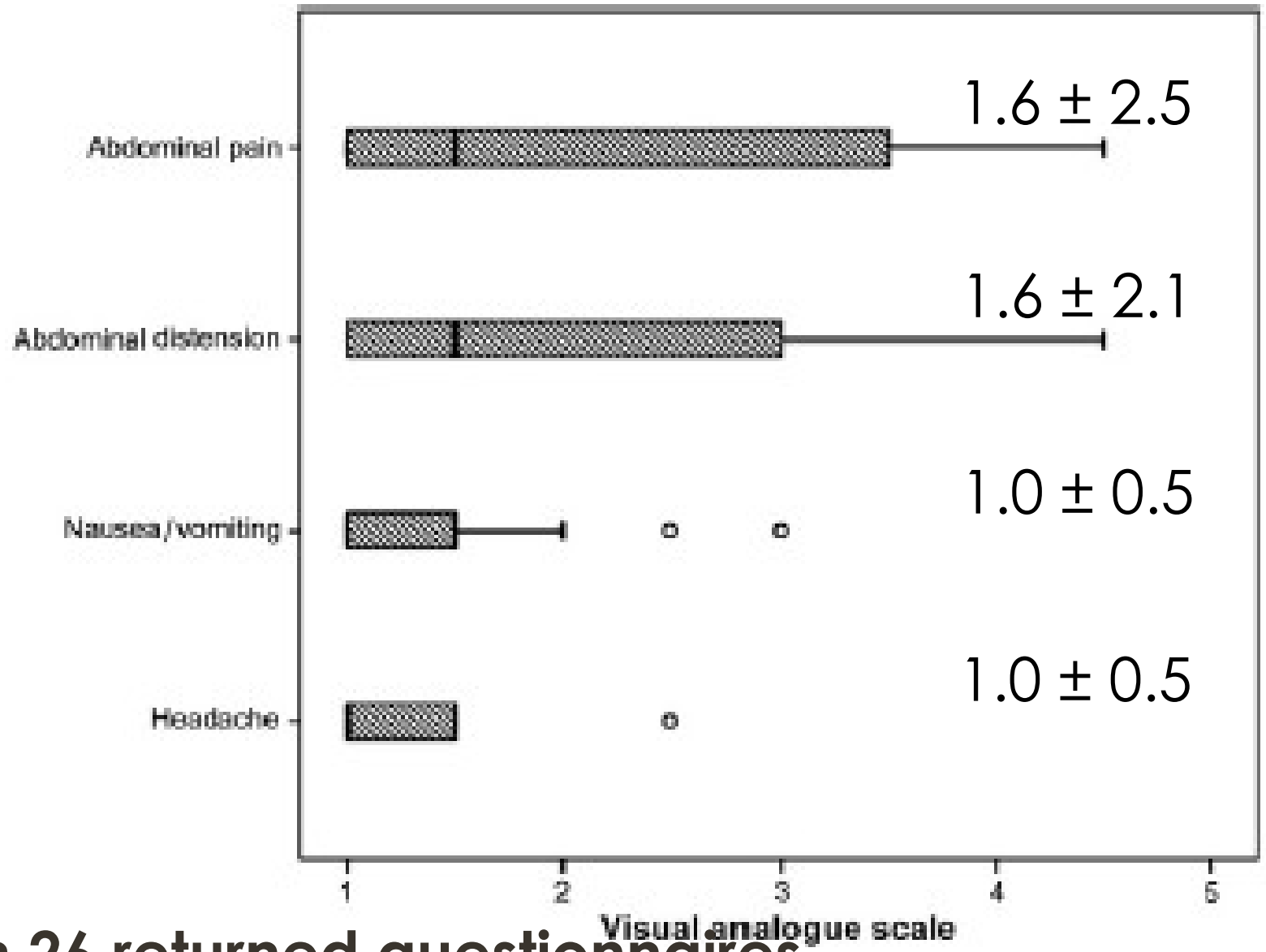


Quadratic equation in the bivariate regression model (R = 0.598;

Tolerability and safety

- No severe OHSS (0%, 95CI 0–11.4%)
- No patient required hospitalization
- *Mean luteal phase* CRP 5.0 mg/l, WBC 9098/μl, progesterone12.9 ng/ml, oestradiol 620 pg/ml, LH 13.3 IU/l, Hct 37.4%
- Total mean ovarian volume: 158 ± 122 mm^3
- Free abdominal fluid: in 32% patients (mean value of the largest diameter of free fluid pocket: 23 ± 7.2 mm)

On D3 or 4 after OR



From 26 returned questionnaires

- Significant correlation
  - Abdominal pain ⇔ distension (Pearson's r 0.75, $P < 0.01$)
  - Abdominal pain ⇔ nausea/vomiting: (Pearson's r 0.51, $P = 0.01$)
- No significant correlation
  - Number of oocytes retrieved ⇔ abdominal pain, distension, nausea or headache

Live birth rate

- 3x not undergo vitrified–warmed ET (1x divorce, 2x spontaneous pregnancy)
- → **26x at least one vitrified–warmed ET**
- → Mean number of transfers: 2.4 ± 1.7
- 0.5x 2PN oocyte → cryopreserved per cumulus–oocyte–complex
- Survival rate after thawing: 96%

Cryopreserved cycle rank	Live birth rate	
	% (n/total)	Confidence interval
1	7.7 (2/26)	2.1–24.1[a]
2	13.6 (3/22)	4.7–33.3[a]
3	9.1 (1/11)	1.6–37.7[a]
4	0.0 (0/7)	0–35.4[b]
5	0.0 (0/3)	0–56.2[b]
6	50.0 (1/2)	9.5–90.5[a]
7	0.0 (0/1)	0–79.3[b]
Live birth rate per total cryopreserved cycles	9.7 (7/72)	4.8–18.7[a]

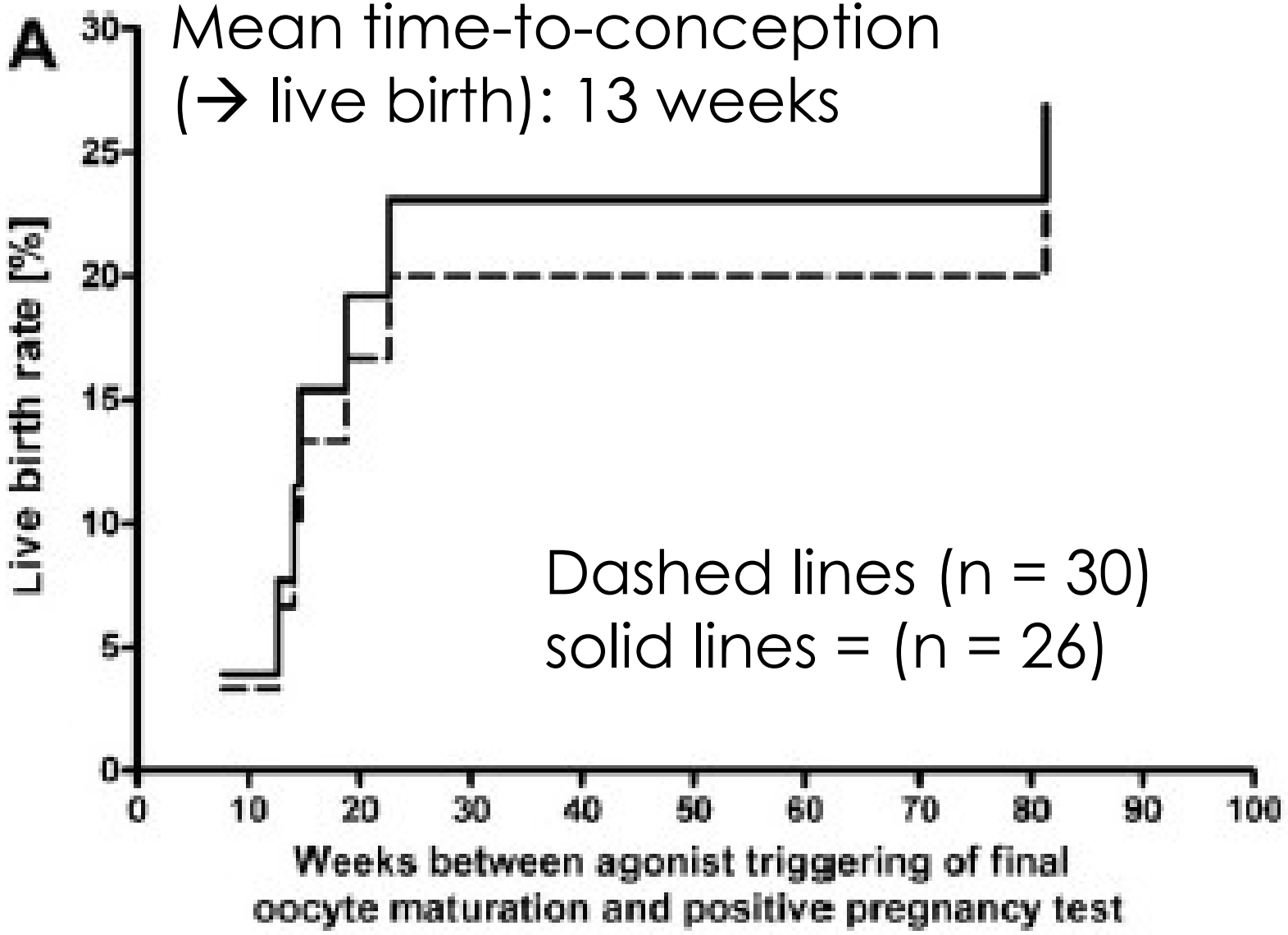
- 72 ET
- → 5 biochemical pregnancies (5/30, 16.7%, 95%CI 16.3–33.6),
- → 2 x first-trimester abortions (2/30, 6.7%, 95% CI 1.8–21.3%)
- → 7 x live birth (7/30, 23.3%, 95% CI 11.8–40.9%)

Cumulative live birth rate

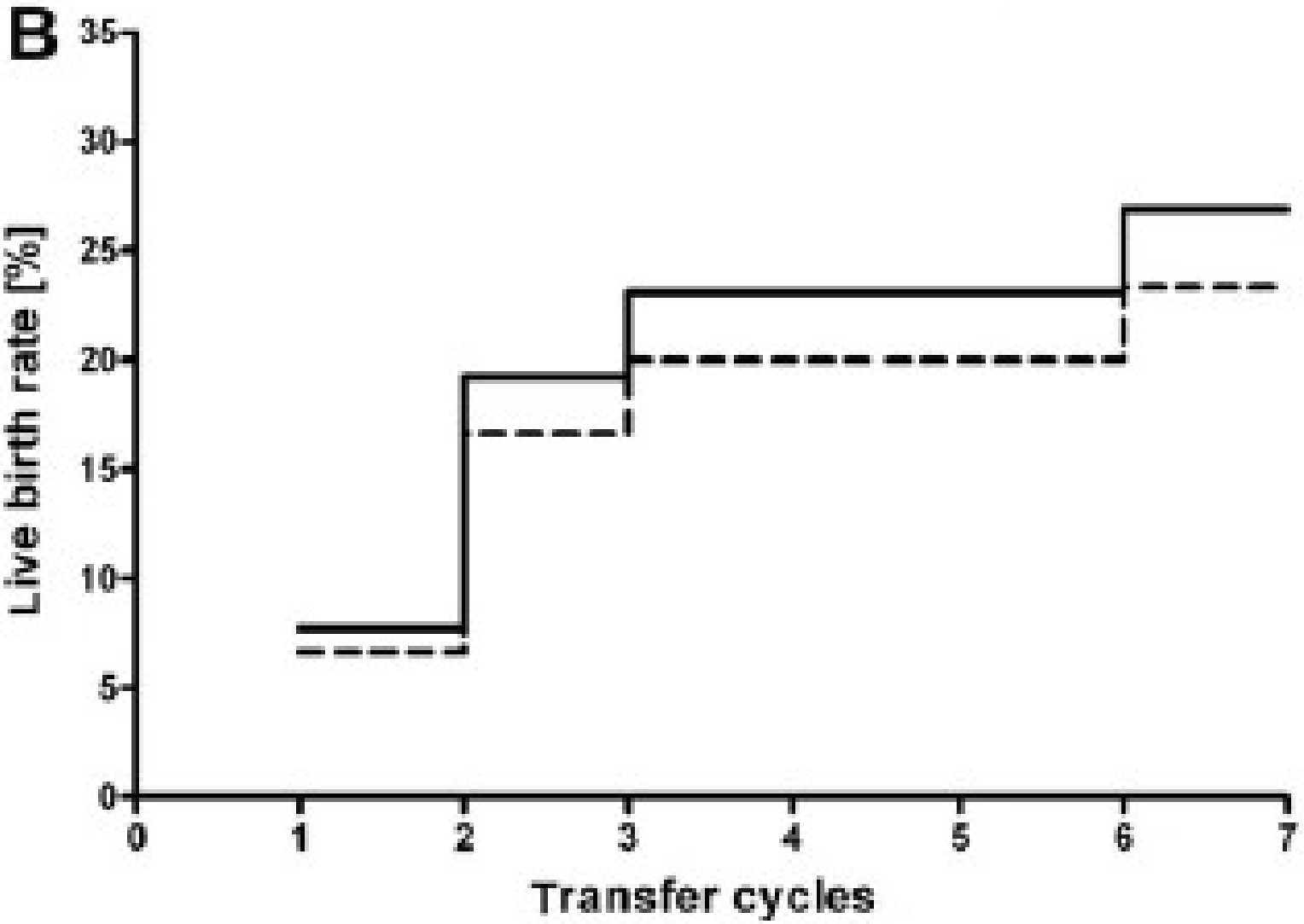
- Strict intention-to-treat approach of analysis (with spontaneous pregnancies) → : 9/30 (30%, 95% CI 16.7–47.9%) = *cumulative clinical pregnancy rate*

- Undergoing at least on vitrified–warmed ET: 26.9% (7/26, 95% CI 13.7–46.1%)

Mean time-to-conception (→ live birth): 13 weeks



Dashed lines (n = 30)
solid lines = (n = 26)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Live birth rate [%]
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
7
Transfer cycles

- End of the f/u period → 14x patient still had mean 5.8 ± 4.1 further 2PN oocytes cryopreserved
- Pregnancy outcome:
  - 5 singleton live births, 2 twins (28.6% twin rate)
  - **All** achieved live births → 2 ET in the successful cycle

Discussion

- Intensified ovarian stimulation
  - → Retrieved oocytes
    - → Average 17 (Relatively young patients)
    - → 9/30 patients → ≥ 20 (maximum: 42)
  - → Safe in terms of OHSS occurrence
    - → Confirm the ability of GnRH agonist triggering → Totally prevent severe OHSS, even in high-risk patients

(Engmann et al., 2006; Griesinger et al., 2007, 2010; Manzanares et al., 2010)

- Maximizing the oocyte yield from a single oocyte retrieval → **Maximize** the chance of the patient becoming pregnant from a **single treatment cycle**
- ↓ the need for subsequent IVF cycles with injections, oocyte retrieval procedures and the associated financial cost

Limitation – 1st

- An uncontrolled study for feasibility of intensifying ovarian stimulation
- No previous experience on intensified ovarian stimulation + agonist triggering + cryopreservation of all available oocytes

➔ Need control group

Limitation – 2nd

- German embryo protection law:
  - ➔**All** 2PN oocytes viable after warming
  - ➔Transfer to the uterus *(regardless of the morphological appearance)*
- ➔**Vitrified–warmed ET:** Ā 2.5 per patient
- ➔**Conceived:** majority within 2x ET, only one from 6x

Limitation – 2nd

- **<u>Maximizing the oocyte yield</u>** → a worthless exercise for some patients (other patients could not be 'pushed' to conceive even by offering them a high number of vitrified– warmed ET)

Limitation – 2nd

- 15/26 patients → only 2 transfers
- 11/26 patients → ≥ 3 ET → only 2 additional live births

→ A relatively large number of oocytes remained cryopreserved at the end of the follow-up period

→ If **embryo selection** → same number of live births by a much smaller number of ET

Limitation – 3rd

- The number of oocytes retrieved → not predictive of pregnancy *(Griesinger et al., 2010; Verberg et al., 2009)*
- ↑ numbers of oocytes → ↑ immature/degenerated oocytes and unfertilized oocytes after IVF or ICSI

→ **Low number of oocytes at retrieval** → not a representation of advanced biological age

Limitation – 3rd

- ↓ Potential benefit of *(Intensifying ovarian stimulation → ↑ the oocyte pool → available for fertilization & later transfer)*
- Mean proportion of fertilized oocytes per cumulus–oocyte–complex: 0.5 ⇔ **Standard GnRH antagonist protocol** with daily 200 IU of FSH and hCG triggering of final oocyte maturation (Devroey et al., 2009)

Higher stimulation dose

- Lower ⇔ higher dose of FSH for ovarian stimulation, (100 IU ⇔ 200 IU) *(Rombauts, 2007)*
- Higher stimulation dose → higher number of oocytes → no differences in pregnancy rates *(Hoomans and Mulder, 2002; Out et al., 1999, 2001)*

→ If higher number of surplus embryos?

→ No cumulative pregnancy rates (including cryopreserved transfers)

‘Patient-friendly’ approach

- Cycle using cryopreserved embryos → less stressful for the patient *(no need for injections, oocyte retrieval and the financial costs associated with a fresh treatment attempt)*
- Each unsuccessful cryopreserved ET → significant psychological burden
- Cumulative live birth rate: 27%, in patient with minimum 1x ET

→Others need furhter treatment attmpts:

‘Patient-friendly’ approach

- **Intensifying** ⇔ ‘conventional’ ⇔ ‘mild’ ovarian stimulation
  - Tolerability, health risks, financial costs, efficacy in terms of cumulative live birth achievement, psychological distress
  - Further Well-designed RCT ideally undergoing a reduced-dose (‘mild’) ovarian stimulation protocol

Thank you for listening