FULL PROJECT TITLE:

Effect of ovarian stimulation on oocyte quality and embryonic aneuploidy: a prospective, randomised controlled trial

(STimulation Resulting in Embryonic Aneuploidy using Menopur (STREAM) Trial)

CHIEF INVESTIGATOR:

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Objective

To determine and compare the mean proportion of oocytes that develop to provide euploid vitrified blastocysts per patient resulting from conventional vs. low dose stimulation protocol.

Primary aim:
To determine whether conventional ovarian stimulation in IVF cycles leads to a lower proportion of euploid embryos per patient.

Secondary aim:
To assess the number and quality of embryos generated using conventional-dose stimulation vs. low-dose stimulation (number of euploid vitrified blastocysts per patient, number of 2PN pre-embryos generated, total number of embryos surviving to D5, total number of blastocysts biopsied, quality of the oocytes generated using mtDNA copy number).
To assess the relative safety of conventional vs. low-dose ovarian stimulation (OHSS resulting in hospitalization, incidence of dose adjustment or cycle cancellation).

Background

Oocyte-derived aneuploidy is the leading cause of IVF failure, early pregnancy loss, and the age-related decline in female fertility. Selection of the dominant follicle during unstimulated cycles is thought to act as a quality control mechanism by selecting the most competent oocyte in a cohort of available follicles. By contrast, controlled ovarian hyperstimulation is used to maximise the number of oocytes collected during IVF cycles and has been implicated as a cause of aneuploidy at the cleavage stage due to recruitment of poor quality oocytes. Primarily, this is based on a randomised controlled trial conducted by Baart et al. (2007) which compared mild stimulation (150IU FSH) with GnRH antagonist co-treatment to conventional high-dose stimulation (225IU FSH) and GnRH agonist co-treatment. The study demonstrated a significantly lower rate group of aneuploidy in the mild stimulation group vs. conventional stimulation with abnormal FISH results at D3 biopsy in 45% vs. 63%

respectively (Baart et al. 2007). Importantly, the authors suggested that the total number of chromosomally normal (i.e. euploid) embryos available for transfer was identical (1.8 in each arm) despite the greater number of oocytes collected in the high stimulation group (12.1 +/- 5.7 versus 8.3 +/- 4.7 in the mild stimulation group, p<0.01).

There have been no studies comparing aneuploidy at D5 of development using comprehensive chromosome screening techniques, now widely considered the gold standard in preimplantation genetic screening. As such, there continues to be variation in clinical practice regarding ovarian stimulation. Some clinicians aim to retrieve fewer oocytes (e.g. <14) and use lower doses while other clinicians aim to retrieve more oocytes (~20+) and use higher doses of gonadotropins routinely, with the strategy of vitrifying all embryos becoming more common. We propose a prospective, multi-centre, randomised controlled trial to compare two different stimulation regimens with universal preimplantation genetic screening using next generation sequencing (NGS). In addition, we will use newer techniques such as mtDNA copy number quantification to provide additional information regarding oocyte quality allowing a more comprehensive assessment of the impact of ovarian stimulation on oocyte quality.

Research Design:
Multicentre prospective randomized controlled trial

Setting:
IVF units across Australia (Monash IVF, IVF Australia, Queensland Fertility Group, Melbourne IVF, Fertility SA, Repromed, Fertility Specialists of Western Australia)

Subjects:
Recruitment of female patients undergoing IVF (see inclusion and exclusion criteria)

Consent:
Prior to undertaking ovarian stimulation

Hypothesis To Be Tested

Controlled ovarian hyperstimulation using conventional ovarian stimulation leads to a lower proportion of euploid embryos per patient than using mild ovarian stimulation when tested using the gold standard of 24 chromosome screening at D5 of development.

Inclusion Criteria

Female age 28-38 years (completed) and projected normal responder based on AMH 8-20 pmol/L on Roche Elecsys measurement, BMI 18.0-32. Additional inclusion criteria include: primary diagnosis of infertility (see exclusion criteria); access to ejaculated sperm suitable for IVF/ICSI (including donor sperm; see exclusion criteria re: severe male factor infertility); trying for pregnancy >12 months before randomization; regular menstrual cycles of 24–35 days; hysterosalpingography, hysteroscopy, or transvaginal ultrasound documenting a uterus consistent with expected normal function; transvaginal ultrasound documenting presence and adequate visualization of both ovaries without evidence of abnormality; the trial cycle being the first or second COS cycle ever or the first or second COS cycle after having achieved pregnancy in a previous COS cycle.

Exclusion Criteria

Those not meeting the inclusion criteria plus patients with significant pre-existing physical or mental health condition inconsistent with ART, unable to give fully informed consent to participation, requiring PGD for single gene disorders or parental chromosomal abnormalities. Additional: Women with polycystic ovaries (defined as ovarian volume > 10 mL or > 25 follicles per ovary) or endometrioma >2 cm diameter, severe male factor defined as <1million/mL total number sperm per ejaculate; poor response in a previous COS cycle, defined as either >16 days of gonadotropin stimulation, cancellation due to limited follicular response, or development of fewer than four follicles >15 mm; severe ovarian hyperstimulation syndrome (OHSS) in a previous COS cycle; history of recurrent miscarriage; current or past (up to 12 months before randomization); abuse of alcohol or drugs; intake of more than 14 units of alcohol per week during the past month or smoking more than ten cigarettes per day within 3 months before randomization. Use of adjuvants recorded and discouraged.

Interventions

Low-dose stimulation: 150IU Menopur for 7 days starting on D2-3 of natural menstruation without OCP pretreatment. GnRH antagonist to commence D5 of Menopur. First bloods and scan D7 of Menopur. Alternate day bloods and scan until trigger criteria reached (see below). No dose adjustment allowed. If >16 days FSH without meeting trigger criteria patient withdrawn from study.

Conventional-dose stimulation: 300IU Menopur for 7 days starting on D2-3 of natural menstruation without OCP pretreatment. GnRH antagonist to commence D5 of Menopur. First bloods and scan D7 of Menopur. Dose adjustment permitted from 300IU to 225 IU per day or cancel if clinician judges unacceptable risk of ovarian hyperstimulation. No other dose adjustment permitted. Use of agonist trigger will minimise risk of OHSS. Alternate day bloods and scan until trigger criteria reached (see below). If >16 days FSH without meeting trigger criteria patient withdrawn from study.

Trigger criteria: Leuprolide acetate or triptorelin acetate 0.2mg within 24 hours of developing 3 follicles of 17mm diameter or greater.

OPU: 34-38 hours after trigger. Aspirate all follicles greater than 12mm. Definition of oocyte retrieved equivalent to COCs.
IVF/ICSI according to unit clinical protocols.
Culture to blastocyst stage then trophectoderm biopsy at D5 all BB stage or above.
Vitrify all biopsied blastocysts.
PGS: VeriSeq protocol as per Illumina.
Transfer euploid embryos sequentially in frozen cycles with appropriate endometrial preparation (data to be collected retrospectively at a later date).

Main Outcome Measures

Primary Outcome

Mean proportion of euploid blastocysts per patient.

Secondary Outcomes

Number of euploid vitrified blastocysts per patient, number of 2PN pre-embryos generated, total number of embryos surviving to D5 and total number of blastocysts biopsied.
Quality of the oocytes generated using conventional vs. low dose stimulation protocols using mtDNA copy number.
OHSS resulting in hospitalisation.
Incidence of dose adjustment or cycle cancellation.

Duration of Study

2 years (Proposed dates 1/9/16 – 31/8/18)

Possible Risks

This is an experimental study in which participants will not undergo any procedures or practices outside the range of normal IVF practice. We therefore consider the project to be low-risk to each participant (no additional risks beyond routine IVF practice). The study will answer an important clinical question which has implications for IVF practice, in particular, the possible benefit of obtaining more chromosomally normal embryos for transfer. A small risk of OHSS exists in all ART cycles and the risk of OHSS in this trial will be minimised by using an agonist trigger and vitrifying all blastocysts for transfer in subsequent cycles.

Statistical Consideration

We have powered the study to show a difference in proportion of euploid embryos per group based on the Baart et al. (2007) study. In addition, we will conduct a post-hoc non-inferiority analysis if possible based on the recorded standard deviation (analysis below).

Study Sample size and power calculations:

To show a difference in proportion of euploid embryos from 37% to 55% (high stimulation versus standard stimulation, based on Baart et al. 2007) with 0.8 power and 0.05 type 1 error rate the study requires 120 patients per group (240 patients total).

Statistical Analysis:

Depending on the recorded variance our study will be able to demonstrate a non-inferiority margin of 0.75 embryos per patient if SD </= 1.75 based on expected recruitment (see table).

Sample size for number of euploid embryos (table shows sample size per group)

Sample size per group - Non-inferiority setting
Assumed equal mean, normal distribution, two-sided 5% level

		Standard deviation						
NI Margin (embryos)	Power	1.00 n	1.25 n	1.50 n	1.75 n	2.00 n	2.50 n	3.00 n
-0.25	80%	253	394	567	771	1006	1571	2262
	90%	338	527	758	1031	1346	2103	3028
-0.50	80%	64	100	143	194	253	394	567
	90%	86	133	191	259	338	527	758
-0.75	80%	29	45	64	87	113	176	253
	90%	39	60	86	116	151	235	338
-1.00	80%	17	26	37	50	64	100	143
	90%	23	34	49	66	86	133	191

n: Samplesize pr group

Data Storage & Disposal

Deidentified study database to be held at UNSW (Professor William Ledger).
Each clinic to store clinical data and CRF securely.
See NEAF for further information.

Feasibility

Fully funded and broad agreement between participating centres (through Ferring IVF Expert panel).

Ethical Issues (include need to involve other institutional Ethics Committees)

See attached NEAF. Other institutional ethics committees will be required for SSA.

Funding Requirements

Fully funded by Ferring Grant for Investigator Initiated Trial ($800470) held by Professor William Ledger.

Additional Comments

Research proposal to be evaluated by Monash Health HREC and site-specific agreements to be generated and evaluated by other centres listed above.

References

Baart EB, Martini E, Eijkemans MJ et al. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Hum Reprod* 2007 22(4):980-8.