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)

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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 or D6, 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

Subjects:
Recruitment of female patients undergoing IVF (see inclusion and exclusion criteria). Randomisation to low dose or conventional ovarian stimulation using a random number generator and stratified recruitment by site for equal recruitment into each arm of the study.

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 or D6 of development.

Inclusion Criteria

Female age 21-40 years (stimulation before patients 41st birthday) and projected normal responder based on AMH 8-25 pmol/L on Roche Elecsys or Beckman measurement, BMI 18.0-35. Patients must consent to undergoing preimplantation genetic screening as part of the trial which would not be a routine part of an IVF cycle but is an available option. 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, second or third COS cycle ever (whether this resulted in a

successful pregnancy or not), 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 (up to 12 months before randomization of at least 3 consecutive miscarriages of a pregnancy beyond 6 weeks gestation. Pregnancy defined as a 'clinical pregnancy' which is confirmed on an early pregnancy ultrasound); 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 D5 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 (Orgalutran or Ganirelix acetate) 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, triptorelin acetate or Buserlin acetate 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 or D6 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

3 years (Proposed dates 1/12/16 – 01/12/19)

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.

Assignment of optimum starting gonadotropin dose is essential to avoid hypo- and hyperovarian response. In conventional ovarian stimulation for IVF, the former is associated with cycle cancellation or poor pregnancy outcome whereas the latter is associated with increased risk of ovarian hyper stimulation syndrome (OHSS). The prediction of "optimum" starting gonadotropin dosing is currently based on physician preference rather than being based on high quality evidence. Follicle-stimulating hormone (recFSH) preparations are currently administered in dosages ranging from 100 to 600 IU/day (Nargund et al, 2007; Malizia et al, 2009).

To evaluate the clinical outcomes in relation to the daily dose of recFSH for ovarian stimulation in IVF among expected normal responders, a systematic review and meta-analysis has been recently conducted (Sterrenburg et al, 2011). The review sought to identify the optimal daily starting dose of recFSH taking into account ovarian response, pregnancy chances, rate of cycle cancellation and the incidence of the potentially life-threatening complication of ovarian hyperstimulation syndrome (OHSS). Published randomized comparative dosage trials were searched in order to identify the recFSH dose with the best clinical efficacy, cost-effectiveness and safety profile. 11 RCTs reporting data on 1967 women undergoing a single IVF cycle. Cumulative pregnancy and live birth rates (from fresh plus frozen embryo transfers) were not assessed. All trials had parallel design and in most studies the treatment was adequately concealed prior to allocation.

Meta-analysis showed that fresh transfer pregnancy rates per started IVF/ICSI cycle did not differ between lower and higher dosages of gonadotropin. However, those treated with higher doses of FSH had more oocytes collected and more embryos cryopreserved in some but not all studies. Hence higher doses may result in a higher cumulative pregnancy rate (fresh plus frozen transfer) than lower dose regimes, but this is not proven.

The studies analysed by Sterrenburg et al. did not use the most up-to date and safest stimulation protocol that is to be employed in our study. Use of a GnRH agonist trigger with antagonist suppression of premature ovulation followed by "freeze all" embryos has practically abolished risk of severe ovarian hyperstimulation syndrome without

deleterious effect on pregnancy rates after later transfer of a frozen embryo (Devroey et al, 2011). Women randomly allocated to the higher dose group in this study may have more embryos available for testing and for cryopreservation and will not be at increased risk of OHSS. Given the lack of consensus in recent literature on the "best" dose of FSH to use to achieve the highest pregnancy rate in GnRH agonist triggered freeze-all cycles, whilst ensuring patient safety, there is equipoise as to whether a dose of 150 or 300 IU per day is better in this protocol.

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

Statistical analysis plan: Chi-square, independent t-test and ANOVA tests will be used to examine the differences between the rates of oocyte derived aneuploidy in patients stimulated with 300 international units compared with 150 international units.

Univariate and multivariate logistic regression models will be employed to estimate the likelihood of dichotomous outcome measurements (e.g. aneuploidy etc.) for women who are stimulated with 300 international units compared with 150 international units. Variables significantly (P<0.05) associated with outcomes on univariate analysis and

other factors identified in the literature as predictive of selected outcomes will be entered into multivariate models. Final models will be determined by taking into account the likely causal pathway, colinearity, statistical significance, and goodness of fit. Cox proportional hazards modelling will be used to estimate the relative (RR) risk of aneuploidy for women stimulated with 300 international units compared with 150 international units.

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.

Sample storage: Most tests taken as part of the study are routine tests required to monitor patient progress in an IVF cycle. Data from these tests will be stored on Laboratory Information Management systems as per routine clinic practice. Biopsied cells from embryos will be lysed prior to undergoing whole genome amplification (WGA) and preimplantation genetic screening as per Illumina Veriseq protocol. WGA products will be stored as per routine laboratory practice in case further verification/retesting is required for clinical purposes. The embryo biopsy itself therefore requires no further storage/disposal.

Feasibility

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

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

As the study involves routine clinical care and does not substantially alter the treatment regimen delivered to participants, UNSW and Ferring Australia will not provide indemnity in the case of an adverse medical event. Insurance however, will be covered by the Fertility clinic providing the service in conducting these procedures.

Funding Requirements

Fully funded by Ferring Grant for Investigator Initiated Trial held by Professor William Ledger. Support in kind from Illumina Inc. and Roche Pty Ltd.

Additional Comments

Approved by Monash Health HREC, Melbourne IVF HREC, and University of Western Australia HREC (documents attached).

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.

Nargund G, Fauser BC, Macklon NS, Rotterdam ISMAAR Consensus Group on Terminology for Ovarian Stimulation for IVF. The ISMAAR proposal on terminology for ovarian stimulation for IVF. *Hum Reprod* 2007 22(11):2801-4.

Malizia BA, Hacker MR, Penzias AS. Cumulative live-birth rates after in vitro fertilization. *NEJM* 2009 360:236-243.

Sterrenburg MD, Veltman-Verhulst SM, Eijkemans MJ et al. Clinical outcomes in relation to the daily dose of recombinant follicle-stimulating hormone for ovarian stimulation in in vitro fertilization in presumed normal responders younger than 39 years: a meta-analysis. *Hum Reprod Update* 2011 17(2):184-96.

Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. *Hum Reprod* 2011 26(10):2593-7.