Human Reproduction, Vol.26, No.5 pp. 1091–1096, 2011
Advanced Access publication on February 28, 2011 doi:10.1093/humrep/der048

human reproduction

ORIGINAL ARTICLE *Infertility*

Timing oocyte collection in GnRH agonists down-regulated IVF and ICSI cycles: a randomized clinical trial

M.H. Mochtar[1,*], I.M. Custers[1], C.A.M. Koks[2], R.E. Bernardus[3], H.R. Verhoeve[4], B.W. Mol[1,2], M. van Wely[1], and F. van der Veen[1]

[1]Department of Obstetrics and Gynaecology, Centre of Reproductive Medicine, Academic Medical Centre, Amsterdam, The Netherlands [2]Máxima Medical Centre, Veldhoven, The Netherlands [3]TerGooiZiekenhuizen, Blaricum, The Netherlands [4]Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands

*Correspondence address. Centre for Reproductive Medicine (Q4), Department of Obstetrics and Gynaecology, PO Box 22660, 1100 DD Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. E-mail: m.h.mochtar@amc.nl

Submitted on September 2, 2010; resubmitted on January 18, 2011; accepted on January 24, 2011

BACKGROUND: The evidence underpinning the timing of an oocyte collection in IVF or ICSI is limited. The aim of this study was to assess the effect of the follicle diameter size of the dominant follicle on ongoing pregnancy rates.

METHODS: We conducted a randomized controlled trial, including women aged between 18 and 43 years who were scheduled for GnRH agonist down-regulated IVF/ICSI treatment in four assisted conception units. Women were randomized between timing oocyte collection when the leading follicle had a diameter of 22 mm or when the leading follicle had a diameter of 18 mm. The primary end-point was ongoing pregnancy, defined as a viable pregnancy at 12 weeks of gestation.

RESULTS: The trial had major problems with recruiting patients and after the planned 2 years of recruiting only half of the aimed 400 inclusions were obtained. We allocated 97 women to the 22-mm group and 93 women to the 18-mm group. In the 22-mm group more women reached an ongoing pregnancy (37 of 97 women, 38%) compared with the 18-mm group (22 of 93 women, 24%) resulting in a relative risk of 1.6 [95% confidence interval (CI): 1.03–2.5]. In a logistic regression analysis, the timing of oocyte collection, adjusted for female age, IVF/ICSI and centre, was still associated with ongoing pregnancy, although the association was no longer statistically significant (OR: 2.0; 95% CI: 0.96–4.2)

CONCLUSIONS: This study suggests that delaying the timing of oocyte collection in IVF or ICSI results in better ongoing pregnancy rates, however, larger studies have to be performed to prove or refute these findings.
Trial registration: ISRCTN24724622.

Key words: IVF / ICSI / follicle diameter / hCG / oocyte triggering / timing oocyte collection

Introduction

In the infancy of IVF, planning an oocyte collection was quite a challenge. Women were admitted to hospital for 3-h measurements of estrogen and luteinizing hormone levels in urine samples. When a significant rise in urinary LH value was detected, an immediate laparoscopic oocyte collection was carried out (Trounson and Wood, 1981). Although IVF is an effective mainstream procedure to date, planning an oocyte collection is still an enigma (Kolibianikis *et al.*, 2004).

It is a widely agreed that follicles need to reach at least 17 mm in diameter before administrating hCG for follicular maturation for oocyte collection (Kolibianikis *et al.*, 2004). This view dates from a period in which the GnRH agonists were not yet introduced in the IVF stimulation protocols. At that time, premature LH surges were very common and it was noticed that these premature LH surges had a deleterious effect on pregnancy rates (Hillier *et al.*, 1985). Cycles with premature LH surges were therefore cancelled. To lower this cancellation rate, hCG was administered at follicle diameters of 17 mm or less. A breakthrough in ovarian hyperstimulation was the introduction of GnRH agonists. The premature LH surges were suppressed and the IVF results improved significantly ever since.

The concept of administering hCG at a follicle diameter of 17 mm thus needed re-evaluation and has so far been addressed in five randomized studies. The results of these studies are not in agreement and their conclusions contradictory. Two studies, one pseudo-randomized study,

which included 325 women and one truly randomized study of 143 women showed a beneficial effect of delaying oocyte collection for 1 or 2 days after the leading follicle had reached a diameter of 18 mm or three follicles had reached a diameter of 17 mm. (Abdalla et al., 1989; Dimitry et al., 1991) Two other studies, one small randomized study of 57 women and one large randomized study of 413 women, showed a deleterious effect of delaying oocyte collection for 1 day after two follicles reached a diameter of 17 mm (Clark et al., 1991; Kolibianikis et al., 2004). One randomized three-arm study of 247 women found no difference of delaying oocyte collection for 1 or for 2 days after the leading follicle had reached a diameter of 18 mm (Tan et al., 1992).

Unfortunately, the designs of these studies were not robust. Four out of five studies used the criterion of a leading follicle with a diameter of at least 17 or 18 mm as a condition for the administration of hCG. This may have lead to possible different sizes of follicle diameters in the standard arm at the day of hCG administration, since 'at least' implies that larger follicles than 17 or 18 mm were also allowed. This problem is even amplified by lack of precise measurements of the cohort of follicles on the day of hCG in the experimental arm. Most studies delayed the hCG administration for 1 or 2 days, instead of reaching a predetermined size of the leading follicle, which makes pooling of the data impossible.

In view of these limitations, we aimed to seek more evidence on the impact of delaying oocyte collection on pregnancy rates. We therefore compared the effect of planning oocyte collection when the leading follicle had a diameter of 18 mm versus planning oocyte collection when the leading follicle had a diameter of 22 mm on ongoing pregnancy rates.

Materials and Methods

Four fertility clinics in the Netherlands participated in this randomized clinical trial between April 2006 and April 2008. The study was approved by the Institutional Review Boards of all clinics and registered in the International Standard Randomized Controlled Trial Number Register (ISRCTN24724622).

Eligible women with an indication for IVF/ICSI treatment were recruited. Inclusion criteria were female's age between 18 and 43 years and a regular menstrual cycle ranging between 24 and 35 days. Excluded from the study were women with polycystic ovarian syndrome because of their known pathological follicular growth, women with ovarian cysts, women with a history of low response to FSH treatment, women with a history of oophorectomy and women with more than two previous IVF/ICSI attempts.

During the stimulation phase, women with an unexpected low response, defined as growth of less than three follicles >12 mm (including the dominant follicle) were also excluded from the study.

At baseline visit, eligible women with a valid indication for IVF or ICSI were asked to participate in the study for one treatment cycle (first or second attempt). Women were eligible when the inclusion and exclusion criteria were met and when written informed consent was given. Controlled ovarian hyperstimulation was started on the fifth or the sixth day of the menstrual cycle, after a baseline transvaginal ultrasound. Stimulation was carried out with 150 or 225 IU recombinant FSH (rFSH), preceded by a GnRH agonist, which was started on the 23rd day of the previous cycle. In case women were pre-treated with an oral contraceptive, the GnRH agonist down-regulation was started 3 days before ceasing the OC. After 7 days of stimulation, a second ultrasound was performed and the dominant follicle was identified. The dosage of rFSH was adjusted if necessary. Two days after the second ultrasound, follicles were monitored every other day and mandatory on the day of hCG administration. As soon as the leading follicle reached a diameter of 18 mm ($\pm$ 1 mm), women were randomized and allocated to administration of 10 000 IU hCG (Pregnyl®; Organon, The Netherlands) for final follicular maturation or were scheduled for a transvaginal ultrasound after 1 or 2 days, with the aim of performing the transvaginal ultrasound at a follicle diameter of 22 mm. Depending on the growth velocity of the leading follicle estimated by previous measurements of the follicle, patients with a fast-growing follicle, i.e. growing faster than 2 mm a day, were asked to come the next day and patients with normal growing follicle, i.e. 2 mm a day, were asked to come in 2 days. As soon as the leading follicle had reached 22 mm ($\pm$ 1 mm) 10 000 IU hCG was administered.

Randomization took place through a web-based randomization programme, stratified for centre. The allocation sequence programme was prepared by the Academic Medial Research Bureau. Patients were recruited by either research nurses or local physicians at baseline visit.

At the day of hCG administration, for follicular maturation, in both groups all follicle diameters were measured as well as the triple layer endometrial thickness in millimetres and a venous blood sample for serum LH and estradiol was taken. The dominant follicle was measured in three planes, the other follicles in two planes (Raine-Fenning et al., 2003; Duijkers et al., 2004) Oocyte retrieval took place 36 h after hCG administration. Luteal support consisted of 400 mg micronized progesterone administered vaginally in two daily doses, starting after oocyte collection for 2 weeks.

Oocyte quality assessments

The quality of the retrieved oocytes was assessed by local embryologists who were blinded for the randomization allocation. They were classified as metaphase II oocytes, metaphase I oocytes, germinal vesicle stage for ICSI patients, or Score 1 (metaphase II), 2 (metaphase I) 3 or 4 (atretic oocytes) for IVF patients (Veeck, 1988).

Semen preparation and insemination

Semen was diluted 1:1 with culture medium and subjected to density gradient centrifugation using 70% PureSperm (Nidacon, Gothenburg, Sweden). The pellet was washed with culture medium and resuspended in 2 ml of culture medium. Subsequently the sample was incubated for 1 h at 37°C in 5% CO_2 in air during which the motile spermatozoa were allowed to swim to the bottom of the tube ("swim-down"). Finally, the pellet was washed and semen parameters were assessed.

Cumulus–oocyte complexes were inseminated individually with 10 000 progressively motile spermatozoa 40 h after hCG (4 h incubation time) in a final volume of 100 µl culture medium, i.e. human tubal fluid medium (BioWhitaker). In case of male factor subfertility, i.e. less than 3 million progressive motile spermatozoa (TMC <3 million) 15 000 progressively motile spermatozoa were used.

ICSI was performed 38 h after hCG (2 h incubation time) when less than 1 million progressively motile spermatozoa (post-wash) were available in the ejaculate at the time of initial fertility work-up or after previous fertilization failure.

Oocytes were inspected for fertilization, which was defined as the presence of two or more pronuclei $\sim$16 to 18 h after insemination. At this time, all embryos were transferred individually to a fresh volume of 75 µl culture medium. Embryos are cultured under oil at 37°C in 5% CO_2 in air.

Embryo quality assessments

Embryo quality was scored according to the criteria defined in the Standard Operating Procedures of the scoring system (Puissant *et al.*, 1987). The morphology was assessed daily by an embryologist/IVF technician, who was blinded to the allocation of the patient. The following parameters were evaluated: Day 1: the size [equal or unequal (>25% difference in size)] and position (central or peripheral) of the pronuclei, the presence of a cytoplasmic halo (a light zone over at least 75% of the circumference of the zygote); Day 2 and Day 3: the number and size [equal, slightly unequal (25–50% difference in size) or unequal >50% difference in size)] of the blastomeres, the degree of fragmentation (grade 0: no fragmentation, grade 1: <10%, grade 2: 10–25%, grade 3: 25–50%, grade 4:>50% fragmentation), the position (local or dispersed) and the size (small or large) of the fragments. Furthermore, the position of the cleavage axes (perpendicular or not) on Day 2 (if a 4 cell embryo was available) and the presence of mono- or multinucleation on Day 2 and Day 3 were evaluated. Embryos were examined using an Olympus IX71 inverted microscope equipped with Relief Contrast optics at a magnification of 320 times.

In the presence of a top-quality embryo, an elective single embryo transfer (eSET) was performed. Embryo transfer followed 72 h after oocyte retrieval and surplus embryos, when eligible, were stored frozen. Treatment was discontinued if the standard criteria of impending ovarian hyperstimulation syndrome (OHSS) were met (NVOG guidelines).

Outcome parameters

The primary outcome of this study was ongoing pregnancy rate, defined as a positive fetal heartbeat by transvaginal ultrasound at least 10 weeks after oocyte retrieval. Secondary outcome measures were total days of controlled ovarian hyperstimulation, total amount of rFSH used, serum estradiol at the day of hCG, total number of retrieved oocytes, OHSS/discontinuation due to a high risk of OHSS, total number of Score I oocytes (IVF only), number of metaphase II oocytes (ICSI only), fertilization rate (number of zygotes), fertilization failure, number and quality of embryos, number of eSET of a high-quality top embryo and number of compulsory single embryo transfer (cSET: meaning that just one embryo was available for transfer, not considering the quality), number of embryos suitable for cryo-preservation, biochemical (defined as any increase in serum HCG >2) and clinical pregnancy rates, defined as a positive heartbeat at transvaginal ultrasound at 7 weeks after oocyte retrieval and live birth rate.

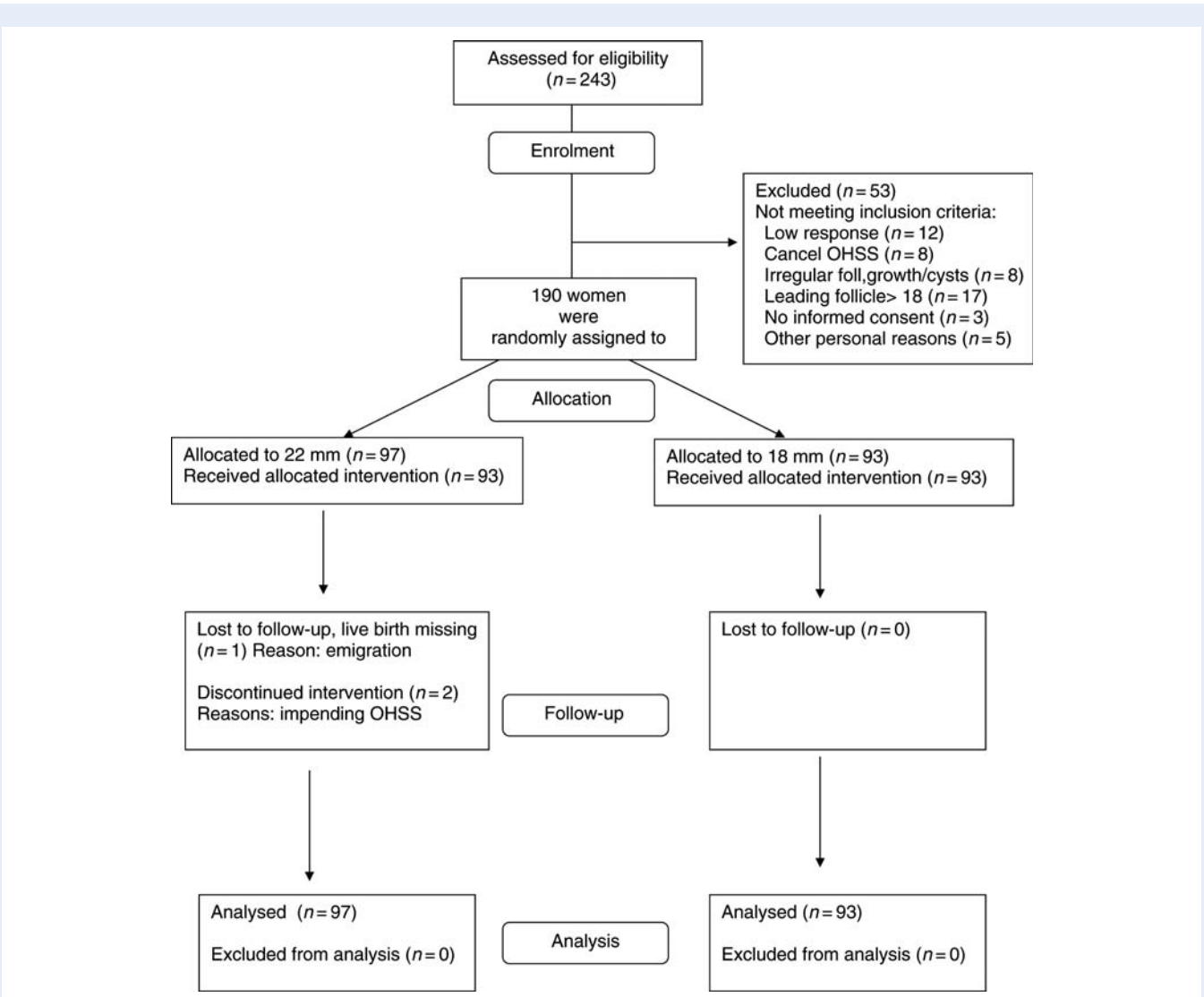

Figure 1 Flow chart.

Analysis

We designed our study as a classical superiority trial for ongoing pregnancy rates because of the anticipated benefit of delaying hCG. For our power analysis, we used the ongoing pregnancy rates of the previous three most comparable studies (Abdalla et al., 1989, Dimitry et al., 1991; Tan et al., 1992) and we thus assumed a 13% difference over a control rate of 25% ongoing pregnancies. To detect this anticipated difference of 13% in ongoing pregnancy rate, with a power of 80% and an alpha of 5%, 200 women per treatment arm were required.

All analyses were performed on an intention-to-treat basis after randomization. The effectiveness of precise timing of hCG administration according to follicle diameter per woman (i.e. 22 mm and 18 mm) was expressed as a risk rate for ongoing pregnancy with corresponding 95% confidence intervals (CI). Continuous data, such as number of oocytes retrieved and number of embryos obtained, were expressed as means with corresponding CI. ANOVA or Mann–Whitney tests on continuous data were performed when appropriate. Dichotomous outcomes were analysed using chi-square (χ^2) tests.

Logistic regression analysis was used to assess whether heterogeneity in female age, IVF/ICSI and centre interfered with the observed association between timing of oocyte collection and ongoing pregnancy rate.

Results

A total of 243 consecutive women agreed to participate in this trial. They were screened for eligibility between April 2006 and April 2008. The last woman was included in September 2008 and the follow-up period lasted until October 2009. Of these, 199 women were found eligible and were subsequently randomized, whilst 53 could not be randomized due to primary exclusion criteria. By recruiting centre, 105 women were included in the academic centre, and 76, 12 and 13 from the other fertility clinics. In total, 97 patients were allocated to the 22-mm group and 93 to the 18-mm group (Fig. 1). Unfortunately, major problems in recruiting patients meant that after the planned 2 years of recruiting only half of the aimed inclusions were obtained. Two women in the 22-mm group cycles had their cycle cancelled because of impending OHSS risk, no such cancelations were found in the 18-mm group.

Patient characteristics are summarized in Table I. After randomization an equal distribution occurred in mean age, BMI, indication, cycle number, IVF/ICSI and starting dose of rFSH between the two groups.

The pregnancy results are presented in Table II. In the 22-mm group more women reached an ongoing pregnancy (37 of 97 women, 38%) compared with the 18-mm group (22 of 93 women, 24%). The resulting relative risk (RR) was 1.6 (95% CI: 1.03–2.5). A statistically significant difference was not reached for the secondary outcomes clinical pregnancy rate and live birth rate. The pregnancy loss from biochemical to ongoing pregnancy was 6/97 in the 22-mm group versus 11/93 in the 18-mm group resulting in an RR of 0.52 (95% CI: 0.20–1.36). This difference is not significantly different. The pregnancy loss from clinical pregnancy rate to ongoing pregnancy rate was 1/97 to 8/93 resulting in an RR of 0.12 (95% 0.015–0.94).

The results of the stimulation and laboratory phase are presented in Table III. As expected, the duration of controlled ovarian hyperstimulation was significantly higher in the 22-mm group compared with the 18-mm group, with a mean difference of 1 day. Also a significantly higher amount of rFSH was used in the 22-mm group, with a mean difference of 250 IU.

In addition, in the 22-mm group, the longer duration of ovarian stimulation yielded a significantly higher mean serum estradiol at the day of hCG administration and significantly more follicles. On the day of hCG, the number of follicles <14 mm was significantly smaller, the number of intermediate follicles 15–19 did not differ and the number of large follicles was significantly higher.

Significantly more oocytes were retrieved, but the overall fertilization rate, i.e. the total mean number of embryos/total number of oocytes, did not differ (52% versus 50%). Total fertilization failure occurred in five cases in the 22-mm group and seven cases in the 18-mm group, respectively.

Table I Baseline characteristics.

Baseline characteristics	22-mm group, *n* = 97	18-mm group, *n* = 93
Mean age (SD)	33.6 (4.3)	34.2 (4.3)
Mean BMI (SD)	24.5 (4.9)	23.5 (4.8)
Primary subfertility (%)	61	59
Duration subfertility (years)	2.8	3.3
Indication IVF/ICSI		
Tubal factor	17	14
Male factor	40	35
Unexplained	21	27
Cervical factor	14	13
Other	5	4
First cycle	91	83
Second cycle	6	10
IVF	60	56
ICSI	37	37

Table II Pregnancy outcome.

Pregnancy outcomes	22-mm group, *n* = 97 (%)	18-mm group, *n* = 93 (%)	RR (95% CI)
Primary outcome			
Ongoing pregnancies	37 (38)	22 (24)	1.6 (1.03–2.51)
Secondary outcomes			
Biochemical pregnancies	43 (44)	33 (35.5)	1.3 (0.88–1.78)
Clinical pregnancies	38	30	1.2 (0.83–1.78)
Live births	34 (35)	21 (23)	1.6 (0.98–2.47)
Singleton	32 (85)	17 (77)	
Twin	2/37 (5)	4/22 (18)	
Lost to follow-up >12 weeks	1		

Table III Stimulation phase and laboratory outcomes.

	22-mm group, *n* = 97	18-mm group, *n* = 93	*P* value
Days of stimulation, mean (SD)	11.7	10.7	0.001
Starting dose rFSH mean IU (SD)	163 (4.8)	164 (6.7)	
Total dose rFSH, mean IU (SD)	1952 (698)	1680 (739)	0.010
Serum estrodial at hCG, mean (SD)	7.8 (4.2)	5.2 (2.7)	0.000
No of follicles at hCG, mm^2 (SD)	13.6 (6.2)	11.9 (4.4)	0.036
<12	1.06 (1.8)	1.99 (2.3)	0.003
13–14	1.59 (2.0)	2.30 (2.5)	0.038
15–16	2.58 (2.9)	3.04 (2.3)	0.2
17–19	3.81 (3.1)	4.2 (2.9)	0.3
20–22	3.95 (3.5)	0.02 (0,1)	0.00
Women with oocyte collections, *n*	96	93	
Zero oocytes	0	1	
Number of oocytes, mean (SD)	11.7 (5.7)	9.7 (4.1)	0.006
Oocyte sc_1 MF II (*n* = 190)	8.15	6.24	0.028[a]
Oocyte sc_2 MF I (*n* = 30)	1.9 (1.3)	1.29 (0.8)	
Oocyte sc_3 (*n* = 58)	5.5 (2.9)	4.9 (3)	
Oocyte sc_4 (*n* = 49)	1.9 (1.5)	1.7 (1.0)	
Germinal vesicle (*n* = 36)	1.87 (0.9)	2.25 (1.8)	
Fertilization rate (tot no embryos/ tot no oocytes)	55%	54%	
Total fertilization failure	4	7	
Number of embryos, mean (SD)	6.5 (4.1)	4.8 (3.3)	0.016
No of top embyos			
>8c1	17	10	
>8c2	38	47	
Embryo score			
Score 1	61 in 31 women	36 in 19 women	0.036[a]
Score 2	240 in 64 women	217 in 59 women	
Score 3	187 in 59 women	122 in 61 women	
Score 4	50 in 26 women	34 in 24 women	
Embryo transfer, *n*	90	85	
Type of embryo transfer, *n* (%)			0.89
No embryo transfer	7	8	
cSET	7 (8)	11 (13)	
eSET	32 (34)	24 (27)	
DET	49 (54)	46 (54)	
TET	3 (3)	5 (6)	

[a]Mann–Whitney test on number of SC1 oocytes and SC1 embryos per woman.
cSET, compulsory single embryo transfer; eSET, elective single embryo transfer; DET, double embryo transfer; TET triple embryo transfer.

In the 22-mm group significantly more embryos were obtained, leading to significantly more excellent/top-quality embryos (embryos with Score 1), but there were no differences in the number of embryos transferred, i.e. SET (single embryo transfer), DET (double embryo transfer) or TET (triple embryo transfer). The mean number of embryos transferred was 1.6 (SD 0.5) and 1.7 (SD 0.6) for the 22- and 18-mm groups, respectively.

In a logistic regression analysis, timing of oocyte collection adjusted for female age, ICSI and centre, was still associated with ongoing pregnancy, although the association was no longer statistically significant (OR: 2.0; 95% CI: 0.96–4.2).

Discussion

The present randomized controlled trial evaluated the timing of oocyte collection when the leading follicle had reached a diameter of 22 mm in comparison to a leading follicle diameter of 18 mm. We found a significantly higher ongoing pregnancy rate and live birth rate when oocyte

collection was planned if the leading follicle had a diameter of 22-mm group compared with 18 mm. In the 22-mm group, there were more follicles and more oocytes were retrieved, resulting in more embryos and in addition, in more top-quality embryos.

The strength of our trial is that the diameter of the leading follicle in both study arms was actually measured and subsequent planning of oocyte collection was planned according to fixed randomization criteria, i.e. when the leading follicle measured 18 mm or when the leading follicle measured 22 mm. The weakness of our trial is that we had to stop the trial prematurely because of difficulty in recruiting patients and as a consequence this trial is underpowered. Patients were found to be very hesitant to participate, for no particular reasons. After 2 years of laborious efforts from the participating doctors to recruit patients, the study had to be stopped because of trial fatigue (Campbell *et al*., 2007).

Besides a higher ongoing pregnancy rate, we also found that more oocytes and more high-quality embryos were obtained in the 22-mm group compared with the 18-mm group. It could be that the better pregnancy rates in the 22-mm group were the result of the sheer increase in number of oocytes and subsequent significant increase in the number of metaphase II oocytes leading to significantly more top quality embryos. As a consequence there were more elective SETs and less compulsatory SETs in the 22-mm group compared with the 18-mm group. In view of the current trend to turn to mild stimulation regimens, it is noteworthy to find that delaying the timing of oocyte collection, instead of using higher dosages of gonadotrophins, may also lead to a greater yield in oocytes. This has never been considered to be an important factor in studies of mild stimulation (Verbers *et al*., 2009). In the present study, all patients received the same, relatively mild stimulation protocol suggesting that the increase in number of retrieved oocytes was due to the delayed timing of oocyte collection rather than a higher dose of rFSH. To obtain better results in IVF, the real paradigm might not be the stimulation regimens (mild or conventional) themselves, but delaying oocyte collection to harvest more oocytes from the growing cohort, which then in turn lead to more high-quality embryos.

Of importance may also be whether pituitary down-regulation is achieved by agonists or antagonists, since a deleterious effect of delaying oocyte collection was seen in a previous study in which patients received GnRH antagonists (Kolibiniakanis, 2004). In that GnRH antagonist study, no increase in oocytes yield by delaying timing of oocyte collection was found, as opposed to this study with an GnRH agonist. Therefore, the results of our study apply solely to ovulatory women with a good ovarian reserve in a regimen using GnRH agonists for ovarian down-regulation.

To avoid underpowered studies in the future in the Netherlands, we are presently working on a fertility consortium (www.studies-obsgyn.nl) in which many specialists in reproductive medicine collaborate to perform studies together.

In conclusion, the present trial although underpowered, suggests that delaying the timing of oocyte collection has a beneficial effect on ongoing pregnancy rates in ovulatory patients. Larger randomized trials should be performed to confirm or refute these findings.

Authors' roles

M.H.M.: trial design, data acquisition, data entry and interpretation, manuscript drafting and corresponding author. I.M.C.: substantial contribution to trial logistics and data managing. C.A.M.K.: substantial contribution to patient recruitment. R.E.B.: contribution to patient recruitment and manuscript revising. H.R.V.: contribution to patient recruitment and manuscript revising. B.W.M.: substantial contribution to trial logistics and important scientific comments. M.W.: trial design, statistical analysis, manuscript drafting. F.V.: substantial contribution to trial design and manuscript drafting.

Funding

We received an unconditional educational grant from Organon Nederland B.V.

References

Abdalla HI, Baber RJ, Leonard T, Kirkland A, Mitchell A, Power M, Owen E, Studd JWW. Timed oocyte collection in an assisted conception programme using GnRH analogue. *Hum Rep* 1989;**4**:927–930.

Campbell MK, Snowdon C, Francis D, Elbourne D, McDonald AM, Knight R, Entwistle V, Garcia J, Roberts I, Grant A. Recruitment to randomised trial: strategies for trial enrolment and participation study. The STEPS study. *Health Technol Assess* 2007;**11**:1–67.

Clark L, Stanger J, Brinsmead M. Prolonged follicle stimulation decreases pregnancy rate after in vitro fertilization. *Fertil Steril* 1991;**55**:1192–1194.

Dimitry ES, Bates SA, Oskarsson T, Margara R, Winston RM. Programming in vitro fertilization for a 5- or 3-day week. *Fertil Steril* 1991;**55**:934–938.

Duijkers IJ, Louwé LA, Braat DD, Klipping C. One, two or three: how many directions are useful in transvaginal ultrasound measurement of ovarian follicles?. *Eur J Obstet Gynecol Reprod Biol* 2004;**117**:60–63.

Hillier SG, Afnan AMM, Margara RA, Winston RML. Superovulation strategy before in vitro fertilization. *Clin Obstet Gynaecol* 1985; **12**:685–723.

Kolibianikis EM, Albano C, Camus M, Tounaye H, Van Steirteghem AC, Devroey P. Prolongation of the follicular phase in in vitro fertilization results in a lower ongoing pregnancy rate in cycles stimulated with recombinant follicle-stimulating hormone and gonadotrophin-releasing hormone antagonists. *Fertil Steril* 2004;**82**:102–107.

Puissant F, Van Rysselberge M, Barlow P, Deweze J, Leroy F. Embryo scoring as a prognostic tool in IVF treatment. *Hum Reprod* 1987; **2**:705–708.

Raine-Fenning NJ, Clewes JS, Kendall NR, Bunkheila AK, Campbell BK, Johnson IR. The interobserver reliability and validity of volume calculation from three-dimensional ultrasound datasets in the in vitro setting. *Ultrasound Obstet Gynecol* 2003;**21**:283–291.

Tan SL, Balen A, El Hussein E, Mills C, Campbell S, Yovich J, Jacob HS. A prospective randomized study of the optimum timing of human chorionic gonadotrophin administration after pituitary desensitisation in in vitro fertilization *Fertil Steril* 1992;**57**:1259–1264.

Trounson AO, Wood C. Extracorporeal fertilization and embryo transfer. *Clin Obstet Gynaecol* 1981;**8**:681–713.

Trounson AO, Mohr LR, Wood C. Effect of delayed insemination on in vitro fertilization, culture and transfer of human embryos. *J Rep Fert* 1982;**64**:284–297.

Veeck LL. Oocyte assessment and biological performance. *Ann N Y Acad Sci* 1988;**541**:259–274. PubMed PMID: 3195909.

Verberg MFG, Eijkemans MJC, Macklon NS, Heijnen EMEW, Baart EB, Hohmann FP, Fauser BCJM, Broekmans FJ. The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: a meta-analysis. *Hum Reprod Update* 2009; **15**:5–12.