# Angionesis and the inception of pregnancy

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## DOES SUBFERTILITY EXPLAIN THE RISK OF POOR PERINATAL OUTCOME AFTER IVF AND OVARIAN HYPERSTIMULATION?

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# Introduction

Singleton pregnancies from assisted conception have a significantly higher risk of (very) low birth weight and (very) preterm birth as compared to naturally conceived controls, although the prevalence of very low birth weight and very preterm birth is low. Confounding factors such as maternal age and parity did not change this outcome[1]. Factors as social economic status, sex of the fetus, delivery date and site can probably not explain the difference[1,2]. However, history of subfertility, irrespective of infertility treatment, has also been found to be associated with perinatal death in a case-control study[3]. Jackson *et al.*[2] summarized the different trials and advised for future research in which treatment biases can be addressed.

Poor perinatal outcome was also observed in 263 singletons born of subfertile patients conceived after only controlled ovarian hyperstimulation (COHS) relative to 5,096 spontaneously conceived controls delivered in the same hospital and period; no difference was seen when the comparison was made between COHS and 162 IVF singletons[4]. After stratification for the number of years of involuntary childlessness, Källén *et al.*[5] still found a significant increased risk of preterm birth (<37 wks) and of low birth weight (<2500 g) in singletons conceived after just COHS as compared to naturally conceived singletons. Gaudoin *et al.*,[6] concluded that "infertility" should be added to the list of recognized factors associated with low birth weight by comparing 97 singletons whose subfertile mothers were treated with COHS & IUI with 35 singletons whose mothers were treated with COHS (although with normal reproductive health) as well as artificial insemination with donor sperm.

In the present study we investigate whether subfertility explains the poor perinatal outcome after assisted conception. We used data from a nation-wide historical cohort of 26,428 women treated for subfertility in the Netherlands between 1980 and 1995 (Klip *et al.*)[7]. Furthermore we tried to answer the question whether COHS with or without IVF adversely affects perinatal outcome.

The observation that birth weight of singletons conceived by implanting a cryopreserved embryo is significantly higher than birth weight after a fresh embryo transfer (ET)[8,9], suggests that the cryopreservation and thawing procedure might essentially differ from the IVF and ICSI procedure, in which the embryo(s) are directly and freshly transferred to the uterus. Since ET of cryopreserved embryos occurs predominantly in a natural cycle, one may hypothesize that COHS it self might influence uterine receptivity. Using the same database as mentioned above, we compared the birth weight and preterm birth of singletons conceived after transfer of thawed embryos with and without COHS.

# Materials and methods

## Study population

Data were obtained from an historical nation-wide cohort study (OMEGA study) of 26,428 women diagnosed with subfertility in all 12 Dutch IVF clinics between 1st of January 1980 and 1st of January 1995. Approval of the ethics committees of all institutions was obtained. Inclusion criteria were at least one year of subfertility[10] and an age older than 18 years at the time of admission to one of the clinics. Women were included in the IVF group when they had completed at least one treatment cycle with COHS and IVF before 1st of January 1995 (n=19,840). A group of 6,588 women unexposed to COHS, whose subfertility was diagnosed after 1980, was recruited from existing computerized databases of 4 out of the 12 clinics. All clinics provided a minimal data set with names, birth dates and addresses of all eligible women. After tracing of the current addresses of all women, they were contacted at their home address and were asked to fill in and return a questionnaire, as well as a written informed consent, asking each participant for permission for data abstraction from their medical records. From the initial 26,428 women, 1,105 women (4.2%) were not approachable for several reasons (for more details see Klip *et al.*[7]. Of the remaining 25,323 women nearly 67% agreed to participate in the study[11].

Some women delivered more than once during the study period. Initially, 29,148 pregnancies were reported in the returned questionnaires. As described in detail by Klip *et al.*[7], 12,148 pregnancies were directly excluded (intrauterine mortality n=10,815; pregnant at the time of returning questionnaire n=404; missing data n=929). This resulted in a total of 17,000 deliveries with a minimal gestational age of 24 weeks. This group consisted of 9,479 pregnancies following assisted reproductive techniques (IVF, ICSI, inseminations and fertility drug use not for IVF/inseminations), 5,862 pregnancies achieved with only IVF; 2,239 of them were IVF singletons. Eighty four singletons were recruited from the group of mothers who were treated with COHS (COHS-only). From the 7,521 subfertile controls, 6,343 were singletons with a full data set.

For the first comparison in this study between the IVF group, the subfertile group and the group with only ovarian hyperstimulation as treatment, pregnancies which resulted from transfer of frozen embryos were excluded. The remaining "ART deliveries" were divided into two groups. The first group consisted of singleton pregnancies which developed after ovarian hyperstimulation with IVF (IVF+COHS; n= 2,239). The second group was the control group and consisted of singleton pregnancies among women with a history of subfertility who conceived spontaneously (n=6,343). The

third group consisted of singleton pregnancies which developed after ovarian hyperstimulation without IVF (COHS-only, n=84).

From the OMEGA data base, 139 pregnancies were the result of cryo-preserved ET. In 66 cases the ET took place after COHS and/or ovulation induction with hCG (Stim+Cryo group). In 56 cases, only human choriongonadotrophin (hCG) was administered and 8 received human menopausal gonadotrophin (hMG) or clomiphene citrate alone or in combination with hCG. In 2 cases the specific type of COHS and/or ovulation induction was not known. In 73 cases (Stim–Cryo group), the ET was performed in an apparently ovulatory cycle or before progesterone administration.

In each participating clinic, research assistants specifically trained for data collection for the OMEGA study abstracted detailed information from the medical records. For each reported child, the questionnaire, completed by the study participants, provided detailed information on the maternal characteristics, method of conception, the duration of gestation in weeks, data of birth, gender and birth weight.

## Definitions

Although the National Institute for Clinical Excellence[34] defines subfertility as failure to conceive after regular unprotected sexual intercourse for 2 years in the absence of known reproductive pathology, the definition currently used in the Netherlands during the period of the cohort followed the one given in the textbook Clinical gynaecologic endocrinology and infertility[10]: 'one year unprotected coitus without conception'.

Gestational age (duration of pregnancy) at birth in case of IVF pregnancies was determined by adding 14 days to the interval between LH administration and delivery. For the control pregnancies, it was calculated as the interval between the first day of the last menstrual period and delivery. International definitions were followed for preterm (<37 weeks), very preterm (< 32 weeks), low birth weight (<2500 g) and very low birth weight (<1500 g).

## Statistics

Differences between groups were assessed by *t*-tests for continuous variables and by $\chi$-square tests for ordinal variables. Multivariate logistic regression analysis was used to determine the odds ratios of (very) low birth weight and (very) preterm birth between the groups. Odds ratios were first adjusted for the confounders, maternal age and primiparity, and thereafter for each of the following potential confounders: BMI, race, education level, smoking, diabetes mellitus and sex of infant. Significance level was set at 5% two-tailed. Analyses were performed with SPSS 12.0.

# Results

Complete perinatal data were obtained from 2,239 singleton IVF+COHS pregnancies, from 6,343 pregnancies in subfertile controls and from 84 COHS-only pregnancies.

## **Maternal characteristics** (Table I)

In the IVF+COHS group the mean maternal age was significantly higher as compared to COHS-only and subfertile controls. The proportion of women with pre-existing diabetes mellitus (for which women used medication during pregnancy) was found significantly higher in the IVF+COHS group as compared to the subfertile control group (Table Ia). The mean BMI was significantly lower and the education level was significantly higher in the IVF+COHS group as compared to the control group. In the COHS-only group, primiparity was significantly more prevalent as compared to the IVF+COHS group and the subfertile controls, whereas there were significantly less women who smoked as compared to the IVF+COHS and subfertile control group. To rule out the possibility that the women with multiple births influenced our results, we also made a comparison (Table 1b) in which we only looked at the first pregnancies of women. No significant changes in the maternal characteristics were seen.

**Table Ia. Maternal characteristics at the onset of and during pregnancy in IVF+COHS, subfertile control and COHS-only pregnancies. Values are means (±SD) or percentages.**

| | IVF+COHS (n=2,239) | Subfertile controls (n=6,343) | COHS-only (n=84) |
|---|---|---|---|
| Age (years) | 34.2 ( 3.7)*) | 30.7 ( 6.0) | 31.9 ( 4.3) |
| Height (cm) | 168.8 ( 6.5)*) | 168.4 ( 6.5) | 168.5 ( 5.4) |
| Weight (kg) | 67.6 (11.2)*) | 68.3 (12.0) | 68.4 (12.7) |
| BMI[1] | 23.7 (3.7)*) | 24.1 ( 4.1) | 24.1 ( 4.2) |
| Caucasian (%) | 97.9 | 97.8 | 98.8 |
| Low education level[2] (%) | 46.6*) | 50.2 | 43.4 |
| Primiparous (%) | 55.1*) | 44.1 | 66.7 |
| Smoking[3] (%) | 64.1 | 65.7 | 51.8 |
| Pre-existent DM[4](%) | 1.1*) | 0.5 | 1.2 |

[1] body mass index (weight (kg) divided by height (m) squared)
[2] only primary school
[3] during pregnancy
[4] diabetes mellitus for which medication was needed also during pregnancy
*) $p<0.05$, IVF+COHS group versus control group
COHS: controlled ovarian hyperstimulation

**Table Ib. Maternal characteristics at the onset of and during first pregnancy of subfertile controls and of women receiving IVF+COHS or COHS-only. Values are means (±SD) or percentages.**

| | IVF+COHS (n=1,576) | Subfertile controls (n=3,754) | COHS-only (n=68) |
|---|---|---|---|
| Age (years) | 33.9 ( 3.7) | 29.6 ( 5.9) | 31.2 ( 4.1) |
| Height (cm) | 169 ( 7) | 169 ( 7) | 168 ( 6) |
| Weight (kg) | 67.8 (11.2) | 68.4 (12.1) | 68.6 (13.7) |
| BMI[1] | 23.8 ( 3.8) | 24.0 ( 4.1) | 24.2 ( 4.5) |
| Caucasian (%) | 97.3 | 97.7 | 98.5 |
| Low education level[2] (%) | 46.8 | 50.6 | 44.2 |
| Primiparous (%) | 70.4 | 68.5 | 77.9 |
| Smoking[3] (%) | 68.3 | 57.1 | 80.9 |
| Pre-existent DM[4](%) | 1.0 | 0.5 | 1.5 |

[1] body mass index (weight (kg) divided by height (m) squared)
[2] only primary school
[3] during pregnancy
[4] diabetes mellitus for which medication was needed also during pregnancy
COHS: controlled ovarian hyperstimulation

**Table II. Birth weight and gestational age of singletons conceived after IVF compared with naturally conceived singletons of subfertile women**

| | | IVF+COHS (n=2,239) | Subfertile controls (n=6,343) | | |
|---|---|---|---|---|---|
| | | | | OR (95%CI) | $OR_{adj}$ (95% CI)[a] |
| Birth weight (g) | | | | | |
| mean | g (SD) | 3,199 (664)* | 3,351 (600) | | |
| > 2500 | n (%) | 1,955 (87.3) | 5,848 (92.2) | 1 | 1 |
| 1500 – 2500 | n (%) | 223 (10.0) | 429 (6.8) | 1.6 (1.3 – 1.8) | 1.7 (1.4 – 2.0) |
| < 1500 | n (%) | 61 (2.7) | 66 (1.0) | 2.8 (1.9 – 3.9) | 2.7 (1.8 – 4.0) |
| Gestational age (wks) | | | | | |
| mean | wks (SD) | 38.9 (2.5)* | 39.4 (2.2) | | |
| ≥ 37 | n (%) | 1,972 (88.0) | 5,842 (92.1) | 1 | 1 |
| 32 – 37 | n (%) | 218 ( 9,8) | 428 (6,7) | 1.5 (1.3 – 1.8) | 1.6 (1.3 – 1.9) |
| < 32 | n (%) | 49 ( 2.2) | 73 (1.2) | 2.0 (1.4 – 2.9) | 2.2 (1.5 – 3.3) |

* $p<0.05$
[a] Adjusted for maternal age and primiparity
COHS: controlled ovarian hyperstimulation

## Perinatal outcome

The mean birth weight and the mean gestational age of the singletons born to the IVF+COHS group were significantly lower and shorter, respectively, as compared to children born to the subfertile control group (Table II). The ORs for of very preterm birth and very low birth weight in the IVF+COHS group in comparison with the subfertile controls were 2.0 and 2.8 respectively, while the ORs of the preterm birth and low birth weight groups were increased to a lesser extent: 1.5 and 1.6 respectively. Only minor changes in the aforementioned ORs were seen after adjustment for potential confounders (maternal age and primiparity and also BMI, race, education, smoking, diabetes mellitus and sex of infant) that may influence birth weight and/or gestational age. When we excluded the multiple births of women and only looked at their first pregnancies, no material changes in the ORs were seen (data not shown). The OR for very low birth weight in the COHS-only group in comparison with the subfertile controls was 3.5 (95%CI 1.1-11.4); however after adjustment for maternal age and primiparity, the association became slightly weaker (Table IIIa).

ORs for preterm birth and low birth weight in the IVF+COHS group compared to the COHS-only group were not significantly different (Table IIIb).

## Cryopreservation

Complete perinatal data were obtained from 66 singleton pregnancies derived from cryopreserved ET after COHS (Stim+Cryo group) and from 73 singleton pregnancies in which the ET was performed in a natural cycle (Stim-Cryo group). When we compared maternal characteristics of the Cryo+ and Cryo- group, no significant differences were found: mean age 34.1 yr vs 33.8 yr, mean height 168.3 cm vs 167.5 cm, mean weight 67.7 kg vs 65.2 kg, mean BMI 24.0 vs 23.3, mean % Caucasian 97.0 vs 98.6, mean % women who enjoyed only primary school 43.9 vs 47.9, mean % primiparous women 36.4 vs 41.1, mean % of women who smoked during pregnancy 37.9 vs 30.1 and in both groups nobody indicated to suffer from pre-existent diabetes mellitus.

The group Stim+Cryo treated women did not have a significantly higher risk of singleton birth with a low birth weight and/or of preterm delivery as compared to when ET had taken place in a natural or progesterone treated cycle (Table IIIc). Correction for the already mentioned confounders did not materially change the results. In the Stim+Cryo group significantly less boys were born (48.5% versus 65.8%, $p=0.03$).

**Table IIIa. Birth weight and gestational age of singletons conceived after controlled ovarian hyperstimulation only compared with naturally conceived singletons of subfertile women**

| | | COHS-only (n=84) | Subfertile controls (n=6,343) | | |
|---|---|---|---|---|---|
| | | | | OR (95%CI) | $OR_{adj}$ (95% CI)[a] |
| Birth weight (g) | | | | | |
| mean | g (SD) | 3,226 (597) | 3,351 (600) | | |
| > 2500 | n (%) | 76 (90.5) | 5,848 (92.2) | 1 | 1 |
| 1500 – 2500 | n (%) | 5 ( 6.0) | 429 ( 6.8) | 0.9 (0.4 – 2.2) | 0.9 (0.4 – 2.2) |
| < 1500 | n (%) | 3 ( 3.5) | 66 ( 1.0) | 3.5 (1.1 – 11.4) | 3.1 (0.9 – 10.2) |
| Gestational age (wks) | | | | | |
| mean | wks (SD) | 39.6 (2.3) | 39.4 (2.2) | | |
| ≥ 37 | n (%) | 79 (94.0) | 5,842 (92.1) | 1 | 1 |
| 32 – 37 | n (%) | 3 ( 3.6) | 428 ( 6.7) | 0.5 (0.2 – 1.7) | 0.5 (0.2 – 1.7) |
| < 32 | n (%) | 2 ( 2.4) | 73 ( 1.2) | 2.0 (0.5 – 8.4) | 1.9 (0.5 – 8.0) |

[a] Adjusted for maternal age and primiparity
COHS: controlled ovarian hyperstimulation

**Table IIIb. Birth weight and gestational age of singletons conceived after controlled ovarian hyperstimulation and IVF compared with singletons conceived after controlled ovarian hyperstimulation alone**

| | | IVF+COHS (n=2,239) | COHS-only (n=84) | | |
|---|---|---|---|---|---|
| | | | | OR (95%CI) | $OR_{adj}$ (95% CI)[a] |
| Birth weight (g) | | | | | |
| mean | g (SD) | 3,199 (664) | 3,226 (597) | | |
| > 2500 | n (%) | 1,955 (87.3) | 76 (90.5) | 1 | 1 |
| 1500 – 2500 | n (%) | 223 (10.0) | 5 ( 6.0) | 1.7 (0.7 – 4.3) | 1.7 (0.7 – 4.4) |
| < 1500 | n (%) | 61 ( 2.7) | 3 ( 3.5) | 0.8 (0.2 – 2.6) | 0.8 (0.3 – 2.7) |
| Gestational age (wks) | | | | | |
| mean | wks (SD) | 38.9 (2.5) | 39.6 (2.3) | | |
| ≥ 37 | n (%) | 1,972 (88.0) | 79 (94.0) | 1 | 1 |
| 32 – 37 | n (%) | 218 ( 9.8) | 3 ( 3.6) | 2.9 (0.9 – 9.3) | 2.7 (0.9 – 8.7) |
| < 32 | n (%) | 49 ( 2.2) | 2 ( 2.4) | 1.0 (0.2 – 4.1) | 0.9 (0.2 – 3.8) |

[a] Adjusted for maternal age and primiparity
COHS: controlled ovarian hyperstimulation

**Table IIIc. Birth weight and gestational age of singleton pregnancies after ET of thawed embryo's in a treated cycle (Stim+Cryo) versus an untreated cycle (Stim-Cryo)**

| | | Stim+Cryo (n=66) | Stim-Cryo (n=73) | | |
|---|---|---|---|---|---|
| | | | | OR (95%CI) | $OR_{adj}$ (95% CI)[a] |
| Birth weight (g) | | | | | |
| mean | g (SD) | 3,396 (621) | 3,319 (641) | | |
| > 2500 | n (%) | 62 (93.9) | 67 (91.8) | 1 | 1 |
| 1500 – 2500 | n (%) | 3 ( 4.6) | 5 ( 6.8) | 0.7 (0.2 – 2.9) | 0.7 (0.2 –2.9) |
| < 1500 | n (%) | 1 ( 1.5) | 1 ( 1.4) | 1.1 (0.1 – 16.7) | 1.0 (0.1 – 16.7) |
| Gestational age (wks) | | | | | |
| mean | wks (SD) | 39.2 (2.3) | 39.2 (2.1) | | |
| ≥ 37 | n (%) | 59 (89.4) | 68 (93.1) | 1 | 1 |
| 32 – 37 | n (%) | 6 ( 9.1) | 4 ( 5.5) | 1.7 (0.5 – 6.3) | 1.7 (0.5 – 6.3) |
| < 32 | n (%) | 1 ( 1.5) | 1 ( 1.4) | 1.2 (0.1 – 20.0) | 1.1 (0.1 – 16.7) |
| Sex of infant | | | | | |
| male | (%) | 48.5*) | 65.8 | | |

* $p<0.05$
[a] Adjusted for maternal age and primiparity
COHS: controlled ovarian hyperstimulation

# Discussion

In this large database of Dutch IVF clinics, singleton IVF pregnancies have significantly worse perinatal outcomes than spontaneously conceived pregnancies in subfertile women. The risk is more pronounced for very preterm and very low birth weight than for preterm and low birth weight. The estimates did not materially change after adjustment for maternal age, primiparity, or other potential confounders.

Randomization is the proper way to evaluate the effect of treatment for subfertility on the perinatal outcome[12]; however, it is difficult and unethical[2] to conduct. Alternative methodological approaches have been followed with different outcomes. In a population-based case-control study, Draper *et al.*[3] showed that history of subfertility, irrespective of treatment, increased the risk of perinatal death, while Basso and Olsen[13] found that the odds of neonatal (and not intra-uterine) death among firstborn singletons was significantly increased in the group of non-treated mothers with >12 months of subfecundity, relative to mothers who became pregnant within 3 months. McElrath *et al.*[14], using logistic regression models, found the risk of very low birth weight among

subfertile, non-treated women to be 1.4 (95% CI 1.1-1.9) and among subfertile, treated women 2.6 (95% CI 2.1-3.2) compared to a national US control group gathered in 1988. Both estimates were slightly lower, but still significantly increased when they were adjusted for effects of multiple gestation, maternal age and a history of miscarriage.

Another approach is to compare different treatments among subfertile couples, with the difficulty of the difference in treatment. Olivennes *et al.*[4] found no difference in the prevalence of (very) preterm and (very) low birth weight among 162 IVF and 263 COHS singletons, Bonduelle *et al.*[15] showed the same results among 1499 ICSI and 1556 IVF singletons. However, the analysis of Ombelet *et al*.[16] showed only a significantly higher risk of preterm birth among 3974 IVF singletons relative to 1655 ICSI singletons. The authors hypothesize that the indication for ICSI is predominantly a male factor. Remarkable is that the two latter studies have been conducted in the relatively circumscriptive, Dutch speaking part of Belgium. The Ombelet study[16] gathered all deliveries in Flanders in the period 1997-2003, whereas the Bonduelle study[15] collected the data of one reproductive centre in the period 1991-1999 for ICSI and 1983-1999 for IVF. Part of these data has been included in the Ombelet study[16]. An explanation for the difference has not been offered by Ombelet *et al.*[16] In the Wang study[17] preterm birth was significantly more often observed in the high technology group (IVF, ICSI, GIFT) than in the low technology group (IUI, donor insemination), with ORs of 2.39 and 1.50, respectively, compared to naturally conceived controls. Two similar studies comparing IVF with IUI singletons[18,19] found no differences in the prevalence of preterm birth and low birth weight.

Our study might suffer from some information bias through the use of a mailed questionnaire to collect perinatal outcome and might be limited by not taking into account pregnancy complications, fetal malformations and a history of previous pregnancy loss, factors that may be associated with adverse pregnancy outcome. However it is unlikely that the IVF+COHS group would report systematically different from the subfertile controls. Therefore we conclude, also based on the data from the above mentioned literature that subfertility might explain part of the association between assisted conception and poor perinatal outcome of singletons, but that still there remains an important effect of assisted reproduction it self.

Is the controlled ovarian hyperstimulation, as part of the assisted technology methods, the culprit or the technique itself as suggested by Olivennes *et al.*[4]? In our study the risk estimates comparing singletons conceived after COHS & IVF versus singletons conceived after COHS only did not differ significantly. Preterm birth and low weight birth were more likely to occur among singletons conceived by transfer of fresh embryos, relative to those conceived by with transfer of frozen embryos, as reported by Wang *et al.*[9] in a retrospective cohort study of Australian data of infants conceived through assisted reproduction. We are not informed in their study whether the transfer of thawed

embryo(s) was performed in a natural or stimulated cycle. Unfortunately, in our study we were not able to test the hypothesis that COHS prior to embryo transfer affects uterine receptivity due to the small number of patients in this database. As female-factor subfertility increased the likelihood of preterm birth and low birth weight significantly more than male-factor subfertility, Wang *et al*.[9] suggested that uterine receptivity might offer us an biological plausibility for the phenomenon, however no difference in prevalence of preterm birth and low birth weight among singletons were seen born after ICSI, representing the male subfertility factor[15,16].

If ovarian stimulation by itself has a negative effect on the pregnancy outcome it may influence oocyte/embryo quality, resulting in impaired implantation and embryonic/fetal development[20]. Other studies suggest that ovarian stimulation is rather associated with an unbalanced endometrium and/or oviductal environment[21,22,23,24]. Supra-physiologic concentrations of estradiol and progesterone during ovarian stimulation may modulate growth factors, cell adhesion molecule profiles, steroid receptors and expression of pinopodes in the endometrium[25,26,28], influencing endometrial receptivity[25,27,28].

Sibug *et al.*[29,30] suggested that the effect of ovarian stimulation on pregnancy outcome might be explained by the modulation of vascular endothelial growth factor (VEGF) affecting angiogenesis during implantation and placentation[31,32,33].

In conclusion, our study shows that the association between assisted conception and poor perinatal outcome can not be explained by subfertility.

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# References


1 Helmerhorst FM, Perquin DA, Donker D and Keirse MJ (2004) Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ 328,261-265.
2 Jackson RA, Gibson KA, Wu YW and Croughan MS (2004) Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol 103,551-563.
3 Draper ES, Kurinczuk JJ, Abrams KR and Clarke M (1999) Assessment of separate contributions to perinatal mortality of infertility history and treatment: a case-control analysis. Lancet 353,1746-1749.
4 Olivennes F, Rufat P, Andre B, Pourade A, Quiros MC and Frydman R (1993) The increased risk of complication observed in singleton pregnancies resulting from in-vitro fertilization (IVF) does not seem to be related to the IVF method itself. Hum Reprod 8,1297-1300.
5 Källén B, Olausson PO and Nygren KG (2002) Neonatal outcome in pregnancies from ovarian stimulation. Obstet Gynecol 100,414-419.
6 Gaudoin M, Dobbie R, Finlayson A, Chalmers J, Cameron IT and Fleming R (2003) Ovulation induction/intra-uterine insemination in infertile couples is associated with low-birth-weight infants. Am J Obstet Gynecol 188,611-616.
7 Klip H, Burger CW, de Kraker J and van Leeuwen FE (2001) Risk of cancer in the offspring of women who underwent ovarian stimulation for IVF. Hum Reprod 16,2451-2458.
8 Wennerholm UB, Bergh C, Hamberger L, Westlander G, Wikland M and Wood M (2000) Obstetric outcome of pregnancies following ICSI, classified according to sperm origin and quality. Hum Reprod 15,1189-1194.
9 Wang YP, Sullivan EA, Black D, Eng JDM, Bryant J and Chapman M (2005) Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. Fertil Steril 83,1650-1658.
10 Speroff L, Glass RH, Kase NG. 6 ed.1999 Lippincott Williams & Wilkins, Baltimore, USA, page 1013.
11 De Boer EJ, den Tonkelaar I, Burger CW and van Leeuwen FE; OMEGA Project Group (2005) Validity of self-reported causes of subfertility. Am J Epidemiol 15,978-986.
12 Buck Louis GM, Schisterman EF, Dukic VM and Schieve LA (2005) Research hurdles complicating the analysis of infertility treatment and child health. Hum Reprod 20,12-18.
13 Basso O and Olsen J (2005) Subfecundity and neonatal mortality: longitudinal study within the Danish national birth cohort. BMJ 330,393-394.
14 McElrath T and Wise PH (1997) Fertility therapy and the risk of very low birth weight. Am J Obstet Gynecol 90,600-605.
15 Bonduelle M, Liebaers I, Deketelare V, Derde MP, camus M, Devroey P, van Steirteghem (2002) Neonatal data on a cohort of 2889 infants born after ICSI (1991-1999) and of 2995 infants born after IVF (1983-1999). Hum Reprod 17,671-694.
16 Ombelet W, Cadron I, Gerris J, de Sutter P, Bosmans E, Martens G, Ruyssinck G, Defoort P, Molenberghs G and Gyselaers W. (2005) obstetric and perinatal outcome of 1655 ICSI and 3974 INF singleton and 1102 ICSI and 2901 IVF twin births: a comparative analysis. Reprod Biomed Online 11,76-85.
17 Wang JX, Norman RJ and Kristiansson P (2002) The effect of various infertility treatments on the risk of preterm birth. Hum Reprod 17,945-949.
18 De Sutter P, Veldeman L, Kok P, Szymczak N, van der Elst J, Dhont M. (2005) Comparison of outcome of pregnancy after intra-uterine insemination (IUI) and IVF. Hum Reprod 20,1642-1646.
19 Nuojua-Huttunen S, Gissler M, Martikainen H and Tuomivaara L (1999) obstetric and perinatal outcome of pregnancies after intrauterine insemination. Hum Reprod 14,2110-2115.
20 Rao CV (2001) Tropic effects of LH and hCG on early pregnancy events in women's reproductive tract. Early Pregnancy 5,18-19.
21 Ertzeid G, Storeng R and Lyberg T (1993) Treatment with gonadotropins impaired implantation and fetal development in mice. J Assist Reprod Genet 10,286-291.
22 Fossum GT, Davidson A and Paulson RJ (1989) Ovarian hyperstimulation inhibits embryo implantation in the mouse. J In Vitro Fert Embryo Transf 6,7-10.
23 Walton EA, Huntley S, Kennedy TG and Armstrong DT (1982) Possible causes of implantation failure in superovulated immature rats. Biol Reprod 27,847-852.
24 Walton EA and Armstrong DT (1983) Oocyte normality after superovulation in immature rats. J Reprod Fertil 67,309-314.
25 Kolb BA, Najmabadi S and Paulson RJ (1997) Ultrastructural characteristics of the luteal phase endometrium in patients undergoing controlled ovarian hyperstimulation. Fertil Steril 67,625-630.
26 Macklon NS and Fauser BC (2000) Impact of ovarian hyperstimulation on the luteal phase. J Reprod Fertil Suppl 55,101-108.

27 Paulson RJ, Sauer MV and Lobo RA (1990) Embryo implantation after human in vitro fertilization: importance of endometrial receptivity. Fertil Steril 53,870-874.
28 Paulson RJ, Sauer MV and Lobo RA (1997) Potential enhancement of endometrial receptivity in cycles using controlled ovarian hyperstimulation with antiprogestins: a hypothesis. Fertil Steril 67,321-325.
29 Sibug RM, Helmerhorst FM, Tijssen AM, de Kloet ER and de Koning J (2002) Gonadotrophin stimulation reduces VEGF(120) expression in the mouse uterus during the peri-implantation period. Hum Reprod 17,1643-1648.
30 Sibug RM, de Koning J, Tijssen AM, de Ruiter MC, de Kloet ER and Helmerhorst FM (2005) Urinary gonadotrophins but not recombinant gonadotrophins reduce expression of VEGF120 and its receptors flt-1 and flk-1 in the mouse uterus during the peri-implantation period. Hum Reprod 20,649-656.
31 Kapiteijn K, Koolwijk P, Van Der Weiden RM, Helmerhorst FM, Kooistra T and van Hinsbergh VW (2001) Steroids and cytokines in endometrial angiogenesis. Anticancer Res 21,4231-4242.
32 Koolwijk P, Kapiteijn K, Molenaar B, van Spronsen E, van der Vecht B, Helmerhorst FM and van Hinsbergh VW (2001) Enhanced angiogenic capacity and urokinase-type plasminogen activator expression by endothelial cells isolated from human endometrium. J Clin Endocrinol Metab 86,3359-3367.
33 Smith SK (1998) Angiogenesis, vascular endothelial growth factor and the endometrium. Hum Reprod Update 4,509-519.
34 National Institute for Clinical Excellence, NHS (2004) Fertility: assessment and treatment for people with fertility problems. London, RCOG Press. http://www.rcog.org.uk/index.asp?PageID=696