University of Groningen

Female reproductive ageing

Haadsma, Maaike Laura

Publication date:
2010

Chapter 4

The predictive value of ovarian reserve tests for miscarriage in a population of subfertile ovulatory women

M.L. Haadsma, H. Groen, V. Fidler, L.H.M. Seinen, F.J.M. Broekmans, M.J. Heineman, A. Hoek

ABSTRACT

Background: The increase in miscarriage rate with female age is attributed to a decline in oocyte quality. This age-related decrease of oocyte quality is accompanied by a decrease in oocyte quantity. Assessment of the number of oocytes by ovarian reserve tests (ORTs) may therefore also represent their quality. The objective of our study was to assess the predictive value of ORTs for miscarriage in subfertile women.

Methods: Subanalysis within a prospective cohort study of 474 subfertile ovulatory couples in two hospitals in Groningen, the Netherlands. The ORTs performed were: antral follicle count, basal and stimulated levels of follicle stimulating hormone (FSH) and inhibin B and the clomiphene citrate challenge test (CCCT). Couples that achieved an ongoing pregnancy (N=233) were compared with couples experiencing miscarriage (N=72) on results of the ORTs and patient characteristics.

Results: In univariate analysis, the outcome of the ORTs did not differ between both groups. Logistic regression analysis including patient characteristics such as female age did not reveal an association between any of the ORTs and miscarriage either.

Conclusion: Neither antral follicle count, basal and stimulated levels of FSH and inhibin B nor the CCCT have a statistically significant predictive value for miscarriage in subfertile ovulatory women.

INTRODUCTION

The chance for a woman to have a live birth declines as her age increases. This is partly due to a diminished chance to conceive, but mainly caused by an increased chance of (very early) pregnancy loss[41]. This process of female reproductive ageing is attributed to a decrease in both oocyte quantity, eventually resulting in menopause, and oocyte quality[8]. The main acknowledged manifestation of decreased quality is the occurrence of chromosomal abnormalities in the oocyte, leading to aneuploidy in the conceptus, which has been established as the reason of pregnancy loss in 35-75% of all cases[7;120;219].

The quality of a woman's oocytes cannot be assessed clinically, but the quantity of the remaining follicle pool can be estimated by so-called ovarian reserve tests (ORTs). Various studies have suggested that the quantitative ovarian reserve is predictive for the chance to miscarry. An elevated level of basal follicle stimulating hormone (FSH), low level of anti-Müllerian hormone (AMH) and low antral follicle count (AFC) have been described to be related to increased miscarriage rates[147-149]. Also, a high incidence of decreased ovarian reserve has been found among women with unexplained recurrent pregnancy loss[220;221]. In line with these findings are publications suggesting a relation between decreased ovarian reserve and chromosomal abnormalities in the conceptus[155;157;161;164].

The studies describing an association between quantitative ovarian reserve and miscarriage or chromosomal abnormalities are challenged by a number of comparable studies that do not demonstrate such a relation[146;150;151;159;162;163;222]. Furthermore, most of the studies on this topic are of retrospective design or include small numbers of subjects. We decided to address the issue in a large prospective cohort study of subfertile ovulatory women to answer the question whether ovarian reserve tests have independent predictive value for the chance to miscarry.

MATERIALS AND METHODS

The present study is part of a prospective cohort study, originally designed to assess the predictive value of ovarian reserve tests for spontaneous pregnancy in an ovulatory subfertile population[223].

Study population

From December 1999 to July 2003 patients were recruited at the tertiary fertility centre of the University Medical Center Groningen and the fertility centre of the Martini Hospital, a teaching hospital, in Groningen, the Netherlands. Patients were asked to participate after routine subfertility evaluation had been completed. This evaluation included assessment of ovulation by sonographic cycle analysis and measurement of midluteal progesterone, semen analysis, post-coital test and hysterosalpingography. The inclusion criteria for study participation were 1) subfertility for at least 12 months, 2) a regular ovulatory cycle with midluteal progesterone of > 30 nmol/l and a mean cycle

length between 21 and 42 days, 3) at least one patent tube at hysterosalpingography, 4) semen analysis with a total motile count $> 1 \times 10^6$ and 5) no sexual disorder leading to a coitus frequency of less than once a month. After basal subfertility evaluation a diagnostic laparoscopy was performed if tubal pathology was suspected clinically (e.g. a history of pelvic inflammatory disease), after an abnormal result of hysterosalpingography and before starting treatment with intra-uterine insemination. Laparoscopy was performed after patients were asked to participate; if two-sided tubal pathology was detected, patients were excluded secondarily. Informed consent was obtained from all participants. The study protocol was reviewed and approved by the Institutional Research Boards of both participating hospitals.

Study protocol
After inclusion patients visited the outpatient clinic in the early follicular phase of the menstrual cycle (cycle day 2, 3 or 4). Transvaginal ultrasound was performed by one of four skilled gynaecologists using a 7.5 MHz vaginal probe on an Aloka SSD-1700 US machine. Follicles were counted and measured in two dimensions. The mean of these measurements was taken to be the follicle size. The numbers of follicles sized 2-6 mm from both ovaries were added for the total antral follicle count (AFC)[215]. Peripheral blood was drawn to measure basal levels of FSH and inhibin B. Subsequently, patients self-administered 100 mg clomiphene citrate on cycle day 5 to 9. One week after the first visit blood samples were drawn again to measure stimulated FSH and inhibin B levels. The result of the clomiphene citrate challenge test (CCCT) was defined as the sum of the values for basal and stimulated FSH.

Hormone assays
For measurements of concentrations of FSH and inhibin B serum was stored at -20°C until processing. Serum FSH levels were measured by fluorimmunometric determination on the AutoDelfia (Wallac/ Perkin Elmer, Turku, Finland). For FSH the inter-assay coefficient of variation was 3.7%, the sensitivity <0.05 IU/l. The lower limit of detection was 0.03 IU/l. The standard of the FSH assay was calibrated against the WHO Second International Reference Preparation for human FSH (78/549). Inhibin B concentrations were assayed with Enzyme Immunoassay (ELISA) from Serotec (Kidlington, Oxford, UK). The inter-assay coefficient of variation for inhibin B was 11% and the sensitivity was <10 pg/ml. The lower limit of detection was 5 pg/ml.

Follow up
After completion of the basal subfertility evaluation, expectant management or treatment was proposed to each couple. The advice was based on duration of subfertility and the estimated chances for spontaneous conception according to the prediction model routinely used in both clinics at the time of study[210]. Couples were advised to start treatment if the estimated chance to conceive was below 30% or duration of subfertility exceeded three years (two years in case of female

age ≥ 37 years). Couples that were primarily advised expectant management and did not conceive spontaneously were also offered treatment as soon as they met these criteria. The kind of treatment offered to the couples with a low chance of spontaneous conception depended on the type of subfertility. Patients were offered treatment by intra-uterine insemination (IUI) with stimulation in case of unexplained subfertility and mild male factor and IUI without stimulation in case of cervical factor up to a maximum of six cycles. If IUI-treatment failed, couples were offered in vitro fertilisation (IVF) treatment, generally up to a maximum of three cycles because of the reimbursement policy of the medical insurance companies. If semen quality was insufficient for IUI, couples were advised treatment with IVF or intracytoplasmic sperm injection (ICSI). Treatment was not generally available for women over the age of 40.

Follow-up started on the day of the first ORT. Data on pregnancies and treatment were recorded. Information was obtained from medical files and from questionnaires completed by the participating couples. Couples were followed until a pregnancy leading to a live-birth was achieved, either after spontaneous conception or after treatment. For the non-pregnant couples follow-up ended after fertility treatment was fully completed. Couples that never started treatment or withdrew from treatment received a questionnaire to complete data. Follow-up also ended when couples started contraceptives or ended their relationship. Last date of follow-up was November 1ˢᵗ 2006. All couples that achieved a pregnancy during the follow-up period were identified. For each couple only the first pregnancy during follow-up was taken into account. Ongoing pregnancies and miscarriages were selected for analysis. An ongoing pregnancy was defined as a viable intra-uterine pregnancy of at least 16 weeks gestation. A miscarriage was defined as a pregnancy loss between 4 and 16 weeks amenorrhoea, with the exception of confirmed extra-uterine pregnancies or artificially terminated pregnancies. Time to pregnancy was defined as the period between the first ORT and the first day of the last menstrual cycle. Assisted conception was defined as conception after treatment with IVF or ICSI as opposed to spontaneous conception or conception after intra-uterine insemination. Female age and duration of subfertility were scored at the first day of the last menstrual cycle. For the other characteristics values at the time of the first ORT were used when applicable.

Statistical analysis

To analyse the relation between ORTs and pregnancy outcome, we performed univariate and multivariate analyses. The univariate analyses compared the miscarriage group to the ongoing pregnancy group with respect to ORTs and patient characteristics, including subfertility features. For this we used the Chi-square test, Mann-Whitney U test and Student's t-test when applicable. The multivariate analysis included logistic regression with miscarriage status as the outcome and the ORTs and patient characteristics as predictors. To explore the nature of the relation with miscarriage of each of the continuous predictors we used basic spline functions[224]. In this way the possibility of a linear and non-linear relationship of each of the variables and miscarriage was assessed, including

4

the presence of cut-off points above or below which miscarriage rates changed.The effect of the variables was described as odds ratios (OR) with 95% confidence intervals (CI). A *P*-value less than or equal to 0.05 was considered statistically significant. Data were analysed with SPSS 14.0 (SPSS Inc., Chicago, IL, USA) and S-plus 7.0 (MathSoft Inc., Seattle, WA, USA).

RESULTS

From the 474 couples included in the prospective cohort study, 320 (67.5%) conceived during follow-up (Figure 1). Of these, 305 pregnant couples were selected for analysis, of which 233 had an ongoing pregnancy and 72 experienced miscarriage. Figure 1 also presents the reasons for exclusion. The grounds for artificial termination were once congenital abnormalities of a foetus with normal karyotype and once personal reasons. The group of 15 excluded couples did not differ from the selected 305 couples with respect to patient characteristics and results of the ORTs (data
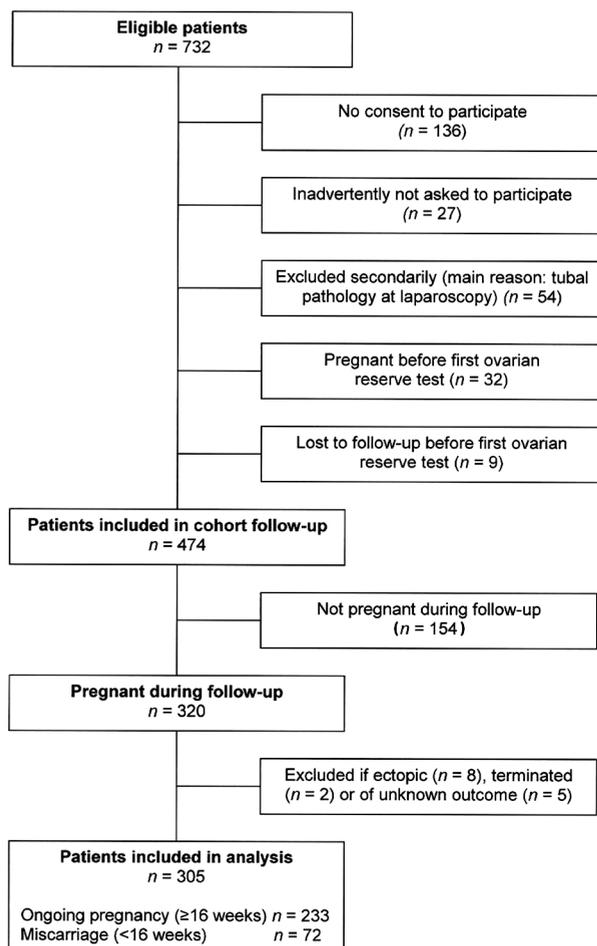

Figure 1. Flowchart of eligible patients

not shown). Of the 305 selected couples 132 (43%) conceived spontaneously, 94 (31%) after IUI, 36 (12%) after IVF and 43 (14%) after ICSI. Of the 132 couples that conceived spontaneously, 37 (28%) had already started therapy and conceived in between treatment cycles or after discontinuing treatment. Of the 154 non-pregnant couples, 19 were lost to follow-up, 14 ended their relationship and 13 started contraceptives during the study period. Median follow-up for the 154 non-pregnant couples was 24.8 months (10th-90th percentiles 2.7 – 46.9 months).

Table I presents a univariate comparison of patient characteristics and results of the ORTs of the groups with ongoing pregnancy and miscarriage. The miscarriage group was older and had more

Table I. Patient characteristics and results of ovarian reserve tests according to pregnancy outcome

	Ongoing pregnancy $N = 233$		Miscarriage $N = 72$		
	Median (*no)	10th-90th percentile (* %)	Median (*no)	10th-90th percentile (* %)	P-value
Patient characteristics					
Age (years)	32.4	27.0 – 38.1	34.2	28.0 – 39.6	<0.01
Body mass index (kg/m²)	22.8	19.4 – 30.1	22.9	19.5 – 33.2	0.82
Smoking habit*	61	28.4%	16	27.1%	0.85
Duration of subfertility (years)	3.3	1.9 – 5.8	3.4	1.9 – 5.4	0.51
Primary subfertility*	162	69.5%	52	72.2%	0.79
Previous miscarriage*	35	15.1%	14	19.4%	0.35
Mean cycle length (days)	28	26 – 33	28	25 – 32	0.52
Semen analysis (TMC, x10⁶)	38.6	4.1 – 179.8	33.6	4.1 – 205.0	0.85
Diagnostic category of subfertility*					0.76
Unexplained	125	53.6%	35	48.6%	
Male factor	99	42.2%	34	47.2%	
Cervical factor	9	3.9%	3	4.2%	
Time to pregnancy (months)	8.8	1.2 – 28.4	10.6	0.8 – 34.4	0.22
Conception after ART*	52	22.3%	27	37.5%	0.01
Results of ovarian reserve tests					
Antral follicle count (n)	11	5 – 23	11	5 – 22	0.86
Basal FSH (IU/l) (bFSH)	6.3	4.5 – 9.6	6.3	4.6 – 10.1	0.71
Stimulated FSH (IU/l) (sFSH)	6.2	3.9 – 10.7	6.5	4.2 – 9.7	0.56
CCCT (bFSH+sFSH) (IU/l)	12.9	9.0 – 19.6	12.8	9.1 – 19.6	0.71
Basal inhibin B (ng/l)	89.0	40.1 – 144.9	79.0	30.0 – 132.0	0.14
Stimulated inhibin B (ng/l)	230.0	98.0 – 144.9	238.5	113.2 – 416.8	0.98

TMC = total motile count, ART = assisted reproductive technology (including in vitro fertilisation and intracytoplasmic sperm injection), FSH = follicle stimulating hormone, CCCT = clomiphene citrate challenge test

often conceived through ART. Logistic regression with splines revealed a statistically significant non-linear association of BMI with miscarriage which could be simplified to a piecewise linear relation: up to a BMI of 32 no change in miscarriage rate was observed, but from a BMI of 32 onwards the probability of miscarriage increased. For none of the ORTs a relation with miscarriage could be demonstrated, both in analyses with and without a correction for possible confounders. The best fitting model included female age (OR per year 1.06; 95% CI 0.99 -1.14), assisted conception (OR yes to no 1.95; 95% CI 1.02 - 3.75) and BMI above 32 (OR per kg/m^2 1.57; 95% CI 1.12 - 2.21). The *c*-statistic (area under the receiver-operator curve) for this model was 0.65.

DISCUSSION

The present study shows that basal and stimulated FSH, the clomiphene citrate challenge test, basal and stimulated inhibin B and antral follicle count have no statistically significant predictive value for the chance to miscarry in a population of subfertile ovulatory women.

This finding is surprising as a decreased quantitative ovarian reserve is considered to be a reflection of advanced ovarian ageing and ovarian ageing is clearly associated with an increased rate of foetal aneuploidy and miscarriage. A possible explanation may be that ORTs basically relate to the number of remaining oocytes and that their quantity is unrelated to their quality. Oocyte quality might predominantly be determined by biological damage accumulated over time and would thus not be related to the number of oocytes left, but mainly to female age[57;61]. This hypothesis is compatible with recent publications demonstrating that female age does predict pregnancy chances, both spontaneous and after assisted conception, but ORTs don't[72;134;136;138]. Also, young women who respond poorly to ovarian hyperstimulation during IVF treatment appear to have clearly better prospects than their older counterparts[140;225]. Alternatively, it has been hypothesized that a biological relation between quantity and quality of oocytes does exist. The so-called production-line theory states that the germ cells produced earliest during foetal life are the least prone to non-disjunction. These oocytes are selected for ovulation first, leaving the oocytes of lesser quality for later years[57;69]. The reason that ORTs do not predict miscarriage could hence be that these tests do not accurately reflect oocyte quantity. For instance, an elevated FSH-level may indeed be due to a decreased number of follicles, but also to a range of other causes including the presence of heterophylic antibodies or FSH-receptor polymorphisms[76;77;226]. Especially when the relation between oocyte quantity and quality is subtle, inaccurate estimation of quantitative ovarian reserve might obscure this relation. Finally, the aetiology of miscarriage is known to be diverse. If any, ovarian reserve is only one of many contributing factors.

Another possible explanation for the absence of a relation between ovarian reserve and the chance to miscarry in our population is that the relation between oocyte quantity and quality does exist,

but only at the very end of the reproductive period, when ovarian reserve is severely diminished. For that reason we also analysed the extremes in our population separately, but in this manner we did not find an indication for a relation between ORTs and miscarriage either. For example, 5% of our population had a basal FSH-level of ≥12 IU/l (n = 15); four of these women (27%) miscarried compared to 23% in the population with FSH <12 IU/l (*P*= 0.73). However, women with a severely decreased ovarian reserve were not likely to be included in our study population, since they often have irregular and anovulatory cycles, which was an exclusion criterion in our study. In general, we cannot exclude the possibility that the relation between ovarian reserve and miscarriage does exist, but was not discovered in our subfertile study population, since differences between the various ORT values may have been too small.

Our findings that miscarriage rates are increased with higher BMI and after conception through ART have been described before[227;228]. Furthermore, we found no effect of male factor on miscarriage rate, which minimises the possibility that a relation between ovarian reserve and miscarriage was obscured by the inclusion of couples with male factor subfertility. Next to analysing a relation between total motile semen count and miscarriage, we assessed the semen parameters volume, concentration, percentage motile sperm and percentage sperm of normal morphology and found no linear or non-linear relation with these factors and miscarriage either (not in table). However, since one of the inclusion criteria of our study was a total motile semen count of at least one million, our results do not exclude the possibility that a very low semen quality actually does influence miscarriage risk.

Limitations

The present study is part of a prospective cohort study, which was originally designed to assess the predictive value of ORTs for the chance of spontaneous pregnancy; no power analysis was performed[223]. However, three acknowledged predictive factors for miscarriage were identified in our study (female age, BMI and assisted conception), which minimises the chance that a major relation between ORTs and miscarriage was missed. Yet, the size of our study population might have been too small to detect a more subtle relation between ORTs and miscarriage. To indicate the size of the effect of the ORTs on miscarriage risk we chose the example of basal FSH. In order to be able to provide confidence intervals (CI), we dichotomised basal FSH and included it in the best fitting model (consisting of age, BMI and conception with or without ART). For example, using FSH=8 IU/l as cut-off point, the effect was OR 1.03 with a 95% CI of 0.48 - 2.2 and *P*-value=0.95. Please note that our analysis of the relation between the ORTs and miscarriage was more refined than shown in this example as we explored the possible linear and non-linear nature of the relationship as well.

The results of most ORTs may vary per cycle in the same woman, especially basal FSH and the CCCT[74;75;84;205]. In our study all ORTs were only performed once per participant. It is not known whether repeating these tests would enhance their predictive value for miscarriage in subfertile populations.

However, in a prospective study among fertile women, Van Montfrans and colleagues[151] could not show a relation between repeatedly measured basal FSH and chance to miscarry.

In our study we did not measure anti-Müllerian hormone (AMH), nowadays a promising ORT[93;229]. Since AMH was not such an acknowledged ORT at the start of the study in 1999, we did not perform this test and unfortunately no serum was stored.

Finally, it has been demonstrated that the shorter the duration of pregnancy before miscarriage, the higher the probability that the loss is caused by aneuploidy[51;119;122]. We defined miscarriage as pregnancy loss between 4 and 16 weeks, which is a wide definition, including late pregnancy loss. In addition, we cannot exclude that we missed several very early pregnancy losses since couples did not routinely perform a pregnancy test every month. Early biochemical pregnancy loss was most likely to be detected in patients receiving ART, since it is usual (though not obligatory) in our IVF clinic to perform a pregnancy test after each ART cycle. This might partly explain the predictive value of assisted conception for miscarriage in our population.

Comparison with other studies

Three small retrospective studies suggest a relation between ORTs and miscarriage. Levi and colleagues found 20 miscarriages among 28 pregnant women with highly elevated basal FSH levels, a significantly increased miscarriage rate (71%) compared to a large control group with normal FSH levels[149]. Levi *et al.* included all subfertile women that visited their clinic, also women with irregular cycles who had possibly already entered menopausal transition. Elter and colleagues compared 28 women that miscarried with 34 women that delivered a healthy baby after ICSI treatment. AFC proved to have predictive value for miscarriage, while female age, basal FSH and estradiol values did not[147]. Most interestingly, Lekamge and colleagues demonstrated a significantly higher miscarriage rate amongst IVF-treated women with low levels of anti-Müllerian hormone (AMH) (5/17, 29.4%) compared to women with high AMH-levels (6/36, 16.7%)[148]. These results are of special interest since their study population was not at risk for severely decreased ovarian reserve: among their inclusion criteria were a basal FSH level <10 IU/l and a proven ovulatory cycle. The findings of Lekamge *et al.* have not yet been confirmed or rejected in other studies.

Among the publications supporting our findings is a prospective study of Van Montfrans and colleagues performed in women over 30 years of age without a history of subfertility, pursuing a spontaneous pregnancy[151]. 41 of the 86 pregnant women (48%) had a miscarriage, including very early pregnancy loss. No relation between miscarriage and basal FSH level was found. Luna *et al.* performed a retrospective study within an IVF population. Miscarriage rate in the group with elevated basal FSH levels was 22.9% (11/48), similar to the controls with normal FSH (19.3%, 226/1169)[150]. Abdalla and Thum also retrospectively evaluated miscarriage rates in an IVF population, selecting the outcome of the first IVF cycle, and found no differences between various groups divided by three different cut-off levels for basal FSH[146].

Clinical implications

The relation between oocyte quantity and oocyte quality is widely discussed, but not agreed on (yet). Therefore caution is required when using ORTs and interpreting 'abnormal' results. The present study shows that ORTs have no predictive value for miscarriage in a subfertile ovulatory population. AMH might prove an exception but was not evaluated in our study. The results of our study are in line with studies showing no predictive value of ORTs for spontaneous and assisted conception in subfertile populations. Since the ORTs evaluated in this study have no apparent predictive value for both the chance to conceive and pregnancy outcome, we recommend not using them in the general subfertile ovulatory population, not only to avoid unnecessary testing itself, but also to prevent unsupported interpretation of their results.

Acknowledgements

4