Female reproductive ageing

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Chapter 8

Summary and general discussion

The primary aim of this research was to determine whether a woman's remaining number of oocytes is related to the quality of those oocytes. To examine this relation, we studied associations between clinical parameters of oocyte quantity and oocyte quality. Secondly, if a clinical relation between oocyte quantity and quality was demonstrated, we aimed to add to the identification of possible predictors for (future) fertility and trisomy risk. This chapter provides an overview of the main results of the studies conducted for this thesis and a discussion in which is reflected on the two aims of the thesis, the possibilities for future research, and the current trend to postpone childbearing.

1. Summary of the findings

Chapter 2 describes a study assessing the relations between age and several endocrine and sonographic ovarian reserve tests in a prospective cohort of 474 subfertile ovulatory women. Specifically, we analysed which sizes of antral follicles were most closely correlated with age and the outcome of endocrine ovarian reserve tests. We found that the number of small antral follicles (2-6 mm) declined with age; the number of larger follicles (7-10 mm) remained constant. Independent of age, the number of small follicles was statistically significantly related to all the endocrine ovarian reserve tests used, including basal levels of follicle stimulating hormone (FSH) and inhibin B, the clomiphene citrate challenge test (CCCT), and inhibin B levels after stimulation with clomiphene citrate ($P<0.001$, except basal inhibin B $P=0.005$). The number of larger follicles was only statistically significantly related to basal inhibin B levels ($P=0.009$).

Chapter 3 aimed to determine whether ovarian reserve tests have predictive value for spontaneous pregnancy. Specifically, we assessed the added value of several ovarian reserve tests to previously identified prognostic factors for spontaneous pregnancy in subfertile ovulatory couples. In a prospective cohort (described in Chapter 2) of 474 couples we determined antral follicle count (AFC), FSH, inhibin B (basal levels and after stimulation with clomiphene citrate) and the clomiphene citrate challenge test. For each couple, the probability of a spontaneous pregnancy was retrospectively calculated using the validated Hunault prediction model, which includes the main known prognostic factors for spontaneous pregnancy. Our outcome measure was time to spontaneous pregnancy resulting in a live birth. When added to the Hunault model, only basal FSH and AFC significantly improved the prediction of spontaneous pregnancy (*P*-values 0.05 and 0.04). However, absolute changes in predicted probabilities after adding basal FSH or AFC were small: the predicted probability of spontaneous pregnancy shifted ≥10% in only 3.8% and 7.9% of the couples, implying that the clinical relevance of adding these tests is limited.

Chapter 4 contains a sub analysis within the prospective cohort of subfertile ovulatory couples used in the previous two chapters. The objective of this study was to assess the predictive value of several ovarian reserve tests for miscarriage. Couples that achieved an ongoing pregnancy (N=233) were compared with couples experiencing miscarriage (N=72) on results of the ovarian reserve

tests and patient characteristics. None of the ovarian reserve tests evaluated, including AFC, FSH, inhibin B (basal levels and after stimulation with clomiphene citrate) and the clomiphene citrate challenge test, had a statistically significant predictive value for the occurrence of miscarriage in this population of subfertile ovulatory women.

Chapter 5 describes a study in a nationwide retrospective cohort of Dutch women undergoing IVF treatment. The aim of the study was to assess whether women who conceived after a poor response to hyperstimulation (defined as an oocyte yield <4 oocytes) had an increased risk of miscarriage. Women who achieved an ongoing pregnancy after their first completed IVF cycle (N=1468) were compared with those who experienced a miscarriage (N=357) with respect to their ovarian response. Logistic regression analysis showed an odds ratio for miscarriage associated with poor response of 1.9 (95% confidence interval 1.3-2.8; *P*= 0.001). Due to interaction, this association became stronger with increasing female age. Among women younger than 36 years of age, miscarriage rates between poor and normal responders did not differ (20% vs. 17%; *P*=0.54), whereas among women of 36 years and older, poor responders had a statistically significant increased miscarriage rate compared to their peers with normal response (47% vs. 25%; *P*=0.001).

Chapter 6 aimed to assess the relation between trisomic pregnancy and three parameters of oocyte quantity. In the same nationwide cohort described in Chapter 5, we identified 28 women with a trisomic pregnancy conceived via, or within one year of, IVF treatment. We performed a case-control study to examine whether trisomy cases more often had a history of ovarian surgery and a lower response to ovarian hyperstimulation than age-matched controls. Subsequently, cases and controls were followed to compare the incidence of signs of menopause at the end of the study period as self-reported by questionnaire. Logistic regression analysis showed an association between trisomic pregnancy and a history of ovarian surgery (odds ratio (OR) 3.3; 95% confidence interval (CI) 1.0-10.5; *P*=0.04) and between trisomic pregnancy and retrieval of ≤4 oocytes during IVF treatment (OR 4.0; 95% CI 1.4-11.5; *P*=0.01). The adjusted OR for signs of menopause associated with trisomic pregnancy was 5.7 (95% CI 1.1-29.9; *P*=0.04).

Chapter 7 describes a prospective cohort study of 294 women aged 35-41 years undergoing their first IVF treatment. The aim of the study was to assess the predictive value of five ovarian reserve parameters, i.e. FSH, inhibin B, anti-Müllerian hormone (AMH), AFC and the number of retrieved oocytes, for embryo morphology. Our main outcome measure was a morphologically top quality embryo on day 3 of development. None of the ovarian reserve parameters had statistically significant predictive value for the chance that an oocyte would develop into a morphologically top quality embryo. The number of retrieved oocytes was strongly related with the chance of a patient having at least one top embryo. In addition, women with high AFC and AMH levels had one or more top embryos significantly more often than women with low AFC or AMH levels.

2. Reflections on the clinical relation between oocyte quantity and quality

Our results show that several ovarian reserve tests, including FSH, inhibin B, CCCT and AFC, have no substantial predictive value for natural conception resulting in a live birth (Chapter 3) or miscarriage (Chapter 4) in a subfertile ovulatory population. This is in line with findings from meta-analyses, that demonstrate no predictive value of these ovarian reserve tests for ongoing pregnancy after IVF treatment[72;134]. These findings suggest that oocyte quantity and quality are not related to each other. In contrast to the ovarian reserve tests, the number of oocytes retrieved in IVF cycles with ovarian hyperstimulation has been shown to have predictive value for ongoing pregnancy: a poor response is highly predictive of low pregnancy chances[112;139;140]. In addition, the work reported in this thesis demonstrates that a low number of retrieved oocytes in IVF treatment is related to miscarriage (Chapter 5) and trisomic pregnancy (Chapter 6). These results suggest there is a relation between oocyte quantity and quality. The findings that a history of ovarian surgery and signs of menopause are related to trisomic pregnancy (Chapter 6) further support a relation between oocyte quantity and quality.

Thus, ovarian reserve tests do not clearly predict oocyte quality, whereas the ovarian response to hyperstimulation does. These observations might be explained by the nature of the ovarian reserve tests. As described in the Introduction (§3.2), the ovaries are capable of long maintaining a regular ovulatory cycle, even when both the number and the quality of the oocytes are already falling. The results from the endocrine ovarian reserve tests studied in this thesis are closely related to the number of small antral follicles (Chapter 2)[88;90]. Biologically, the results of these tests represent the quantity of functional granulosa cell tissue. Endocrine ovarian reserve tests may therefore represent granulosa cell function rather than the number of remaining oocytes. In line with this assumption is the fact that the only study that actually compared the results of endocrine ovarian reserve tests with the findings at histological study of the ovaries failed to demonstrate a statistically significant relation between the number of counted follicles and basal FSH, CCCT and a second dynamic ovarian reserve test, the GnRH agonist stimulation test[102].

In addition, the antral follicle count supposedly overestimates the total number of follicles in women with a reduced ovarian reserve, as a relatively larger proportion of follicles is recruited monthly[19;107]. As the size of the total follicle pool is reduced, the proportion of visible growing follicles increases.

In contrast to the ovarian reserve tests, for the ovarian response to IVF treatment actual oocytes are counted. The ovarian response to hyperstimulation may be the 'dynamic ovarian reserve test' that approaches the true ovarian reserve best. Since the findings supporting a relation between oocyte quantity and quality in this thesis come from studies performed in IVF populations, caution is required when interpreting the results since IVF patients are unlikely to represent women of reproductive age in general.

After adjusting for the oocyte yield in IVF treatment, female age still plays an important role in predicting oocyte quality. Younger poor responders have better ongoing pregnancy chances than

their older counterparts[139;140]. This thesis shows a clear interaction of age with the relation between poor response and miscarriage: the predictive value of poor response for miscarriage increases with increasing female age (Chapter 5). These observations support the assumption that – next to oocyte quantity – age plays a vital role in determining the oocyte quality.

It is also noteworthy that women with a poor response due to their decreased ovarian reserve and their peers with a 'coincidental' poor response have comparably low pregnancy chances in the IVF cycle that the oocytes are retrieved from[112;139]. The differences lie in their prospects: women with decreased ovarian reserve are more likely to have another poor response and thus again low pregnancy chances in following IVF cycles. In line with these findings, the thesis demonstrates that the proportion of morphologically top quality embryos in IVF treatment is not affected by a woman's ovarian reserve. This implies that, independent of the ovarian reserve, the number of top embryos is simply determined by the number of oocytes retrieved in that particular cycle (Chapter 7). The observations that a poor response to hyperstimulation is predictive of low pregnancy chances and a low number of top quality embryos, independent of whether the poor response is due to a decreased ovarian reserve or not, supports the hypothesis that the chance that a good quality oocyte is available decreases if there are fewer oocytes to choose from.

In conclusion, ovarian reserve tests have no substantial predictive value for oocyte quality, but the ovarian response to hyperstimulation has. A low response to hyperstimulation is predictive of low oocyte quality, independent of whether the low response is due to decreased ovarian reserve or not. In addition to the oocyte yield, the woman's age plays an important role in predicting her oocyte quality.

3. Reflections on possible predictors for (future) fertility and trisomy risk

The studies reported in this thesis did not reveal possible predictors for (future) fertility and trisomy risk that are generally applicable. Ovarian reserve tests, including FSH, inhibin B, CCCT and AFC, have not shown substantial predictive value for ongoing pregnancy in subfertile (this thesis) or IVF-treated populations[72;134]. Further evaluation of the use of these tests for the prediction of future fertility is therefore not advocated. The predictive value of ovarian reserve tests for the risk of a trisomic pregnancy was not assessed in the current thesis. The few studies performed on this subject, as described in the Introduction (§5.3), show conflicting results. Future research is needed to reveal whether ovarian reserve tests have predictive value for the risk of trisomic pregnancy or not; an example of how this can be done is described below in §4.4.

In this thesis, an increased miscarriage rate was demonstrated in women with a poor response to ovarian hyperstimulation in IVF treatment, which is in line with the known association of poor response and lower ongoing pregnancy chances[112;139;140]. This implies that the ovarian

response may be a useful predictor for future fertility. In a prospective cohort of 222 IVF-treated women, Hendriks *et al*. showed that a model including female age and the oocyte yield in the first IVF cycle, had statistically significant predictive value for ongoing pregnancy chances in two following IVF cycles[139]. In addition, poor responders were classified as 'expected' (signs of decreased ovarian reserve) and 'unexpected' (no signs of decreased ovarian reserve) based on the results of three ovarian reserve tests (FSH, inhibin B and AFC). Adding the type of poor response further refined the model: unexpected poor responders had similar future pregnancy chances compared to their peers with a normal response, whereas expected poor responders had poor prospects of pregnancy, even more so if they were of advanced reproductive age. Two other studies demonstrated comparable results using FSH[112] or CCCT[259] to identify the type of poor response. The model proposed by Hendriks *et al.* has not yet been validated in a larger cohort.

A history of ovarian surgery and low response to IVF treatment (<5 oocytes) were associated with an increased risk of a trisomic pregnancy (Chapter 5). In line with our findings, one small case-control study also demonstrated that women with a child with trisomy 21 had a history of ovarian surgery or congenital absence of an ovary significantly more often than controls[164]. The relation between ovarian response to hyperstimulation in IVF treatment and trisomic risk has not been studied before. Our findings suggest that a history of ovarian surgery and the response to IVF treatment may add to the risk estimate of having a trisomic pregnancy, although these factors are only of relevance for a small part of the population of reproductive women. Confirmation of our findings is needed and the exact change in risk of trisomic pregnancy should be determined before these variables can be applied in clinical practice (see also §4.3).

4. Future research

The aim of this thesis was to determine whether a woman's remaining number of oocytes is related to their quality and if so, to add to the identification of possible predictors for (future) fertility and trisomy risk. In future work addressing these questions, animal studies and basic laboratory research on oocytes and embryos will play a vital role in elucidating the various processes of ovarian ageing. But also for the clinician, there are still many other issues for further research on this subject: several possibilities are given below. Outside the scope of this thesis, but obviously of profound interest, is the fact that increasing knowledge about reproductive ageing processes may eventually lead to tools for conserving fertility.

4.1 End of fertility and age at natural menopause

The fundamental question in clinical research on the relation between oocyte quantity and quality is whether the interval between the end of fertility and the onset of menopause in a woman is fixed or not. This issue can only be studied in populations not using contraceptives, such as isolated

populations or strictly religious groups, which are self-evidently hard to approach. Demographers with access to these populations could collect data on childbirth and menopause in a longitudinal study design. Another option would be to retrospectively collect data on menopause and age of last childbirth in all women of, for instance, 60 years or older. The latter approach is, however, prone to reporting bias.

4.2 Genes and the onset of natural menopause

Genome-wide association studies are being conducted to unravel the genetics of age at menopause. In these studies the phenotype of the participants is of crucial importance. This is true not only for the timing of menopause itself, which is complicated by the use of oral contraceptives and hormonal replacement therapy, for instance, but also for confounding factors such as smoking. If, indeed, age at menopause and the end of fertility are related, the 'genetic menopausal profile' of a woman could have predictive value for her future fertility. Ideally, this genetic profile would be integrated into a fertility prediction model including several prognostic factors. Women with a likely early reduction in fertility might adjust their views on family planning and decide not to postpone motherhood. However, the development and especially the validation of such a model will be complex.

4.3 Confirmation of the observed association of ovarian response in IVF treatment and a history of ovarian surgery with the risk of trisomic pregnancy

The findings from this thesis suggest that the response to IVF treatment and a history of ovarian surgery may add to the risk estimate of having a trisomic pregnancy. Our results need confirmation in a different, and preferably larger, IVF cohort. In collaboration with a Danish research group we are currently exploring the options for this. The extensive and high quality medical registries in the Nordic countries may provide the opportunity to link response to IVF treatment and previous ovarian surgery to the outcome of pregnancy on a larger scale. If our initial observations are confirmed, the second step would be to determine the exact change in risk of trisomic pregnancy for both ovarian response (IVF population) and a history of ovarian surgery (IVF population and general population). Since a recognised trisomic pregnancy is a relatively infrequent event, prospective studies on the relation between ovarian reserve and trisomic pregnancy are time consuming. In the north of the Netherlands, data from all women undergoing ultrasound examination any time during their pregnancy are now registered. At present, the possibility of adding information on previous ovarian surgery to this registry is being explored; in this manner we would, in due course, be able to assess the relation between a history of ovarian surgery and the outcome of pregnancy in the general fertile population.

4.4 Complementary studies on the relation between ovarian reserve and the risk of trisomic pregnancy

If a reduced ovarian reserve does in fact increase the risk of a trisomic pregnancy, the addition of a generally applicable measure for ovarian reserve may refine the risk estimate, which is currently

based on age and, if performed, on serum screening and measurement of nuchal translucency in the first trimester of pregnancy. Compared to other ovarian reserve tests, AMH seems to reflect the gradual decrease in ovarian reserve with age best, including in the general fertile population (see also Introduction, §3.2 and Figure 9)[91-95]. One case-control study showed no statistically significant relation between AMH serum levels in the first trimester of pregnancy and trisomy 21 in the foetus (N=25 cases), but with such a small sample size, a more subtle relation of AMH and trisomic pregnancy could have been missed[160]. We are currently working on a larger case-control study to assess the relation between AMH serum levels in the first trimester and trisomic pregnancy.

A different approach for studying the relation between ovarian reserve and trisomic pregnancy is to assess the relation between trisomic pregnancy and future age at menopause. Two studies on this subject demonstrated a non-significant decrease in age at menopause in women with a history of trisomic pregnancy compared to controls[155;156] (see also Introduction, §5.3). To further elucidate this issue we are currently involved in a follow-up study of mothers of a child with Down syndrome and controls, initiated by researchers at the Division of Reproductive Medicine, Vrije Universiteit Medical Center, Amsterdam.

4.5 The influence of ovarian surgery on reproductive lifespan

Iatrogenic loss of ovarian reserve due to ovarian surgery cannot readily be compared with age-related loss of ovarian reserve. Remarkably, the relation between ovarian surgery and age at menopause has hardly ever been studied. Our group aims to assess the chances of IVF-related ongoing pregnancy, miscarriage and early menopause in women with a history of ovarian surgery and in controls, using the OMEGA database described in Chapters 5 and 6.

5. Reflections on the postponement of childbearing

The increased age of childbearing in Western societies is the main reason for the current interest in the phenomenon of female reproductive ageing. Dutch women have a mean age at the birth of the first child of 29.4 years, which gives them the questionable honour of being among the world leaders in this respect[5]. A report by the Dutch Council for Public Health and Health Care in 2007 described the main reported reasons for the postponement of childbearing[260]. Before starting a family, both men and women stated they wanted to have completed their education, started and preferably secured their careers, exploited their freedom, achieved a financially stable situation and have adequate housing. Not having a stable partner during the years of optimal fertility is also a major reason to postpone parenthood: for most men and women a stable relationship is the basis for starting a family. Differences between men's and women's views also play a vital role: when women are ready to start a family it takes them, on average, one to two years to convince their partners. Although many women proclaim that an equal division between both partners in the care for their children is ideal, in the Netherlands women still generally play the major role in the

children's upbringing and work fewer hours outside the home. This prospect of 'taking a step back' deters them from early motherhood. Moreover, having children after the age of 30 seems to have clear advantages for the mother's social and financial position.

The report further states that the government and the medical branch have an important role in educating the public, both men and women, on the risks associated with postponing childbearing [260]. The awareness of the possible consequences of such a delay is already increasing, although many people do not realise that the increased risks apply from age 30 years onwards. An essential role for the government lies in simplifying the combination of work and childcare for both men and women, especially since mothers in the Netherlands work fewer hours than most of their European counterparts. Measures to help combine having a career and a family could slightly influence the maternal age at childbirth, but are also vital in the light of the general ageing population. The younger generations will have to provide for an extensive number of elderly people and the government will need the women of reproductive age both to have children and a substantial job outside the home.

Not having a partner during the reproductive years is one of the most under-exposed reasons for involuntary childlessness; this may be true for both men and women. The Academic Medical Center in Amsterdam recently offered single women around the age of 35 years the opportunity to freeze some tens of oocytes in order to possibly preserve their fertility options. A public debate has now been started on this subject.

The good news is that the trend to postpone parenthood and the decreasing number of children born per woman in the most developed countries seems to have reached a halt[261]. In the Netherlands, the average maternal age of 29.4 years at the birth of the first child has been the same for the past 5 years[5]. And fortunately, most couples who want to start a family at or after this age do succeed in doing so. To only illustrate worst-case scenarios would thus be an exaggeration. But having said this, the suffering of those couples on the wrong side of the line should not to be underestimated. Being dependent on fertility treatment is burdensome; involuntary childlessness is a private disaster. If women with a likely early reduction in fertility could be identified and warned in good time, this may be regarded as a substantial benefit of knowledge on reproductive ageing.