# Chapter 1

## Introduction

The position of women in Western societies has changed fundamentally during the last few decades. Higher education and a growing participation in the workforce has led to the increasing self-awareness and economic independence of women[1]. Prosperity and the increasing availability of spare time has enabled the development of a more hedonistic lifestyle for both men and women. The introduction of oral contraception in the 1960s played a key role in the emancipation of women, by untying the age-old knot between sexuality and reproduction[2]. Rather than being an unavoidable destiny, motherhood became a choice.

One of the consequences of these changes is a deliberate reduction of family size and postponement of childbearing[2-4]. In the Netherlands the mean maternal age at the birth of the first child has risen from 24.2 years in 1970 to 29.4 at present (Figure 1)[5]. Generally, the higher the woman's level of education, the higher the age at which she will give birth to her first child[3;4;6]. The current mean age of lower educated Dutch women at the birth of their first child is 27 years, whereas for women with a higher education the mean age is 34 years[5].

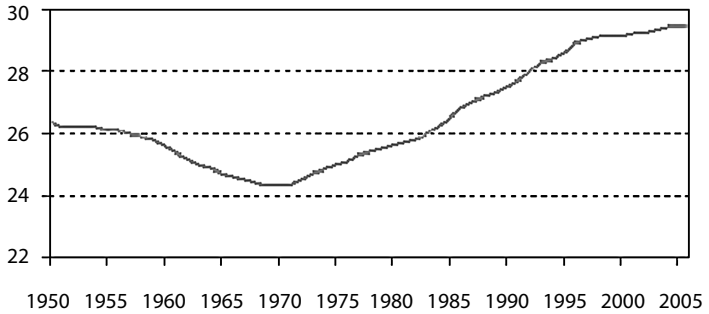


**Figure 1.** Increase of the mean maternal age at first child birth (Y-axis, in years) in the Netherlands during the last decades (X-axis).
*(Adapted from CBS, the Dutch National Institute for Statistics)*

### 1. Female reproductive ageing

However, the trend of delaying motherhood has its price. Monthly pregnancy rates decline with a woman's age, while the chance of pregnancy loss increases[7;8]. It is assumed that the general postponement in childbearing leads to an increased incidence of fertility problems, smaller family size than desired, and unwanted childlessness[8]. Together with voluntary childlessness and reduction in family size, in many Western societies the postponement of childbearing has resulted in the average number of children born per family being too low to ensure replenishment of the population[9;10]. In addition, higher maternal age is an important risk factor for perinatal complications affecting both mother and child[11-13]. The effect of the increasing paternal age on reproductive potential is small compared to the contribution of female ageing[14].

*1.1 Decrease in the number of oocytes*

The phenomenon of female reproductive ageing is attributed to a decrease in both the quantity and quality of a woman's oocytes[7;8]. In contrast to the male situation, the general view is that women are born with their stock of gametes that will have to serve their entire reproductive life[15;16]. However, recent mice studies have challenged this view, suggesting the presence of germline stem cells that give rise to new oocytes[17]. These findings have been fiercely debated: it is not clear whether these 'adult' oocytes indeed exist and if so, what these findings mean for humans. Therefore, the assumption that women's total number of oocytes are determined before birth has served as the basis for this thesis.

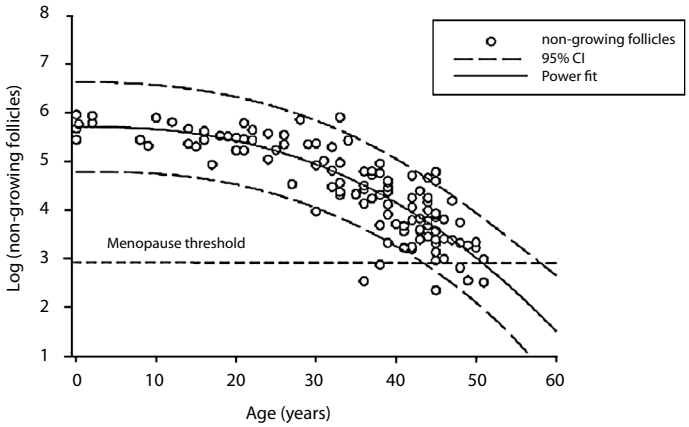


**Figure 2.** Power model of the decrease in the number of non-growing follicles in the ovaries with female age. *(Adapted from Hansen et al., Human Reproduction 2008;23:699-708)*

In the foetal ovaries, millions of primordial follicles are formed, consisting of oocytes surrounded by a flat layer of supporting granulosa cells. The original size of this foetal follicle pool reduces prenatally from about 7 million to an estimated 1 or 2 million at birth[15;18-20]. After birth the follicle pool size steadily decreases further, and more rapidly from the end of the fourth decade onwards (Figure 2)[21;22]. Finally, the remaining number of follicles drops below a threshold of an estimated thousand follicles, resulting in menopause[23;24]. The mean age at menopause in various populations is around 51 years, but ranges from before the age of 40 up to over 60 years (Figure 3, right-hand curve)[25-28]. The timing of natural menopause is considered to be mainly heritable, based on a large agreement between the ages at menopause of mothers and daughters as well as sister pairs; it has been suggested that more than 80% of the differences in age at menopause have a genetic origin[29-31]. Recent studies have revealed several genes and loci presumed to be involved in the age at menopause[32-35]. The wide age range for the onset of menopause is assumed to reflect differences between women in the original size of the follicle pool and the atresia rate of these follicles. In addition, iatrogenic interventions and environmental influences may also affect oocyte quantity.

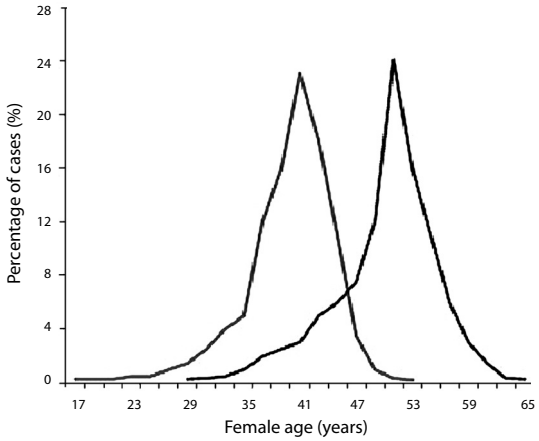


**Figure 3.** Distribution curves for the observed maternal age at last child birth (left-hand curve) and age at menopause (right-hand curve).
*(Adapted from Lambalk et al., Maturitas, 2009;63:280-91)*

An evident medical intervention reducing the size of the ovarian follicle pool is ovarian surgery. Other examples of medical interventions renowned for their often devastating effect on oocyte quantity include cancer treatments, i.e. chemotherapy and abdominal radiation[36;37]. The best-known environmental factor influencing follicle pool size is smoking. Women who smoke enter menopause at an earlier age than their non-smoking peers[38;39].

*1.2 Decrease in the quality of oocytes*

Obviously, a natural pregnancy cannot be achieved when there are no oocytes left. But several years before menopause, fertility is already severely impaired, even when the menstrual cycle is still regular and ovulatory[7]. In so-called natural fertility populations, where no contraception is used, the age of the mother at the birth of her last child is on average 41 years, i.e. ten years before the mean onset of menopause (Figure 3, left-hand curve)[40;41]. The birth of the last child rather than menopause seems to mark the end of female fertility. Moreover, in the decade preceding the birth of the last child, monthly fecundity rates gradually drop[42;43].This observed reduction in pregnancy chances from a mean age of 31 years onwards has been confirmed in female partners of azoospermic men undergoing donor insemination; this excludes the possibility of decreased sexual activity with age or a male factor as single causes (Figure 4)[44;45]. At the age of 38 years, the average monthly probability of conception leading to a live-born child has dropped to one quarter of that in women aged 30 years. The same trend is observed in the results of artificial reproductive techniques: success rates drop markedly with increasing age of the female partner[46-49]. If donor eggs are used, success rates largely depend on the age of the donor, which further underlines the ovarian contribution to female reproductive ageing (Figure 5).

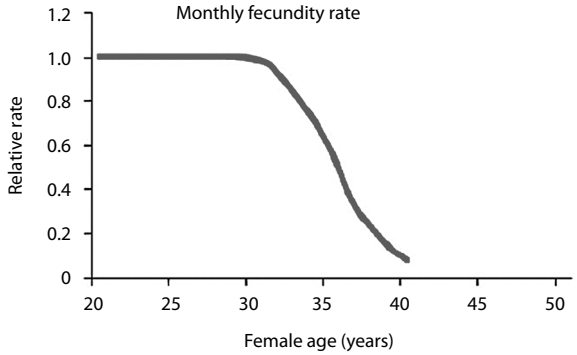


**Figure 4.** The decrease in monthly fecundity rate (rate of live born children) relative to the fecundity rate of women in the 20-30 year age group.
*(Adapted from Broekmans et al., Trends in Endocrinology and Metabolism, 2007;18:58-65)*


**Figure 5.** Effect of female age upon the average singleton live birth rate per started IVF cycle. The dotted line represents the average singleton live birth rate after oocyte donation as a function of the recipient's age.
*(Adapted from Broekmans et al., Trends in Endocrinology and Metabolism, 2007;18:58-65)*

Next to the decreasing monthly pregnancy rates, the chance of a chromosomally abnormal conception has been demonstrated to increase with age, leading to higher rates of early pregnancy loss and a higher incidence of children with a chromosomal abnormality (Figure 6)[50;51]. Moreover, very early loss of the conceptus, before a pregnancy is recognised as such, is held responsible for the major part, if not all, of the decreased monthly pregnancy rate with age[41]. The decreased pregnancy chances and increased miscarriage rates with a woman's age are attributed to a reduced oocyte quality[7;8].

A clear denominator of decreased quality is the observed increased rate of meiotic non-disjunction in oocytes with age[52-55]. During early foetal development the oocytes enter meiosis, a process requiring two subsequent cell divisions, meiosis 1 and 2, to reduce the number of chromosomes

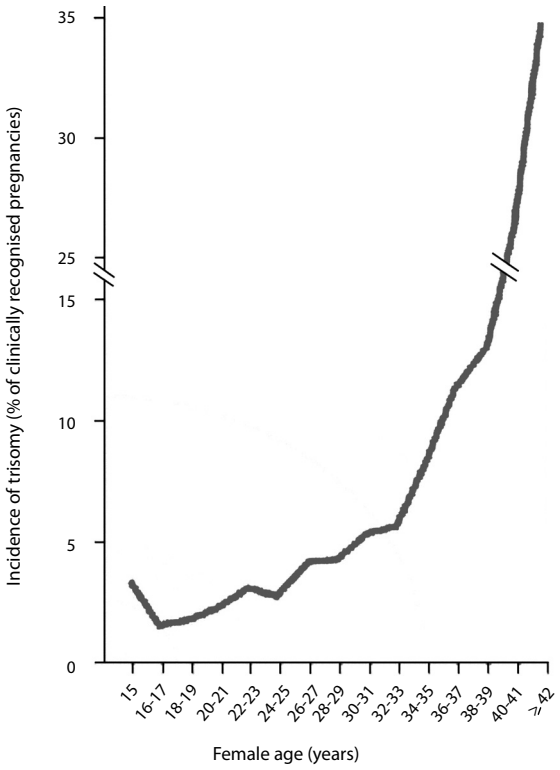


**Figure 6.** Increase in the incidence of trisomy in clinically recognised pregnancies with female age.
*(Adapted from Hassold and Hunt, Nature Reviews Genetics 2001;2:280-291)*

from the diploid number of 46 to the haploid number of 23. At the beginning of meiosis 1, DNA is replicated and each of the 46 chromosomes is duplicated into sister chromatids. Homologous chromosomes then align themselves in pairs and genetic material is exchanged (recombination). At this point the process is arrested for years. Meiosis 1 is only completed if the oocyte is selected for ovulation: the homologous pairs then separate into two daughter cells. Meiosis 2 subsequently separates the sister chromatids and is completed if fertilisation occurs. In total four haploid cells are formed during meiosis, one mature oocyte and three polar bodies.

In meiotic non-disjunction, which may occur either during the first or second meiotic division, separation of a homologous chromosome pair or pair of sister chromatids fails (Figure 7). Both members of one pair move into one cell, resulting in one cell with a chromosome too many and one cell with a chromosome too few. This results in an age-related increased incidence of aneuploidic conceptions, mainly monosomies and trisomies[56]. Autosomal monosomic conceptions are hardly ever recognised and are presumed to result in pre-clinical very early pregnancy loss[57]. Trisomy 21, or Down syndrome, is the most common trisomic condition in ongoing pregnancies. In over 90% of all cases of clinically recognised trisomy 21, the origin of the aneuploidy is maternal[58]. The trisomy may result from a variety of types of meiotic errors, both occurring during meiosis 1 and 2[51;58].

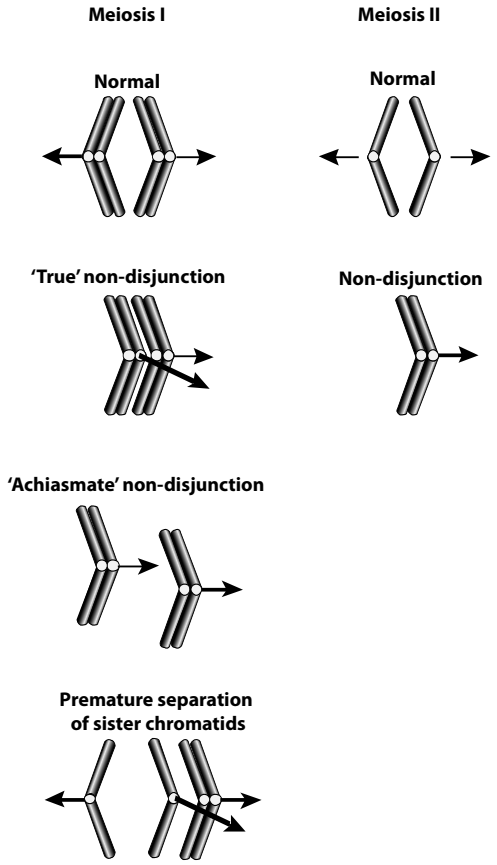


**Figure 7.** Meiotic non-disjunction.
A normal meiosis I (MI) division results in the segregation of homologous chromosomes. There are several possible patterns of abnormal MI segregation: including 'true' non-disjunction, in which homologues travel together to the same pole; 'achiasmate' non-disjunction, in which homologues that have failed to pair and/or recombine travel independently to the same pole; and premature separation of sister chromatids, in which chromatids — rather than homologues — segregate from one another.
A normal meiosis II (MII) division involves the segregation of sister chromatids. Non-disjunction at MII is assumed to result from failure of the sisters to separate, although more complicated errors that involve sequential abnormalities at MI and MII have been proposed.
*(Adapted from Hassold and Hunt, Nature Reviews Genetics 2001;2:280-291)*

Next to the relation with female age, a clear association of trisomic pregnancy and recombination during oocyte formation in foetal life has been established[59;60]. Absent, reduced or sub optimally positioned recombination may result in various meiotic errors later in life. Exactly how deviant recombination eventually leads to aneuploidy is still unclear.

*1.3 The relation between oocyte quantity and oocyte quality*

The biological rationale behind the limits of the reproductive life span of women and the exact mechanisms underlying female reproductive ageing are not yet understood. For instance, the nature of the relation between oocyte quantity and oocyte quality as the two components determining reproductive capacity is so far unclear. Such a relation is readily expected if we look at the exponential increase in aneuploid conceptions with age, which parallels the exponential decrease in follicle number (Figures 2 and 6). Likewise, the distributions of the ages at menopause and the ages at the birth of the last child in natural fertility populations are remarkably alike (Figure 3). However, although these patterns resemble each other, this does not prove a causal relation between the two processes. For example, it has not been confirmed whether women having their last child at

35 and 46 years, respectively, are the same women who enter menopause ten years later around 45 and 56 years. In other words, it is not clear whether the interval between the end of fertility and the onset of menopause in a woman is fixed. It may well be that the processes of oocyte quantity loss and oocyte quality loss simply occur in parallel with increasing age and that they have no causal interrelationship.



**2. Suggested processes responsible for decreased oocyte quality with age**

Several theories have been developed that aim to explain the age-related decrease in oocyte quality. An introduction to the suggested processes involved is given below.

*2.1 Accumulation of biological damage over time*

The older a woman gets, the longer time her ovaries are exposed to environmental hazards and ageing processes. The oocyte is likely to be vulnerable for accumulation of biological damage over time, since it is arrested in the meiotic prophase from foetal life until time to ovulation (or atresia). The chance that individual oocytes are harmed would thus increase with age[57;61]. This theory is supported by studies showing ageing effects on the meiotic machinery of the oocytes, such as defective spindle formation and chromosome alignment, and of the supporting granulosa cells[52;57;62;63]. These processes would be influenced by age and environmental factors, but not by follicle pool size.

*2.2 Factors related to both a decrease in oocyte quantity and oocyte quality*

As the number of follicles decline, the endocrine and paracrine changes accompanying follicle loss may harm the remaining oocytes. Increased levels of follicle stimulating hormone have been shown to have unfavourable effects on oocyte quality (see also section 4.2)[64-66]. A second theory suggests that an age-related decline in the vascularisation of the ovaries may damage the remaining follicles, causing both a decrease in the number of remaining oocytes and in their quality. As the blood flow to the ovaries becomes impaired, an accumulation of factors accompanying oxidative stress may occur within the follicle, causing both atresia and non-disjunction[61;67;68].

*2.3 The selection of oocytes may become impaired when fewer follicles are available*

The 'production line theory' stipulates that the oocytes produced first in foetal life are the least prone to non-disjunction[69]. According to the principle 'first in, first out' these oocytes of highest quality are selected for ovulation first, leaving the oocytes of lesser quality for later years. Since deviant recombination in foetal life is related to increased rates of non-disjunction later in life, at least part of the quality of an oocyte is indeed established prenatally. However, whether these oocytes of lesser quality are indeed selected only later in life is not clear, as is the hypothesis that a 'second hit', for instance age-related damage, is necessary to cause the actual non-disjunction in these error prone oocytes[59].

The 'limited pool theory' states that the chance that a good quality follicle is available for selection during the natural cycle in exactly the right developmental phase is lower if the number of follicles to choose from is reduced[63]. This theory can also be viewed from a broader perspective: it is conceivable that, in some cycles, good quality oocytes are simply not available if there are fewer follicles to choose from. It is unknown whether the selection procedure itself is subject to ageing processes.

When reflecting on the summary of processes described above, which are all biologically plausible and partly supported by scientific evidence, it is easy to think that the age-related reduction in oocyte quality is caused by more than one process. Sections 2.2 and 2.3 describe processes that support a relation between the reduction in oocyte quantity and quality. Studies on the relation between these two entities are not only of basic scientific interest, but may be highly relevant clinically. If oocyte quantity and oocyte quality are related, the estimated size of the remaining follicle pool may be predictive for (future) fertility and trisomy risk. Especially since the age range in the onset of menopause shows that oocyte quantity differs widely between women, the size of the remaining follicle pool may be more informative than age alone. To study the clinical relation between oocyte quantity and oocyte quality, associations between parameters reflecting properties of both these entities should be analysed.

**3. Parameters of oocyte quantity**

The ultimate assessment of oocyte quantity, in clinical practice often referred to as the 'ovarian reserve', is by histological study of the ovaries after surgical removal[19]. Besides being very laborious, for obvious reasons this parameter cannot be used clinically. A summary of clinically applicable parameters for the number of remaining oocytes that are relevant for the work included in this thesis is presented below.

*3.1 Menopause, cycle length and menopausal transition*

Next to histological studies, a second fundamental, but obligatory retrospective measure for oocyte quantity is the moment of menopause, the reproductive event reflecting near depletion of the ovarian follicle pool. In the years preceding menopause the length of the menstrual cycle first shortens by 2-3 days[43]. When the number of remaining follicles further decreases and can no longer sustain a regular ovulatory cycle, menstrual cycles lengthen and become increasingly irregular, a phenomenon called menopausal transition[70]. The interval between the start of cycle irregularity and menopause seems to be relatively constant at 5-6 years, irrespective of the eventual age at menopause[71]. Cycle disturbances are, however, no unique marker for oocyte quantity since cycle irregularity may also occur due to hormonal dysregulation unrelated to ovarian reserve.

*3.2 Endocrine and sonographic ovarian reserve tests*

A different approach to estimating the size of the remaining follicle pool is based on the observed endocrine changes accompanying follicle loss. Levels of the pituitary-produced *follicle stimulating hormone* (FSH) in the early follicular phase of the menstrual cycle rise with age as a result of decreasing endocrine feedback from the partially depleted ovaries (see Figure 8). The basal FSH level was the first 'ovarian reserve test' (ORT) to be widely applied in clinical practice in general subfertile and IVF populations[72;73]. The interpretation of FSH values is hampered by the fact that FSH levels not only fluctuate within but also between cycles of the same woman[74;75]. In addition, it has been shown that raised FSH-levels may originate from a variety of causes apart from decreased ovarian reserve, including the presence of FSH-receptor polymorphisms and heterophylic antibodies interfering with the test assay used[76;77].

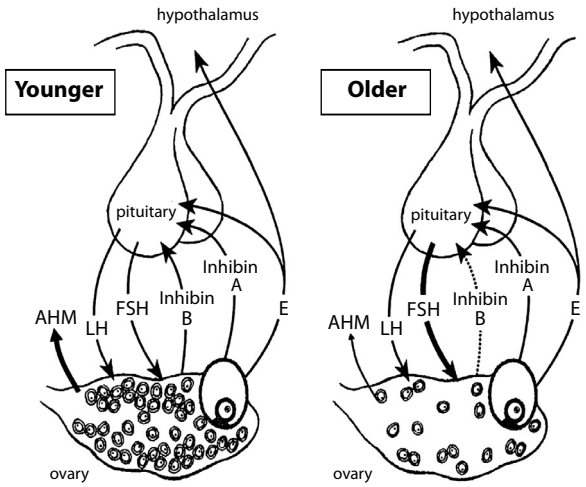


**Figure 8**. Schematic representation of the effect of the change in ovarian reserve with age upon several endocrine factors, i.e. AMH (anti-Müllerian hormone), LH (luteinizing hormone), FSH (follicle stimulating hormone), inhibin A and B and estradiol (E).
*(Adapted from Broekmans et al., Endocrine Reviews 2009;30:465-493)*

One of the presumed causes of increased basal FSH levels with age is the reduced secretion of *inhibin B* by the granulosa cells of the developing antral follicles (Figure 8). Since inhibin B is produced by the ovarian follicles themselves, its measurement in the follicular phase of the menstrual cycle was expected to reflect ovarian reserve more directly than FSH. However, despite a statistically significant relation of both basal FSH and inhibin B levels with age, a large overlap between the values for older and younger women has been observed[78;79]; this reduces the applicability of FSH and inhibin B as ovarian reserve tests. In addition, both basal FSH and inhibin B levels have been shown to change only relatively late in a woman's reproductive life span, approximately ten years before menopause, when fertility is already severely reduced[78-80]. These observations show that despite reducing follicle

numbers, the ovaries are apparently capable of long maintaining a regular ovulatory cycle with unchanged baseline endocrine levels.

To unmask such subclinical decreased ovarian reserve, dynamic ovarian reserve tests have been developed, which assess the endocrine response of the ovaries to exogenous endocrine stimuli. Ovaries with a limited number of remaining follicles are assumed to be unable to produce proper negative feedback to the challenge of supra physiological levels of FSH. An example of a dynamic ovarian reserve test is the *Clomiphene Citrate Challenge Test* (CCCT)[81;82]. For this test, the basal level of FSH is determined on day 3 of a natural cycle. From cycle day 5 to 9 patients self-administer 100 mg of the anti-oestrogen clomiphene citrate, which results in a reactive increase in endogenous FSH. On cycle day 10 FSH is measured again. In women with only few remaining follicles, the endocrine reaction of the ovaries to the endogenous FSH increase will be insufficient to return it to the basal level. The fact that results of the CCCT, just like basal FSH levels, may show substantial intercycle variability hampers interpretation of the test values with respect to ovarian reserve[83;84].

In recent years *anti-Müllerian hormone* (AMH) has been shown to be a promising new endocrine ovarian reserve test[85;86]. AMH is a dimeric glycoprotein exclusively produced by the granulosa cells of pre-antral and smaller antral follicles and is postulated to inhibit the recruitment of primordial follicles[87-90]. A gradual age-related decrease of AMH has been demonstrated, with peak levels during late puberty, a gradual decline during the reproductive years, and undetectable levels after menopause or bilateral ovariectomy[91-94]. Recent studies suggest a positive relation between AMH and time to menopausal transition and menopause[94;95]. Furthermore, AMH shows relatively steady values throughout the natural cycle[96;97], which simplifies the timing of the test and interpretation of the results.
The rationale behind the use of the above endocrine tests for assessing the ovarian reserve is based on their relation with female age and moment of menopause (see Figure 9)[93;94;98-101]. Studies relating the values of the endocrine ORTs with histological assessment of the ovaries, the 'gold standard' for assessing ovarian reserve, have hardly been performed. In one small, but well-documented study, three ovarian reserve tests were performed in 22 parous women, aged 36-42 years, in the cycle before they would undergo oophorectomy. After surgery, the number of follicles in the ovarian tissue was assessed. No statistically significant relation was observed between the number of counted follicles and basal FSH, CCCT and a second dynamic ovarian reserve test, the GnRH agonist stimulation test[102].

The second group of ovarian reserve tests consists of sonographic parameters of which *antral follicle count* (AFC) is the main one. Antral follicles can be made visible by transvaginal ultrasound and range in size from 2-10 mm. The number of antral follicles corresponds well with the number of primordial follicles in histological analysis[21;103]: the more follicles are available, the more follicles will

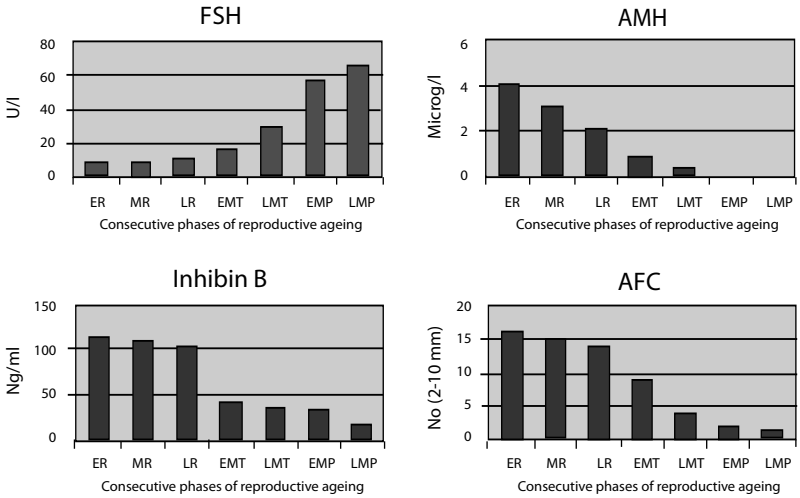


**Figure 9.** Schematic representation of the average levels in the early follicular phase of the menstrual cycle of 1) follicle stimulating hormone (FSH), 2) antral follicle count (AFC), 3) inhibin B and 4) anti-Müllerian hormone (AMH) in the various phases of reproductive ageing, i.e. early, mid and late reproductive phase (ER, MR and LR), early and late menstrual transition (EMT and LMT) and early and late postmenopausal phase (EMP and LMP). *(Adapted from Broekmans et al., Endocrine Reviews 2009;30:465-493)*

grow. Studies in fertile and IVF-treated populations show a close negative correlation between AFC and age, a positive relation between AFC and time to menopause (see Figure 9), and a positive relation between AFC and AMH (93;104-106). A disadvantage of this test is that AFC supposedly overestimates the total number of follicles in women with reduced ovarian reserve as a relatively larger proportion of follicles is recruited monthly[19;107]: as the total follicle pool size reduces, the proportion of visible growing follicles increases.

*3.3 Response to ovarian hyperstimulation in IVF treatment*

In *in vitro fertilisation* (IVF) treatment with controlled ovarian hyperstimulation, gonadotrophins are given in order to stimulate the growth of multiple follicles. The response to ovarian hyperstimulation may be regarded as a dynamic ovarian reserve test[72]. The number of retrieved oocytes in IVF decreases with female age (Figure 10)[108]. Compared to a 25-year old woman, the number of oocytes retrieved from a 41-year old woman is halved. The oocyte yield after hyperstimulation has been shown to be related to the size of the remaining follicle pool: women with a so-called 'poor response', often defined as an oocyte yield of <4 oocytes, are at increased risk of early menopausal transition and menopause[109;110]. Both AMH and AFC are positively related to the ovarian response in IVF treatment[111]. However, the exact number of oocytes retrieved from the same woman during consecutive IVF cycles may vary. In addition, a poor response is no proof of decreased ovarian reserve: younger women with an ovarian reserve within the normal range may occasionally experience a poor response, for instance due to relative underdosing of medication[112].

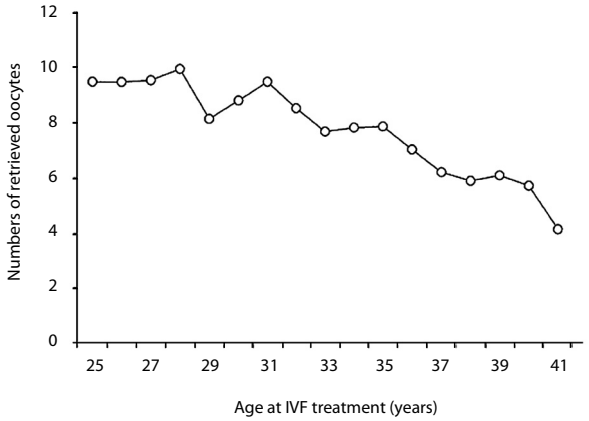


**Figure 10.** The mean number of retrieved oocytes with age at IVF treatment.
*(Adapted from De Boer et al., Human Reproduction 2004;19:58-65)*

*3.4 Iatrogenic and environmental factors influencing ovarian reserve*

In the physiological situation the number of oocytes present in the ovaries at any time during a woman's reproductive lifespan depends on the original size of the follicle pool and the atresia rate of those follicles. However, as stated earlier, multiple iatrogenic interventions and environmental influences may also affect oocyte quantity. The most tangible medical intervention reducing the size of the ovarian follicle pool is ovarian surgery. Women with a history of unilateral ovarian surgery have been suggested to have a lower response to IVF treatment and to enter menopause sooner[113;114]. In this thesis, a *history of ovarian surgery* is used as a dichotomous measure for decreased ovarian reserve. The best-known environmental factor influencing follicle pool size is *smoking*. Women who smoke have lower antral follicle counts, lower ovarian response to IVF treatment, and enter menopause earlier than their non-smoking peers[38;39;115]. These observations make clear that smoking reduces oocyte quantity. It is conceivable that smoking may also compromise oocyte quality, for instance by direct toxic effects[116]. Therefore, in studies on the relation between oocyte quantity and oocyte quality, measures relating to smoking (for instance, the number of pack years) cannot readily be used as parameters for oocyte quantity alone. In these studies, the possible confounding effect of smoking habits should be taken into account.

**4. Parameters of oocyte quality**

As described above, the concept of reproductive ageing stipulates that long before a woman's ovaries are depleted of follicles, a reduction in oocyte quality already compromises her fertility. It is assumed that the most important aspect of reduced oocyte quality with age is the occurrence of meiotic non-disjunction. A list of clinical parameters reflecting oocyte quality is given below.

*4.1 Live birth*

Both in the natural situation and in IVF cycles, pregnancy chances drop with female age (Figures 4 and 5). This trend is attributed to increased embryo loss due to chromosomal abnormalities[41]. The birth of a healthy child may be regarded as the ultimate proof of good oocyte quality. Obviously the opposite is not always true: in couples trying to conceive not all good quality oocytes will result in an ongoing pregnancy.

*4.2 Pregnancy loss*

The incidence of miscarriage increases exponentially with age (Figure 11)[117]. Miscarriage rates vary from 10% in a woman's early twenties up to over 80% in her late forties. Overall, cytogenetic abnormalities are found in 35-75% of all spontaneous abortion specimens[118-121]. The most common karyotypic abnormalities are autosomal trisomies[51]. The older the woman and the shorter the duration of pregnancy before miscarriage, the higher is the probability that the loss is caused by aneuploidy[50;51;119;122].


**Figure 11.** Increased risk of spontaneous abortion with female age.
*(Adapted from Nybo Andersen et al., British Medical Journal 2000;320:1708-1712)*

*4.3 Trisomic pregnancy*

In line with the findings in miscarriage tissue, the results of chorionic villus biopsy and amniocentesis show that the incidence of trisomic pregnancy decreases with the duration of pregnancy. The earlier the timing of prenatal diagnosis, the higher the number of trisomic pregnancies found[123;124]. Although most aneuploid conceptions result in early pregnancy loss, some lead to ongoing pregnancies. In total, 0.3% of live-born children are aneuploid[119]. With increasing female age, the incidence of live-born children with trisomy 13, 18 and most notably 21 (Down syndrome) rises[125]. Next to non-disjunction in the oocyte, trisomies may be caused by an unbalanced translocation or may be of paternal origin, but this is true for only a minority of cases (<10%)[126].

*4.4 Embryo quality*

IVF treatment offers a unique possibility of direct clinical assessment of oocytes. The oocyte has a key role in early embryo development, since the genome of the embryo is not activated during the first days after fertilisation and development up to that point largely depends on proteins and RNA stored in the oocyte[127]. Therefore, the morphology of the developing embryo may reflect oocyte quality. Embryos can be scored on a number of morphological features, including number and regularity of blastomeres, percentage of fragmentation, and the presence of multi-nucleated blastomeres; these scores may be added to form the cumulative embryo score[128]. *Embryo morphology scores* may be a useful marker for oocyte quality.

In recent years there has been a focus on aneuploidy detection in embryos as an expression of decreased oocyte quality. Indeed, with increasing female age, biopsied blastomeres from human embryos in IVF more often show aneuploidy[56;129;130]. However, these embryos also commonly show mosaicism, i.e. blastomeres of the same embryo differ in chromosomal content, implying that chromosomal analysis of one or two blastomeres does not adequately reflect the ploidy status of the oocyte the embryo originated from[56;131;132]. Blastomere biopsy is therefore not a suitable marker for oocyte quality.

**5. The clinical relation between parameters of oocyte quantity and quality**

Several studies have been performed assessing possible associations between the clinical parameters of oocyte quantity and quality described above. A systematic introduction to the findings thus far is given below.

*5.1 Ovarian reserve and ongoing pregnancy*

It is not clear whether age at menopause has a fixed relation with pregnancy chances earlier in life. One retrospective cohort study of 2,393 women indirectly suggests the existence of such a relation by revealing an association between self-reported fertility problems and earlier age at menopause[133]; the odds ratio for unwanted childlessness was 0.78 (95% confidence interval (CI) 0.64-0.94) per five years' higher age at menopause.

The relation between various ovarian reserve tests and ongoing pregnancy chances has been studied extensively in IVF populations. Recent meta-analyses on the subject show no clear predictive value of these tests (including FSH, inhibin B, various dynamic endocrine ORTs, AMH and AFC) for success rates in IVF treatment, independent of female age[72;134].

Less information is available on ovarian reserve tests and natural pregnancy chances. Five studies have been performed on this subject in general subfertile populations with conflicting results[82;135-138]. Scott *et al.* found that FSH and the CCCT had a statistically significant predictive value for spontaneous and treatment-related pregnancy, using cut-off levels determined in their own study population, which they assessed both prospectively and retrospectively[82;135]. Van Montfrans *et al.* could not confirm the predictive value of FSH for spontaneous or treatment-related pregnancy

in a small, nested case-control study[137]. The prospective cohort study of Van Rooij *et al.* showed no linear relation between FSH, inhibin B, AMH and AFC for spontaneous pregnancy[93]. In a large prospective cohort study of 3,519 subfertile couples, Van der Steeg *et al*. found a statistically significant increase in time to spontaneous pregnancy for basal FSH values from 8 IU/l onwards[136]. However, they labelled the clinical relevance of their findings as limited, when compared to other prognostic factors for spontaneous pregnancy, including female age.



In contrast to the ovarian reserve tests, the number of oocytes retrieved in IVF treatment with ovarian hyperstimulation indisputably has a predictive value for ongoing pregnancy. A poor response to hyperstimulation is highly predictive of low pregnancy chances[112;139;140]. If, next to the poor response, there is another sign of diminished ovarian reserve (e.g. previous poor response, abnormal result of an ovarian reserve test or advanced reproductive age) the prospects of an ongoing pregnancy decrease further compared to age-matched women with a normal response[112;139].

Less is known about the predictive value of a history of ovarian surgery for ongoing pregnancy. No definite conclusions can be drawn, but pregnancy chances do not seem to be clearly decreased for IVF-treated women with a history of unilateral ovariectomy[113]. The relation between smoking and pregnancy chances is better established: smokers have statistically significantly decreased pregnancy chances compared to non-smokers; this is true for both natural and IVF-related conception[141-145].

*5.2 Ovarian reserve and miscarriage*

Whether there is an association between the occurrence of miscarriage and the age at onset of menopause later in life has hardly been studied. In one large cohort study among menopausal women, the odds ratio for a self-reported history of at least one miscarriage was 0.89 (95% CI 0.79-1.01) per five years' later menopause. This implies that having ever had a miscarriage was associated, though not statistically significant, with earlier age at menopause[133].

Several studies are available on the relation between ovarian reserve tests and miscarriage[146-151]. From the results of these studies it is not clear whether FSH, AMH and AFC have a predictive value for miscarriage or not. The studies were performed in various populations of fertile, subfertile or IVF-treated women, were mostly of retrospective design, and/or included small numbers of subjects. Studies on the relation between ovarian response to IVF treatment with hyperstimulation and miscarriage are scarce and provide no evidence of the existence of such a relation[152-154]. No data are available on ovarian surgery and miscarriage risk. Finally, smoking has been shown to increase miscarriage rates[144;145].

*5.3 Ovarian reserve and trisomic pregnancy*

Two recent studies have been published on the occurrence of a trisomic pregnancy and age at menopause. Kline *et al.* compared 111 women with a spontaneous abortion with a trisomic karyotype with 226 women with a healthy live birth and 157 women with a chromosomally normal

pregnancy loss. Compared to the other two groups, the women with a history of trisomic pregnancy entered menopause approximately 1 year earlier (0.96 years, 95% CI -0.18–2.10)[155]. In the second study, 104 mothers of a child with Down syndrome entered menopause 0.7 years earlier than 121 control mothers with a healthy child[156]. In both studies the difference in menopausal age between cases and controls was not statistically significant.

Contradictory findings have been reported on the relation between trisomic pregnancy and the results of ovarian reserve tests. Van Montfrans *et al*. found that mothers of a child with trisomy 21 had significantly higher levels of FSH and lower levels of inhibin B than age-matched controls[157;158]. Kline *et al.* did not find statistical differences in FSH, inhibin B or AFC between women with a spontaneous abortion of a trisomic pregnancy than women with a chromosomally normal pregnancy[159]. In a third study, AMH-levels were measured in samples from a prenatal screening programme[160]. No statistically significant difference was found between 25 women with a Down syndrome pregnancy compared to 125 matched controls. Nasseri *et al*. karyotyped miscarriage tissue and found higher FSH and/or estradiol levels in women with an aneuploid spontaneous abortion than in women with a euploid spontaneous abortion[161]. These findings were, however, not confirmed by two other studies on the same subject[162;163]. The relation between ovarian response to IVF treatment and trisomy risk has not been studied yet.

In a case-control study, women with a child with trisomy 21 significantly more often had a history of ovarian surgery or congenital absence of an ovary than controls (7/189 cases versus 1/329 controls)[164]. These findings correspond with classic mouse studies, which showed an increased incidence of aneuploid embryos in ageing mice after unilateral ovariectomy[165]. Finally, the relation between smoking and trisomic pregnancy has been studied widely, yielding conflicting results but, in general, there does not seem to be a clear-cut association[125].

*5.4 Ovarian reserve and embryo morphology*

Although there is no information on embryo morphology and age at menopause, three recent studies have shown that AMH is not related to the proportion of morphologically top quality embryos in IVF-treated women[148;166;167]. In addition, Smeenk *et al.* found no predictive value of various ovarian reserve tests for the morphology of transferred embryos[168]. In contrast, Silberstein *et al.* found that AMH, but not FSH, was associated with the morphological qualities of the embryos transferred[169]. The last two studies did not comment on the relation between ovarian reserve test results and the proportion of morphologically top quality embryos.

Devreker *et al*. found no correlation between the number of retrieved oocytes and mean morphological embryo score[170]. Likewise, De Sutter *et al.* found no difference in the proportion of embryos of good morphological quality between women with a low number of retrieved oocytes in conventional IVF (<5) compared to normal responders[152].

Embryo morphology in relation to a history of ovarian surgery has not been studied. Finally, the morphological features of the embryo do not seem to be related to women's smoking habits[116;171-173].

## 6. Aim and outline of the thesis

From the above it is clear that there is no consensus in the literature on the clinical relation between oocyte quantity and oocyte quality. If, indeed, oocyte quantity and quality are related, the estimated size of the remaining follicle pool may have predictive value for (future) fertility and trisomy risk. Especially since the age range in the onset of menopause shows that oocyte quantity differs widely between peers, the size of the remaining follicle pool may be more informative than age alone. To examine this relation we decided to study associations between clinical parameters of oocyte quantity and quality that have not been completely elucidated yet.

The primary *aim of this thesis* was to determine whether a woman's remaining number of oocytes is related to the quality of those oocytes. Secondly, if there is a relation, we aimed to add to the identification of possible predictors for (future) fertility and trisomy risk. An overview of the chapters in this thesis is given below and is depicted in Figure 12.

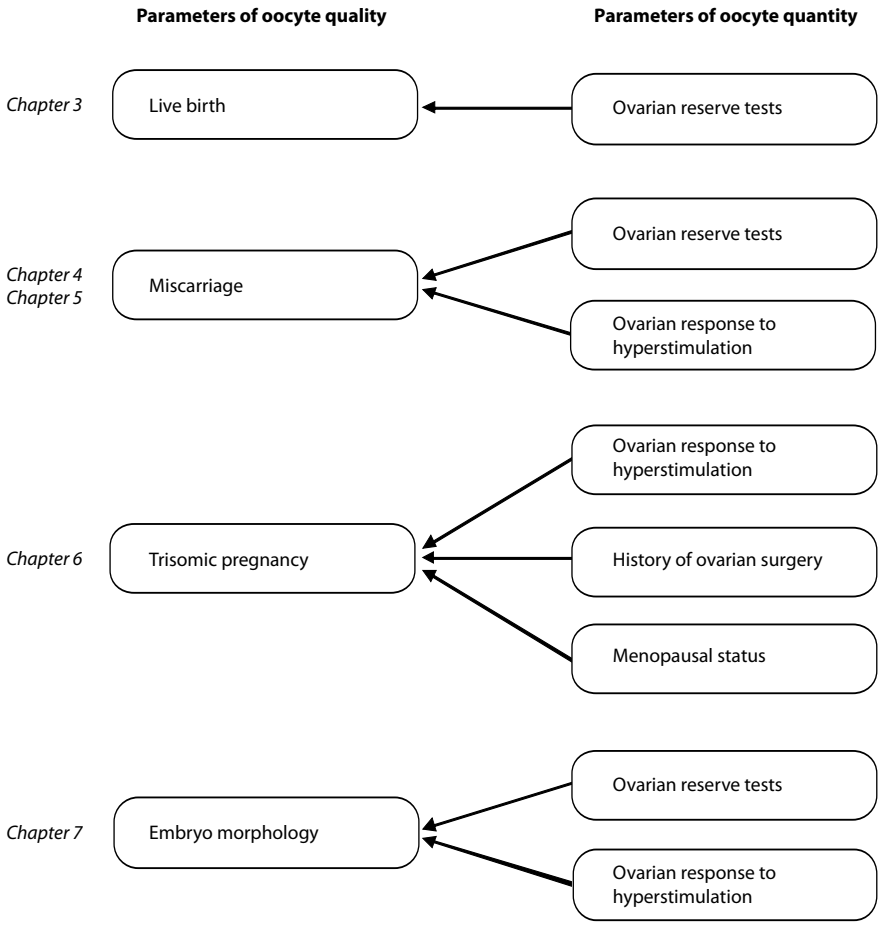


**Figure 12.** Graphical depiction of the associations between parameters of oocyte quantity and quality studied in the current thesis.

*Chapter 2* provides background information on the relations between several ovarian reserve tests in subfertile ovulatory women. The focus of the study is on the relation between various antral follicle sizes, female age and the results of endocrine ovarian reserve tests, including FSH, inhibin B and the CCCT.

*Chapter 3* describes a prospective cohort study in a subfertile ovulatory population. It was designed to determine whether FSH, inhibin B, CCCT and AFC have predictive value for the occurrence of natural conception resulting in live birth and, if so, whether they add value to known prognostic factors for natural pregnancy, including female age.

*Chapter 4* describes a subanalysis in the prospective cohort used in the previous two chapters. The aim was to assess the predictive value of FSH, inhibin B, CCCT and AFC for the occurrence of miscarriage.

*Chapter 5* describes a study in a nationwide retrospective cohort of Dutch women undergoing IVF treatment. The aim was to evaluate whether women who conceive after a poor response in their first completed IVF cycle have an increased risk of miscarriage compared to their peers with normal response.

*Chapter 6* describes a case-control study in the same nationwide retrospective cohort (Chapter 5). The study aimed to determine whether IVF-treated women with a trisomic pregnancy more often had a history of ovarian surgery and a lower response to ovarian hyperstimulation than controls. Subsequently, these women were followed to assess whether the trisomy cases had more often reached menopausal transition or menopause at the end of the study period than controls.

*Chapter 7* describes a prospective cohort study of IVF-treated women of advanced reproductive age. The aim was to determine whether FSH, inhibin B, AMH, AFC and the ovarian response to hyperstimulation have predictive value for embryo morphology.

*Chapter 8* provides an overview of the main results of the studies presented in this thesis and a discussion in which is reflected on the two aims of this thesis, the possibilities for future research, and the current trend to postpone childbearing.