DETERMINANTS OF FEMALE FECUNDITY AND OUTCOME OF PREGNANCY

epidemiological cohort studies to the effects of age, biometry and life-style habits

Boukje Zaadstra

Correspondence:
B.M. Zaadstra
TNO Institute for Preventive Health Care, Child Health Division,
P.O. Box 124
2300 AC Leiden
the Netherlands

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DETERMINANTEN VAN VROUWELIJKE VRUCHTBAARHEID

EN ZWANGERSCHAPSRESULTAAT

epidemiologische cohort studies naar effecten
van leeftijd, biometrie en leefgewoontes

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The teacher opens the door
..... and you walk through yourself.
(Chinese proverb)

Voor alle vrouwen
die nog moeder
willen worden .....

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Chapter 1

INTRODUCTION

1. INTRODUCTION

Becoming a mother is no more a matter of destiny only, but increasingly a matter of choice as well. Until recently, having children was as natural as eating, drinking and dying. Children just "happened" during the course of (married) life. However in modern societies life does not just "happen" any more; life is organized around the assumption that men and women should feel free to choose the goals in their lives and the ways to achieve such a goal, in other words: to plan their lives ahead. With the general availability of education, it was possible for women also to achieve a professional goal through a career path.
With the availability of contraception, especially since the introduction of the pill in the sixties, it was possible to *design* a reproductive "career" as to the question: Do I want children at all; if yes how many and when?

In order to observe changes in societal trends, the disciplines of epidemiology and demography are more suitable than the medical sciences. A medical doctor has been educated for the "exception", the individual patient approach and not for the "rule".
Demographic data show that of those Dutch females who are now fifty years of age or older, about 10% have remained childless. Of those, now 40 years old, 15% has not had a child yet. The Netherlands Central Bureau of Statistics predicts a further increase in childlessness at 40 to about 20-25% for females born after 1965. Because there are no indications that infertility among females, who are currently 40 years of age, is higher than in previous generations, it seems that voluntary childlessness has risen considerably among women.[1] Thus an increasing number of women appear to plan a career of *non*-reproduction.
However the women who do plan a reproductive "career" do not only opt for fewer children than in the past, but also that the child should be perfect, in impeccable state. A realm of prenatal screening services are developed to distinguish during early pregnancy between the affected and unaffected fetus; leaving the subsequent decision to carry the pregnancy to term to the individual parents. In other words a desired pregnancy does not necessarily lead to a desireful pregnancy outcome.

In modern life reproduction itself is broken up in several decisions tracks. Reproduction has now become "plannable", meaning active intervention by individuals to prevent fate and discomfort now and in the future. However planning and biology do not necessarily match: the planning capacity for pregnancy is also determined by the biological potential of both partners; namely the fecundity of the woman and of her partner and the subsequent ability to carry

a pregnancy to term. This is particularly of importance in delaying childbearing, as an increasing number of women do in western societies. In the Netherlands, the average age at which a woman bears her first child has risen by 3 years since 1970 and is now 28 years. The Netherlands is unique in this respect, nowhere else in the world do women become a first time mother so late. Postponing pregnancy or deciding never to have children at all seems to be highly correlated with participation in the labour force and the woman's level of education. Initially, postponing pregnancy or deciding against parenthood mainly occurred among the most highly educated, but this trend can now also be detected among women with lower education.[1]

Demographically this leads to two main effects. Childbearing is compressed into the later half of the reproductive life-span; the phase where increasing age works against the probability of conception. Thus leading to amounting pressure to shift the endpoint of the reproductive life span even beyond menopause. The technological possibilities, oocyte donation and the like, are already there to fulfil this scenario.
The other effect is that the childbearing period will be relatively short; with an average of 2 children, the birth interval between children will be only a few years, so that professional responsibilities can be resumed after a couple of years "when little hands are not demanding".
So two "compression" mechanisms account here: compression of reproduction into the later life-span and compression of reproduction into a shorter time-interval within a woman's reproductive career. The explanation for these mechanisms lies clearly in the virtual impossible combination, under the conditions in the Netherlands, of motherhood with a job or a study, and in the threatened loss of personal freedom. Due to this, many women decide at younger ages not to have children at all. However, over time the biological clock ticks away towards the point of no return (the menopause), therefore many women do change their minds at a later age towards childbearing.

The so called 'obstetric geriatrics' will become common place in the future. One great advantage in being older and therefore more experienced in coping with people can be that a woman is more skilled and confident in finding out what she needs to know about the medical system, manoeuvring her way through it and getting what she wants. She is more likely to know where to go to get accurate information and how to sift the evidence.[2]
Popular belief says that 'evidence' should not be available to a woman contemplating pregnancy, because it makes her unnecessarily anxious. However if she is to understand treatment or investigations proposed, she needs information

if she is to act as a self-conscious adult. Part of preparing for a baby is to think through the way she lives, so she can provide the best start possible for a new human being. This is where epidemiology falls into place.

Terris describes epidemiology as the study of health of human populations. Its functions are to discover the factors which affect health, in order to provide the scientific basis for the prevention of disease and injury and the promotion of health. Prevention is far more important than treatment.[3] Therefore public health epidemiology is geared towards identifying determinants, which are in potential modifiable and do not serve the purpose of clarification or explanation only. Thus the question is not primarily: whether disease Z is caused by factor A, B or C, but whether by manipulating factor A the risk on disease Z is altered (i.c. lowered), within a population so that health can be promoted.[4] Within epidemiology of reproductive health the same holds true. The search is for modifiable determinants of reproductive health of women. The choice for women as a study-group is a pragmatic one: first of all it are women who can get pregnant and carry a pregnancy to term; secondly the epidemiology of reproductive health of men is still in its infancy.

The goal of this thesis is to investigate the effect of modifiable determinants on probability of conception and outcome of the pregnancy. Three domains of modifiable determinants were chosen, all of relevance to women in affluent societies: maternal age, biometry and life-style habits.

1. **Maternal age:**
   Female fecundity is generally acknowledged to decrease with increasing age, but the beginning of the fall in fecundity has not been pinpointed to a specific age.[5] Such information is important to the increasing number of women who are delaying childbearing. The objective was to study the age of the start of the fall (critical age) in fecundity; the probability of a pregnancy leading to a healthy baby taking into account the age of the woman; and combining these results, the relation between age and the probability for a non-pregnant woman to achieve a pregnancy leading to a healthy baby in the next menstrual cycle.

2. **Biometry:** (Body fat and fat distribution)
   Human obesity, which is generally defined as an excessive storage of body fat, is a major public health problem in affluent societies. Estimates of its prevalence range from 10-50% or more in the adult population, depending upon the criteria used to define obesity. It is well known that

obesity is associated with a large number of metabolic complications, including coronary heart disease, cerebrovascular diseases, non-insulin dependent diabetes mellitus, gallbladder disease, hormone dependent neoplasms such as carcinoma of endometrium.
The distribution of body fat plays a critical role in this context. An abundance of visceral fat (i.e. intra abdominal fat as indicated by a high waist to hip ratio) is a stronger predictor of specific metabolic aberrations than overall body fatness. It has been suggested that sex hormones may play an important role in the regulation of body fat distribution, due to their specific effects on adipocyte metabolism in the different regions of the body.[6,7]
Menstrual disorders have been found to be related to obesity as well as to a predominance of fat in the abdominal region. It is not known however, whether body fat distribution has any effect on the ability to conceive, independent of weight and menstrual disorders.
Therefore the purpose of this study was to investigate the effect of body fat distribution in women of reproductive age on fecundity.

3. **Life-style habits:**
Alcohol drinking, cigarette smoking and coffee drinking.
These life-styles are typical of women in affluent societies. In 1986, at the start of the study, app. 45% of women in the Netherlands of reproductive age did smoke. It are especially the younger women (< 25 yrs) who do smoke the most in comparison with older age groups.
However, older and higher educated women do drink more than younger and lower educated.[8] Caffeine drinking has been widely accepted by all age groups of women. These life-style habits have so far been studied during pregnancy as to their effect on pregnancy outcome. In particular smoking decreases the birthweight of the baby and excessive alcohol drinking can lead to the so called fetal alcohol syndrome. The effect of caffeine varies from negligible to inconsistent.
Little is known however about the effects of these life-styles on fecundity itself. Smoking has been associated with delayed conception; the effect of excessive alcohol drinking seems to increase subfecundity and the reports on effect of caffeine intake are inconsistent. The purpose of this study was to investigate whether these life-styles affected the probability of conception and the outcomes of pregnancy as well.

What makes these determinants in potential modifiable? Maternal age, biometry and life-style habits are all components of behaviour and therefore inducible for

change through intervention, whether it be on the societal and/or on the individual level.
The choice for early or late childbearing could change by altering societal conditions. Fatness and fat-distribution could change through intervention by weight loss programs (though the effect of visceral fat loss on conception rates warrants further investigation). Life-style habits like smoking or drinking are changeable too under certain conditions.

To study whether these determinants affect female fecundity and subsequent outcome of pregnancy, a study design is required following women in time from wanting and trying to become pregnant until the eventual outcome of pregnancy. However, women of reproductive age wanting to become pregnant cannot be distinguished, by some characteristic, from other women. In depth interviews of a large population of women and close monitoring of every subsequent menstrual cycle would be needed, leading to costly, time-consuming studies. Other possibilities like carrying out a retrospective study among women already having given birth to a child leads to over-representation of fecund women in the study population, while studies of women attending fertility clinics will lead towards a selection of the subfecund. A choice for one of these study populations would lead to selection bias, posing a methodological problem because the composition of the study population is directly related to the outcome variable at hand. This cannot be corrected by any analysis whatsoever.
In contrast, women wanting to become pregnant but not having achieved a pregnancy because of their partners' infertility and therefore seeking donor insemination treatment, provide an excellent opportunity to prospectively study determinants of female fecundity and subsequent outcome of pregnancy.
The advantage is that such a study population is representative for the reproductive health of women of reproductive age because they come to treatment due to infertility of their *partner*. The exposure to the "male" factor (insemination treatment) is more or less standardized; so that the "pure" effect of the determinants can be studied.[5]

Three cohort studies were undertaken

1. The effect of maternal age on fecundity and pregnancy outcome was studied by a retrospective cohort design of the medical records of 1637 women entering artificial insemination programs in 2 clinics from 1973 onwards until the protocols changed from treatment with fresh semen to frozen semen. Of the 1637 women, 751 women fulfilled the selection criteria: being married to an azoospermic husband, nulliparous and never having received donor insemination before. The results are presented in

chapter 3. However biometrical parameters as well as life-styles were not systematically recorded at the start of treatment at that time.

2. Therefore a second cohort study was set up prospectively in one clinic, where all women and their partners requesting donor insemination from 1986 on were asked to participate in the study (to fill in life-style questionnaires and to have biometric measures taken). Up to 1990, the closing year of intake, about 1000 women and their partners participated; blood and early morning urine samples were taken to be stored in a biological bank for future research purposes.
Due to limited resources we could only follow the 500 women entering in 1986 and 1987 (2 year cohorts) during their subsequent insemination cycles and verify whether conception occurred. The results are presented in chapters 4 and 5.

3. Among the 259 women with confirmed conceptions in the second cohort study, another study was carried out. In October 1990 a self administered questionnaire was sent to retrospectively assess alcohol drinking, smoking and coffee drinking before and during pregnancy, as well as to validate information about the course, duration and outcome of pregnancy, including information about the health of the child after birth into the present time. The effect of the determinants on fecundity as well as pregnancy outcome are presented in chapter 6.

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Chapter 2

THE STUDY POPULATION

THE STUDY OF RISK HABITS IN REPRODUCTIVE AND PERINATAL EPIDEMIOLOGIC RESEARCH:
the use of a donor inseminated population of women

Boukje M. Zaadstra[1]
Jan Karbaat[2]
Egbert R. te Velde[3]
J. Dik F. Habbema[4]
Paul J. van der Maas[4]

1 TNO Institute of Preventive Health Care, Child Health Division, Leiden, the Netherlands
2 Cryo Biological Laboratories Bijdorp, Barendrecht, the Netherlands
3 Department of Obstetrics & Gynaecology, University Hospital, Utrecht, the Netherlands
4 Department of Public Health and Social Medicine, Erasmus University Medical School, Rotterdam, the Netherlands

2. THE STUDY POPULATION

SUMMARY

In this study we assess whether a population of donor inseminated women (ADI) provides an efficient alternative to an open population of women of reproductive age to study the effects of risk habits (cigarette smoking, alcohol consumption and other determinants) on reproductive and perinatal health. An ADI population can be prospectively monitored before and after pregnancy; women enter the programme because of their husband's infertility. In the pilot clinic every couple asking for first insemination was requested to fill in a self-administered questionnaire on risk habits and the use of medicine. Medical histories of both the man and woman were taken. In 1986 and 1987, 519 women entered the programme. The response rate was 95%. Distribution of the exposure variables of the women: currently smoking (52%) and currently drinking (68%) compared well with a general population survey from the town of Rotterdam. With regard to their risk habits and reproductive health, the population of women married to infertile men seems representative of women of reproductive age from an open population.

INTRODUCTION

We are interested in studying the effect of risk habits (life-styles and other determinants) on reproductive health of women and their pregnancies. It is known already that smoking by the pregnant mother results in low birthweight of the baby.[1,2] Less is known about the effect of smoking itself on conception and the early phases of gestation.[3,4] To address this and related questions we require a population which could serve as a data-base for studying effects on the reproductive life-span, before and after conception.
In this paper we explain why a population of donor-inseminated women (ADI) can serve this purpose. (The abbreviation ADI is preferred to AID because recent experience shows that at first glance there is confusion between AID and AIDS).

General theoretical considerations

The optimal design for studying processes which develop over time is to set up a prospective study: start the study before conception and extend the follow-up of the women until a reproductive event takes place.
The greatest problem in the set-up of such a prospective study in an open (natural) population is the acquisition of complete and reliable information

related to reproductive health. Data are needed with regard to infertility of men and women before the woman becomes pregnant. This implies that every month or cycle the investigator needs to gather information about intercourse timing and frequency during the cycle, menstrual patterns of the woman and onset of ovulation, cycle irregularity, etc. Apart from the fact that such information belongs to the domain of privacy, the information itself can only be collected with enormous effort and expense.

A practical solution is to find a population of women of reproductive age, who are themselves normal in respect of reproduction and are already being monitored before and after conception. Women with infertile husbands requesting artificial donor insemination fulfill these criteria. Therefore, this population of women may provide a unique chance to study the effects of various exposures on reproductive and perinatal health.

Information readily available in a donor inseminated population of women

1 A well-defined population; the denominator is known and consists of women who all want to become pregnant.

2 There are no undesired pregnancies, no induced abortions occur for that reason. The number of spontaneous abortions will usually be known; unless occurring very early, within 2 weeks of gestation, before a pregnancy test is performed.

3 Normally a woman is fertile during a limited period before ovulation. Since in most ADI programmes the menstrual cycle is monitored, the fertile period can be estimated retrospectively.

4 The times of insemination in the course of the cycle are known and can retrospectively be related to the fertile period.

5 The quality of the donor semen, in terms of density and motility, is usually known.

6 The day of conception is approximately known and the conception itself is confirmed within 14 days by pregnancy tests.

7 The course of pregnancy from conception until delivery is usually known. This gives the possibility of creating a fetal life-table.

8 The moment of birth is known. In subtracting day of birth from day of conception one can get an exact figure of the duration of the pregnancy itself.

9 The course and mode of delivery and the health of the baby at birth are usually known.

The composition of a donor inseminated population of women

Women married to infertile (azoospermic) men, have no chance of becoming pregnant by their husbands. Therefore, the distribution of fertility potential among women presenting themselves for donor insemination, is likely to reflect the distribution of fertility potential in a natural female population of reproductive age.

Women married to subfertile men (oligo-asthenospermia) have a small chance of becoming pregnant by the husband. One may expect that a proportion of women with a high fertility potential may compensate for the low fertility potential of their husband and become pregnant. Therefore, the proportion of women left to present themselves for donor insemination is likely to represent a proportion of women with lower fertility potential in comparison to the distribution in an open, natural population. This will lead to selection bias.

From a clinical point of view it seems reasonable to perform a thorough fertility-check-up of the women before they start ADI. From an epidemiological point of view, this routine will lead to an increase in the proportion of fertile women starting treatment. Only presumably highly fertile women will enter the programme whereas the less fertile might be discouraged from entering the ADI-programme at all. This will contribute to selection bias.

THE ADI POPULATION UNDER STUDY

We decided that the advantages of studying a donor inseminated population were attractive enough to contact an ADI clinic for collaboration. This clinic, Bijdorp Centre near Rotterdam, is the biggest of its kind in the Netherlands and adheres to the following policy: the real patient is the man. It is the husband who has a fertility problem, and not the wife. Therefore women are accepted into the ADI-programme under the principle: 'she is fertile, unless proven otherwise in the course of donor insemination therapy'. This means that after six unsuccessul cycle the woman is referred back for a thorough fertility check-up. After a number of unsuccessful cycles the results of the fertility check-up offer the investigators the possibility of subdividing the total population of women at a later date into fertile, subfertile and probably infertile groups.

Donor semen is used only from married men with proven fertility with at least one healthy child. Extensive blood and semen testing is done before a donor is accepted. Four to six weeks after the intake an appointment is made for the first insemination.

If after 2 weeks the expected menses does not occur, a pregnancy test is carried out to confirm a pregnancy. If the test is positive the woman is referred back to

her specialist, with a request for information about the course and result of the pregnancy.

The prospective study protocol for the study of risk habits

In collaboration with the clinic 'Bijdorp Centre', a prospective cohort study was designed and started in January 1986. Approximately 250 couples are referred each year for a first ADI treatment.

All new couples coming to the clinic are requested to complete a questionnaire about their smoking and drinking habits and their medicine use. In addition, questions are asked about occupational exposures, coffee and tea consumption, the prior use of contraceptives, known infertility problems of relatives, and stress-related factors as perceived by the woman.

A structured medical history and anthropometric measurements of each man and woman were taken. This includes a short physical examination. Information on the extent of male infertility is given by the referring specialist. The woman is asked about her cycle characteristics, basal body temperature charts, parity, medical history of diseases and abdominal operations. Blood testing is done to detect viral and other infectious diseases (Chlamydiae, Hepatitis B, Toxoplasma, HIV, etc.). One sample of serum is kept for storage in a biological bank at -20°C. Unfortunately, so far no satisfactory marker for alcohol intake in stored blood is known. We plan to keep the blood stored for other reasons, including the possibility of testing future hypotheses in relation to reproductive events.[5]

At the first insemination we collect urine samples and toenail clippings from the women; these will be stored in a biological bank. The collected urine samples of the women provide us with the means of measuring the concentration of cotinine (the main metabolite of nicotine) in the urines of smokers, non-smokers and passive smokers; the results enable validation of the answers received on the questionnaire.

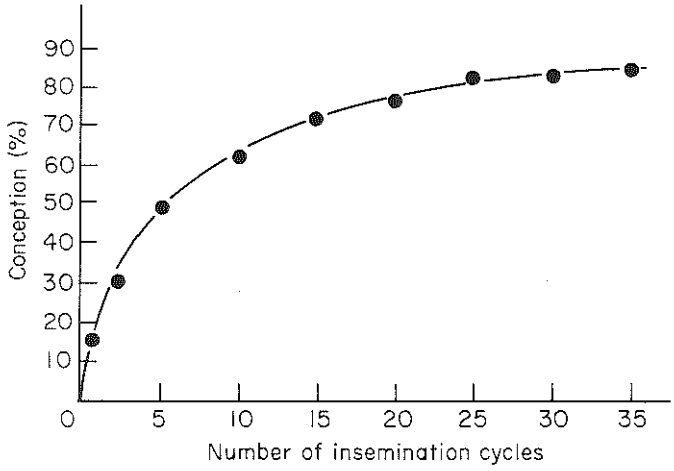
From toenail clippings we can measure the concentration of trace-elements, specifically cadmium and selenium. This gives us the ability to test hypotheses relating concentrations of trace-elements to subsequent conception or reproductive outcomes. Toenail clippings of the two big toes represent the metabolised trace-element situation prevailing 6 months before the clipping date.

PILOT STUDIES

The plans for this prospective study were based on the results of two pilot studies. The first concerned a retrospective analysis of 659 women in the period 1974-1978, starting ADI therapy for the first time,[6] the second concerned the distribution of risk-habits in a population of donor inseminated women in 1986-1987.

From the first pilot study, we calculated the cumulative probability of a first ADI conception by life-table analysis (fig. 2.1). It can be seen that after six insemination cycles a woman has close to a 50% probability of becoming pregnant; alternatively one may say: of 100 women starting ADI therapy, after 6 cycles about 50 will be pregnant.

Figure 2.1 Cumulative probability of conception in 659 women starting ADI therapy

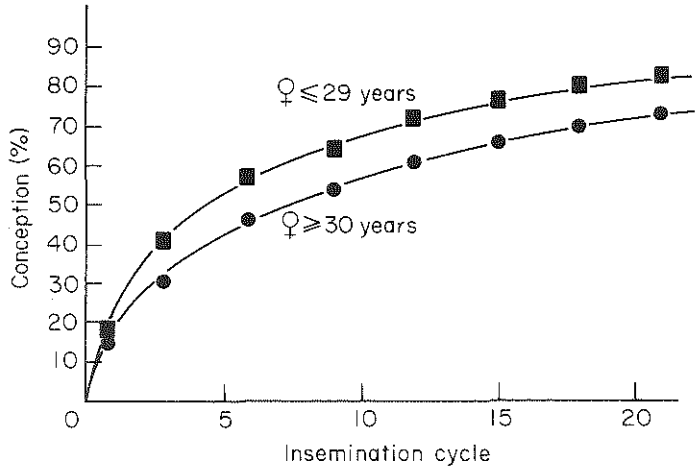


Up to the sixth cycle the cumulative probability of conception rises very fast; up to the 15th cycle the curve slows down and after the 15th cycle the curve more or less stabilises at approximately 70% to 80%. The slope of the curve is determined by the distribution of fertility-related factors in the starting population for ADI therapy. This means that a clinic with very stringent women fertility-related entry criteria will have a steeper slope than we found in our clinic, where fertility has not been investigated at the start, but after six unsuccessful cycles.

We were also interested in the effect of age of the woman on the probability of conception, as a possible confounder. The youngest woman starting ADI treatment was 19 years old, the oldest 42. We divided the population into two age

categories: 406 women younger than 30 years old, 253 women aged 30 or older (fig. 2.2).

Figure 2.2 The effect of age of the woman on the probability of conception



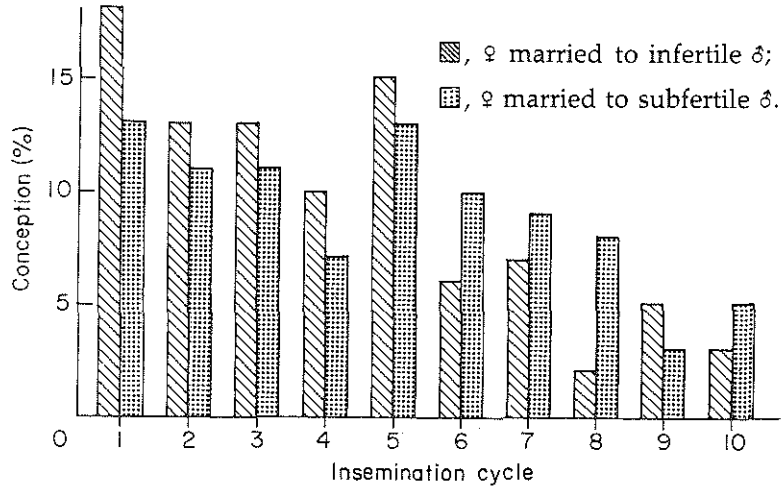
Women younger than 30 years old had a systematically higher probability of conception than older women.[7,8]
If we compare the cumulative conception probabilities after six insemination cycles between four age groups, the relationship with age is even greater (table 2.1). Women aged 35 or more seem to be at the greatest disadvantage.

In figure 2.3 we compare the conception probability per cycle of 369 women married to absolutely infertile (azoospermic/aspermic) husbands and 253 women married to subfertile (oligo/asthenospermic) husbands. Omitted are 37 women in the programme for other reasons.

Table 2.1 The effect of age of the woman on the probability of conception

Age group	Number of women	Cumulative conception probability after 6 cycles
≤ 24	81	58%
25-29	325	56%
30-34	204	48%
≥ 35	49	38%
Total	659	53%

Figure 2.3 Time to conceive and the degree of infertility of husband



During the first five insemination cycles women married to infertile partners have a higher conception probability than women married to subfertile partners. From the fifth cycle onwards, the subfertile 'catch up'. Thus women married to subfertile partners do indeed get pregnant, but need far more time; so 'time to conceive' is longer than for women married to infertile husbands. (In consequence, if a similar clinic were to adopt a policy of stopping ADI treatment after, say, six cycles, women married to subfertile husbands would be at a disadvantage).

Our purpose in the second pilot study was to assess the distribution of exposure variables such as smoking and drinking in the population starting ADI for the first time.[9] In 1986 and 1987, 519 women entered the programme: the response rate to the questionnaire was 95%.

One could argue that women who are so anxious to become pregnant that they go to the trouble of entering an ADI programme, will avoid any risk habit that is known to be inversely related to health, such as smoking and drinking. Thus, the exposure variable might be present only in a small proportion of women entering an ADI programme. However, to the question at intake 'Do you currently smoke or drink alcoholic beverages?' we received the following answers: smoking, yes in 52% (270/519); and drinking, yes in 68% (355/519).

These figures compare reasonably well with the results of a general population survey in Rotterdam in 1983[10] (41% of women smoked; 71% of women drank alcoholic beverages).

CONCLUSIONS

The study of the effects of risk habits on reproductive and perinatal health requires a well-described, well-monitored population; open populations do not lend themselves easily to such studies. Our conclusion, based on the results of the two pilot studies, is that such a study is feasible and practical with a donor inseminated population of women.

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Chapter 3

MATERNAL AGE

DELAYING CHILDBEARING:

effect of age on fecundity and outcome of pregnancy

Boukje M. Zaadstra[1]
Caspar W.N. Looman[2]
Hans Alsbach[3]
J. Dik F. Habbema[2]
Egbert R. te Velde[3]
Jan Karbaat[4]

1 TNO Institute of Preventive Health Care, Child Health Division, Leiden, the Netherlands
2 Department of Public Health and Social Medicine, Erasmus University Medical School, Rotterdam, the Netherlands
3 Department of Obstetrics & Gynaecology, University Hospital, Utrecht, the Netherlands
4 Cryo Biological Laboratories Bijdorp, Barendrecht, the Netherlands.

3. MATERNAL AGE

ABSTRACT

Objectives
To study the age of the start of the fall (critical age) in fecundity; the probability of a pregnancy leading to a healthy baby taking into account the age of the woman; and, combining these results, to determine the age dependent probability of getting a healthy baby.

Design
Cohort study of all women who had entered a donor insemination programme.

Setting
Two fertility clinics serving a large part of The Netherlands.

Subjects
Of 1637 women attending for artificial insemination 751 fulfilled the selection criteria, being married to an azoospermic husband and nulliparous and never having received donor insemination before.

Main outcome measures
The number of cycles before pregnancy (a positive pregnancy test result) or stopping treatment; and result of the pregnancy (successful outcome).

Results
Of the 751 women, 555 became pregnant and 461 had healthy babies. The fall in fecundity was estimated to start at around 31 years (critical age); after 12 cycles the probability of pregnancy in a woman aged > 31 was 0.54 compared with 0.74 in a woman aged 20-31. After 24 cycles this difference had decreased (probability of conception 0.75 in women > 31 and 0.85 in women 20-31). The probability of having a healthy baby also decreased - by 3.5% a year after the age of 30. Combining both these age effects, the chance of a woman aged 35 having a healthy baby was about half that of a women aged 25.

Conclusion
After the age of 31 the probability of conception falls rapidly, but this can be partly compensated for by continuing insemination for more cycles. In addition,

the probability of an adverse pregnancy outcome starts to increase at about the same age.

INTRODUCTION

Female fecundity (the ability to conceive) is generally acknowledged to decrease with increasing age, but the beginning of the fall in fecundity has not been pinpointed to a specific age. Such information is of importance to the increasing number of women who are delaying childbearing. In naturally selected populations studying the decrease in fecundity caused by biological factors is confounded by diminished sexual activity with age and possibly also by a decrease in male fertility. Schwartz and Mayaux[1] studied the age effect in women treated by artificial donor insemination. Their data suggest that reduced fecundity starts around the age range 31-35, but their follow up time of 12 cycles was relatively short. Older women who continue treatment for a much longer period may eventually conceive[2]. Moreover there is evidence that advancing maternal age has an adverse effect on the outcome of pregnancy because of a higher abortion and perinatal mortality.[3]

Our study of a cohort of women receiving donor insemination was undertaken to examine the age of the start of the fall in fecundity (critical age), the probability of a pregnancy leading to a healthy baby taking into account the age of the woman, and the age dependent probability of getting a healthy baby, by combining the critical age and the probability of a pregnancy leading to a healthy baby.

SUBJECTS AND METHODS

Two fertility clinics (A and B) serving different geographic areas participated in the study. Women were referred to the clinics by a general practitioner or a specialist. The source population consisted of all women who attended the clinics for artificial donor insemination between January 1973 and the years when the protocols were changed from treatment with fresh semen to frozen semen (1980 for clinic A and 1986 for clinic B).

A total of 1637 women entered the artificial insemination programme in the two clinics, 1036 in clinic A and 601 in clinic B. Out of these, 751 women satisfied the selection criteria - that is, women who were married to an azoospermic husband, were nulliparous, and had never received artificial insemination before.

Insemination procedures were usually conducted in every subsequent menstrual cycle. Women were inseminated intracervically, and timing of insemination was estimated on the basis of the basal body temperature chart and by judging the quality of cervical mucus. On average the number of inseminations per cycle was three for clinic A and two for clinic B. Fresh semen was used from donors aged 25-45 with a proved fertility (having fathered at least one child) and with sperm properties satisfying the World Health Organisation criteria.
In clinic A the specialists were free to prescribe supplementary treatment such as induction of ovulation by clomiphene if a woman did not conceive after a few cycles.
In clinic B induction of ovulation was largely confined to women who proved to have anovulatory cycles or who had very long cycles during an observation period before treatment.
If induction of ovulation was started the treatment was continued in subsequent insemination cycles. If a woman did not conceive hysterosalpingography was performed after the sixth cycle and laparoscopy after the twelfth cycle.
All women were followed until the end of treatment with artificial insemination. The end was defined as the result of each woman's last insemination cycle, being either a confirmed pregnancy (success) or stopping treatment without pregnancy (failure). Only first conceptions as a result of the artificial insemination were used for the analysis. Fifteen women who dit not report the results of their last insemination cycle were recorded as a failure up to their last but one cycle.
If menstruation did not start after two weeks the women were instructed to contact the clinic for a pregnancy test. If the test was positive the woman was assumed to be pregnant and was referred to her family doctor. Women were asked to report the outcome of pregnancy. Successful pregnancy was defined as a pregnancy leading to the birth of a healthy child.

Methods of analysis

The cumulative probability of conception by insemination cycle was calculated using Kaplan-Meier estimates for the two clinics separately and for four age groups[4]. Proportional hazard regression analysis was used for analysing the relation between age at first insemination and probability of conception[5]. We have used the term hazard as shorthand for the more informative but longer term "pregnancy rate per cycle." We fitted models with a gradual fall of fertility (see model 1.2 in appendix) and a model that estimates a constant initial rate of pregnancy, an age at which the decrease begins (critical age), and the rate of decrease after that age (see model 1.3 in appendix). Though model 1.2 describes the biological pattern of the fall in fertility, model 1.3 would answer the question of the practising clinician - namely, at what age does the fall start?

The dependency of the probability of a successful pregnancy on age was analysed by logistic regression methods[6] (see model 2.1 in appendix).

RESULTS

Fecundity

All 751 women were followed until the end of treatment; 316 (71%) women in clinic A and 239 (78%) women in clinic B became pregnant. Only 15 women did not report the result of their last insemination cycle. The last intake of women treated with fresh donor semen took place in 1985. None of these women were still being treated at the end of 1989. The women in clinic B were on average 1.5 years older than those in clinic A; this difference was significant ($p<0.05$). There was a systematic difference between clinic A and clinic B in overall cumulative probability of conception. The cumulative pregnancy rates, corrected for censoring, after 12 and 24 cycles were 67% and 80% respectively for clinic A and 76% and 91% respectively for clinic B.

Few women in clinic B received insemination after 24 cycles; clinic A continued treatment for longer and after 35 cycles had a cumulative pregnancy rate of 90%. The systematic difference in cumulative pregnancy rates between the clinics was however not related to differential treatment with age by clinic. Therefore all 751 women were used for the analysis of the effects of age.

The youngest woman entering the study was 18 years old, the oldest 42. Table 3.1 gives the age distribution of all the women.

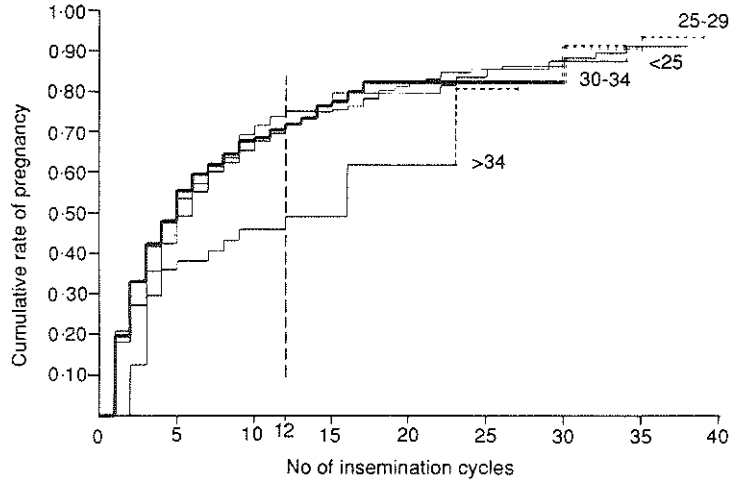
Table 3.1 Characteristics per age group

age group	number of patients		number and % pregnant	
18-24	111	(15%)	86	(78%)
25-29	390	(52%)	298	(76%)
30-34	201	(27%)	146	(73%)
35-42	49	(7%)	25	(51%)
Total	751	(100%)	555	(74%)

Figure 3.1 gives the curves of cumulative probability of conception for the four age groups. The cumulative pregnancy rates after 12 cycles were 0.75 (women aged <24), 0.72 (25-29), 0.72 (30-34), and 0.49 (>34). The probability of conception for each age group was also calculated with the proportional hazard

model (see model 1.1 in appendix). The difference in probability of conception between the oldest and the other age groups was significant (likelihood ratio test). The oldest age group had about half the hazard (pregnancy rate/cycle) of the other age groups (95% confidence interval 0.31 to 0.77).

Figure 3.1 Cumulative pregnancy rate by age group
The age group 30-34 is emphasized by the darker line. (A dotted line indicates that less than 5 women were still receiving ADI).
The cumulative pregnancy rates after 12 cycles were 0.75, 0.72, 0.72 and 0.49 for increasing age groups respectively.



In a more detailed analysis we estimated the pregnancy chances for each separate year of age (fig. 3.2). This gave a dispersed plot as many points were based on a small number of observations. The general shape, however, corroborates the use of a model in which the probability of conception falls after a certain age. The model with a gradual fall seemed to fit the data quite well (model 1.2). At age 31 the probability of conception was 95% of the initial level, and at older ages the fall became increasingly steeper. The choice of 95%, however, was quite arbitrary. For the model with the critical age (model 1.3) the fit was better than for model 1.2. Our estimation method gave a critical age of 31 years. After the age of 31 the chance of conception per cycle fell by about 12% each year of age (model 1.3).

To assess the goodness of fit we investigated three aspects of the regression models. Firstly, the models 1.2 and 1.3 should be adequate for describing the relation with age. Figure 3.2 shows that both models are valid in this respect.

The critical age model performed only slightly better. Secondly, the proportional hazards should be stable for subsequent insemination cycles. We performed the analysis for the first three cycles and for later cycles separately. In both subsets the start of the fall in fertility was at age 31. This proves the stability to be adequate. Thirdly, according to the models the difference between the clinics should be constant with age. When the data set was divided into clinics the critical age for clinic A was 31 and for clinic B 33 years. This difference, however, was not significant.

Figure 3.2 Relative pregnancy rates by age (Cox regression estimates)
Relationship between pregnancy rate per cycle (hazard) and year of age. The mean hazard for women with age between 20 and 30 was scaled as 1.00. The hazards by year of age are indicated by blocks; the size of the blocks refers to the number of observations. The hazards fitted by the "critical age" model are indicated by a line. The dotted line indicates the age range where the number of observations is too small for reliable estimates.
An example of the use of this figure: a value of 0.75 (for 33 years) indicates that a woman of that age has in each cycle 75% of the chance of a woman aged 20-30 in getting pregnant.

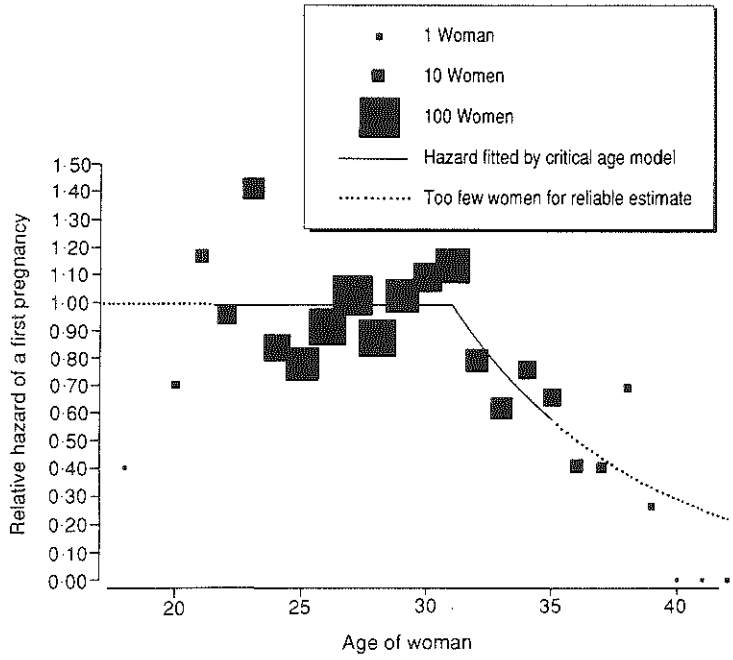
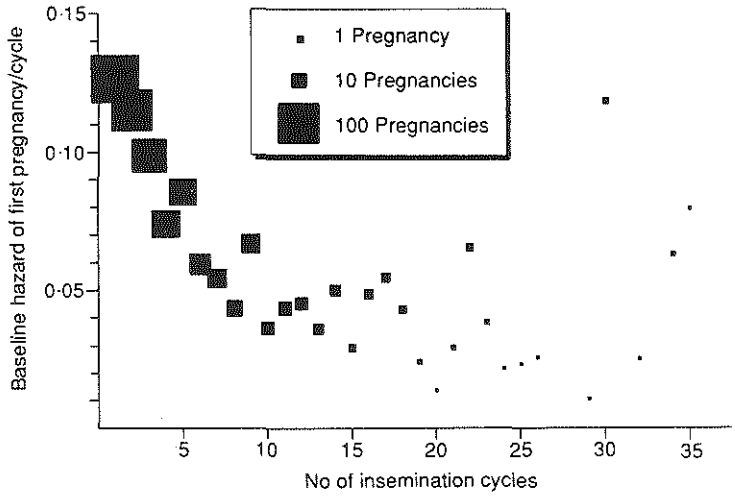


Figure 3.3 shows how the chance of pregnancy falls in each subsequent cycle in the whole group. This fall results from the selection process by which highly fecund women tend to get pregnant earlier than less fecund women.

Figure 3.3 Baseline chance of a first pregnancy (hazard) in each cycle.
The hazard is the pregnancy chance in a cycle after failures in the previous cycles. The size of the blocks refers to the number of pregnancies per cycle. The decline reflects the selection process towards lower fecund women with ongoing cycles. The figure can be interpreted as showing the pregnancy rate of women of age under 31 of clinic A in subsequent cycles.



Outcome of pregnancy

Of the 555 women who conceived, 532 women reported a result of pregnancy and 23 (5%) did not. Table 3.2 gives the reproductive outcomes according to age group. The fall in the probability of a healthy child related to age was estimated by logistic regression. Analysis was done on all 555 women who conceived, 461 women reporting a healthy child and 71 women reporting adverse outcomes. The 23 women with unknown outcome were first analysed as successes, assuming that adverse outcomes would have been reported. There was a significant decrease in the chance of having a healthy child after the age of 30 (see model 2.1 in appendix). If a pregnancy occurred women aged under 30 had an 89% chance of having a healthy baby. Thereafter this chance decreased by about 3.5% each year.

Table 3.2 Pregnancy outcome per age group

pregnancy outcome	age group								total	
	< 25		25-29		30-34		>34			
spontaneous abortions	9	12%	30	10%	20	14%	6	25%	65	12%
stillbirth	0	-	1	0%	2	1%	0	-	3	1%
congenital anomalies	0	-	1	0%	1	0%	1	4%	3	1%
healthy child	69	88%	257	89%	118	84%	17	71%	461	87%
known outcome	78	100%	289	100%	141	100%	24	100%	532	100%
unknown outcome	8		9		5		1		23	
Total	86		298		146		25		555	

There was no significant difference between clinic A and clinic B in the probability of a baby being healthy, after correction for the difference in age between clinics (hazard ratio 1.01; 95% confidence interval 0.61 to 1.69). No significant difference was found in successful pregnancy among women who took different times to conceive (hazard ratio 1.01/completed cycle; 0.96 to 1.06) nor between women with pregnancies that ensued after induction of ovulation and those with pregnancies occurring during spontaneous cycles (hazard ratio 1.03; 0.55 to 1.94).

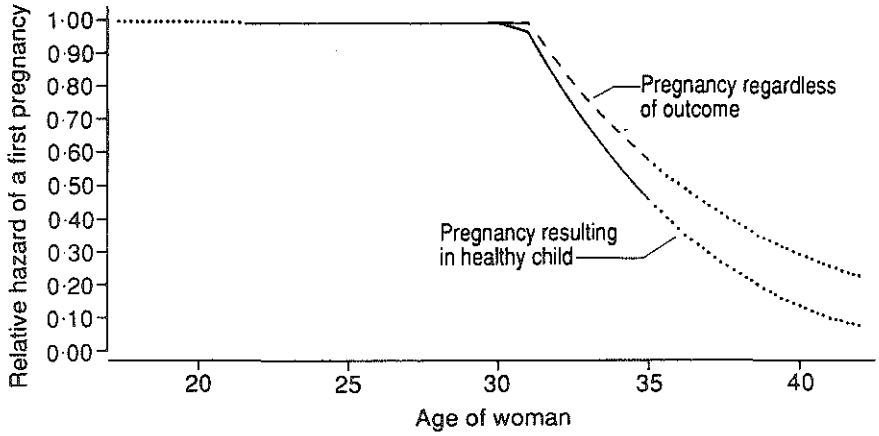
We plotted the probabilities for year of age in the same way as in figure 3.2. This showed the critical age model to be adequate for modelling outcome of pregnancy. Combining the chances of pregnancy and of a pregnancy being successful, gives a curve of the falling chance of successful pregnancy with age (fig. 3.4).

Figure 3.4 Relative pregnancy rates by age with regard to successful outcome

The figure shows the decline in hazard of pregnancy and of healthy child related to year of age. The dashed line shows the differences per ongoing age in hazard of pregnancy regardless the outcome (identical with figure 3.2). The lower line is the combination of

- the dashed line: the hazard of getting pregnant per ongoing age and
- the probability of a healthy child per ongoing age, given a pregnancy (calculated by logistic regression).

This combination yields the differences per ongoing age in hazard of successful pregnancy i.e., with healthy child as outcome.



DISCUSSION

We studied the effect of age on female fecundity and outcome of pregnancy in women receiving donor insemination. By excluding confounding variables, such as diminished sexual activity with age and various degrees of male subfertility, these women provide a better opportunity to study potentially predictive variables with regard to fecundity and outcome than do naturally selected populations.[7,8] To prevent selection bias toward a population of lower fecundity, only women married to azoospermic husbands and who had not been treated elsewhere were admitted to the study.[9]

Although we cannot fully explain the difference in cumulative conception rates between clinics A en B, the difference in policy on induction of ovulation is obvious. As cycles with and without ovulation induced by clomiphene were not randomly compared we can only speculate on the adverse effect of clomiphene in women with normal ovulatory cycles.[10]

We added a model with a continuous fall in fecundity with age (model 1.2); we proved this to be no improvement on the critical age model (1.3). The model with an abrupt start of fall performed slightly better and, more importantly, determined when the fall in fecundity starts.

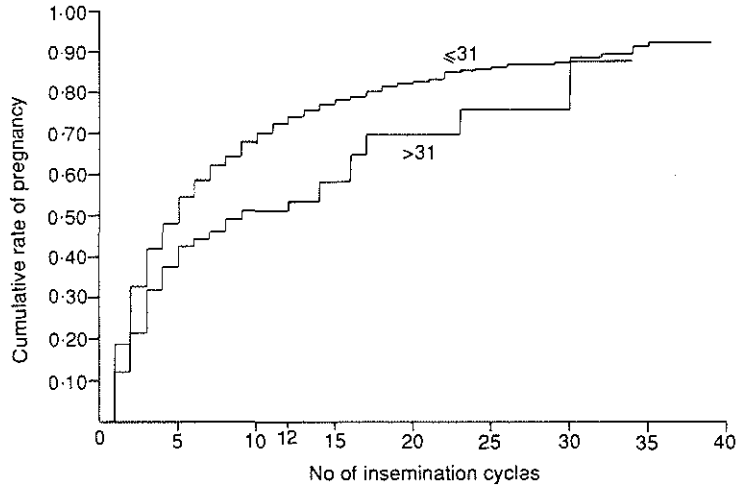
The finding of a critical age of 31 years does not seem to agree with figure 3.1, which shows a significant decrease of fecundity only in women older than 34 and not in those aged 30-34. However, the good result of the 30-34 group is caused by the fact that the pregnancy rates in women aged 30 and 31 were (possibly by chance) rather high (fig. 3.2). We could not account for the effect of possible confounders such as smoking, alcohol, or coffee consumption because this information was not systematically available. Therefore, to be cautious we will interpret the critical age to be around 31.

The critical age around 31 for decreasing fecundity falls within the ranges found by Howe et al in women stopping contraception,[11] by Schwartz and Mayaux in a population of women undergoing donor insemination,[1] and by Menken et al in historical populations.[12] The critical age did not change after correcting for the use of ovulation induction, nor did length of menstrual cycle affect our finding.

What are the reasons for decreasing female fertility with age? In vitro fertilisation clearly shows that the number of oocytes retrieved and the rates of pregnancy obtained decrease with age.[13,14] Lower pregnancy rates may be due to an uterine factor interfering with implantation. Reports on successful oocyte donation in women over 40, however, suggest that oocyte quality rather than uterine environment is the limiting factor in older women.[15] Subtle deterioration of oocytes probably starts before the age of 35. Anovulation, oligomenorrhoea, or

cycle irregularities apparently are later reflections of the same process of deterioration.
The models that we used do not account for the fact that during a series of insemination cycles the ages of the women increase. However, the age effect was only a 1% fall per cycle after the age of 31. Because few women received artificial insemination for a long series of cycles we would not expect including aging during the series to improve the models.
The start of the fall around age 31 means that women older than 31 will take longer to become pregnant (eventually) than would younger women. We divided the population into groups younger or equal and older than 31 (fig. 3.5).

Figure 3.5 Cumulative pregnancy rate by 2 age groups
Women were divided in groups older (n=131) and younger or equal (n=620) the critical age. The cumulative pregnancy rates after 12 cycles were 0.74 and 0.54 for the younger and older age group.



The pattern of the curve suggests that a policy of stopping treatment in the older age group is not advisable; treatment for longer than 12 cycles seems to be worth while. After 12 cycles the pregnancy rate in the older women increased from 54% to over 75% at 24 cycles; the pregnancy rate in the younger women increased in the same period from 74% to 85%. Because of the small number of women completing 24 cycles or more, however, the suggestion in our data that older women will eventually have the same pregnancy rate as younger women must be interpreted with caution.

Apart from the fecundity we also found that the chance of successful pregnancy (resulting in a healthy baby) in nulliparous women decreased after the age of 30. Classifying pregnancies in women in whom the outcome was unknown as unsuccessful did not alter this conclusion. Most fertility specialists are aware of the fall in fecundity and the chance of successful pregnancy with increasing age. When counselling women considering delaying childbearing we should know the combined effect on the likelihood of giving birth to a healthy child. We estimate that the relative chance per cycle of a 35 year old women giving birth to a healthy baby is 50% that of women of 25 (fig. 3.4).

Older women who do not get pregnant in the first cycle can get pregnant in one of the next cycles; as time taken to conceive was not related to outcome of pregnancy the differences between older and younger women in the cumulative probability of having a healthy child will become smaller after every subsequent cycle. Recently Berkowitz et al. found that delayed childbearing poses little, if any, increased risk of adverse neonatal outcome,[16] but they did not assess spontaneous abortions and chromosomal abnormalities. The positive association of unsuccessful pregnancy with age greater than 30 in our study was largely due to the contribution of spontaneous abortions. Study designs ascertaining the reproductive outcome after the gestational period when spontaneous abortions are most likely to occur will be less prone to show an age effect.

Our results are based on a cohort of women who received donor insemination. The number of women conceiving in this population is known to be lower than in a random population.[2] In our analysis, however, it is the critical age and the decrease with age in the probability of pregnancy and of pregnancy being successful that are at issue. We cannot find any reason why the critical age in this population would differ from that of a population conceiving naturally. Women receiving artificial insemination still represent the most feasible way to study the age effect on female fecundity and outcome of pregnancy. The question of how long women can wait can now be answered: around the age of 31 the probability of pregnancy in nulliparous women starts to fall. Older women can get pregnant, but at a slower rate than younger women. Women over 30 face a decreasing chance of having a healthy child.

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APPENDIX: mathematical details

The regression models used in this paper were estimated using GLIM software.[6] For non-linear models (1.2 and 1.3) only estimates can be calculated, that is no standard errors. The three models for the Cox regression calculations were as follows:

1.1 The model for age groups

$$h = \lambda e^{\alpha_2 L_2 + \alpha_3 L_3 + \alpha_4 L_4 + \kappa K}$$

where

h hazard of pregnancy in cycle t, that is the probability of pregnancy in cycle t assuming that no pregnancy has occurred until then

λ base-line hazard in cycle t (not modelled). The baseline group consists of women from age group 1 and clinic A

α_i parameter for the difference between the hazards for age group i and age group 1

L_i dummy variable, which takes the value 1 when the woman belongs to age group i and zero otherwise

κ parameter for the difference between the two clinics

K dummy variable, which takes the value 1 when the woman is treated by clinic B and zero otherwise

The age groups are here: less then 25 (1), 25 to 29 (2), 30 to 34 (3) and 35 and over (4). The baseline hazard (λ) was found to fall with increasing number of cycle. It reflects a selection effect: women with high fecundity get pregnant leaving after some cycles a higher proportion of women with average low fecundity to remain in the population. Parameter estimates are $\alpha_2 = -0.09$, $\alpha_3 = -0.07$, $\alpha_4 = -0.71$, $\kappa = 0.28$.

When the number of age groups is equal to the number of different ages in the study population this model estimates the hazards as indicated by the blocks in figure 3.2.

1.2 The model with age as a continuous variable

$$h = \lambda e^{\beta . l^{\pi} + \kappa . K}$$

where:

β	parameter for the strength of the effect of age
π	parameter for the abruptness of the start of fall
l	age (now a continuous explanatory variable)

This model fits a smooth curve that is almost constant at first and then starts to fall with an abruptness depending on the value of π. The best estimate for π was 13.1; addition of lower order polynomials did not improve the fit, which means that a rise in fecundity of younger women is not supported by our data. This log-likelihood was 3493.8.

1.3 The critical age model

$$h = \lambda e^{\beta . m + \kappa . K}$$

$$m = (1 > \gamma \mid l - \gamma)$$

where:

γ	critical age (age where the fall starts)
β	rate of fall after the critical age
m	number of years older then critical age, or zero if younger

Only discrete values of the critical age were investigated. The value with the maximum likelihood was selected. Therefore standard errors could not be calculated.

The baseline hazards (λ) are shown in figure 3.3. The relative hazards ($e^{\beta . m + \kappa . K}$) are indicated by the line in figure 3.2. The estimates were $\gamma = 31$, $\beta = -0.136$, $\kappa = 0.272$. The log-likelihood was 3491.9 with the same degrees of freedom as model 1.2. The estimated hazard of a certain woman in a certain cycle (h) can be calculated by multiplication of the baseline hazard (λ) depending on the number of the cycle and the relative hazard ($e^{\beta . m + \kappa . K}$) depending on the age and clinic.

We realize that the fact that our population consisted of a mixture of fertile and infertile women is theoretically incompatible with the use of the Cox regression model.[17] The mixed character of the population should lead to a constant shift in

the proportional hazards between young and old with time (insemination cycle). However, modelling interaction of the parameters with time (that is i.e. assuming β and γ are different in a first and second period of time) does not lead to a significantly better fit. Therefore we conclude that the theoretical incompatibility does not cause problems in this particular analysis.

2.1 The logistic model for probability of successful pregnancy

$$p = \frac{e^{\alpha+\beta.m+\kappa.K}}{1 + e^{\alpha+\beta.m+\kappa.K}}$$

Where p is the probability of successful pregnancy and α indicator for the baseline level. The other symbols have the same meaning as in model 1.3. The estimator for difference between clinics (κ) was not significant. The parameters of the model without κ were $\alpha = 2.13$, ,$\beta = -0.25$, $\gamma = 30$.

Chapter 4

BIOMETRY

FAT AND FEMALE FECUNDITY:
a prospective study of effect of body fat distribution on conception rates

Boukje M. Zaadstra[1]
Jacob C. Seidell[2]
Paul A.H. van Noord[3]
Egbert R. te Velde[4]
J. Dik F. Habbema[5]
Baukje Vrieswijk[2]
Jan Karbaat[6]

1 TNO Institute of Preventive Health Care, Child Health Division, Leiden, the Netherlands
2 Department of Human Nutrition, Agricultural University, Wageningen, the Netherlands
3 Department of Epidemiology, University of Utrecht Medical School, Utrecht, the Netherlands
4 Department of Obstretrics & Gynaecology, University Hospital, Utrecht, the Netherlands
5 Department of Public Health and Social Medicine, Erasmus University Medical School, Rotterdam, the Netherlands
6 Cryo Biological Laboratories Bijdorp, Barendrecht, the Netherlands.

4. BIOMETRY

ABSTRACT

Objectives:
To study the effect of body-fat distribution in women of reproductive age on fecundity.

Design:
Prospective cohort study of all women who had entered a donor insemination programme.

Setting:
One fertility clinic serving a large part of the midwest of the Netherlands.

Subjects:
Of all 542 women attending the clinic for artificial insemination for the first time, 500 women were eligible for study.

Main outcome measures:
Probability of conception per cycle and number of insemination cycles before pregnancy or stopping treatment.

Results:
A 0.1 unit increase in waist-hip ratio led to a 30% decrease in probability of conception per cycle (hazard ratio 0.706; 95% confidence interval 0.562 to 0.887) after adjustment for age, fatness, reasons for artificial insemination, cycle length and regularity, smoking, and parity. Increasing age was significantly related to lower fecundity ($p<0.05$); very lean and obese women were less likely to conceive ($p<0.10$) as were women with subfertile partners ($p<0.10$). All other exposure variables were not significantly related to fecundity.

Conclusions:
Increasing waist-hip ratio is negatively associated with the probability of conception per cycle, before and after adjustment for confounding factors. Body fat distribution in women of reproductive age seems to have more impact on fertility than age or obesity.

INTRODUCTION

Little is known about the effects of body fat distribution on reproductive function in women. Obesity is common among multiparous women[1] but this may indicate that weight gain is the result of multiple pregnancies rather than a prerequisite for conception.[2] Menstrual disorders have been found to be related to obesity as well as to a predominance of fat in the abdominal region.[3,4] An abdominal type of fat distribution may be related to increased androgenicity of these women.[5,6] Frisch suggested that underweight women would also have impaired reproductive function owing to a lack of oestrogen produced in adipose tissue.[7] It is not known, however, whether body fat distribution has any effect on the ability to conceive (fecundity) independent of weight and menstrual disorders.

A population of women presenting for artificial donor insemination provides an opportunity to study prospectively determinants of fecundity while controlling for possible confounders.[8] We studied 500 women aged 20 to 42 years presenting for artificial insemination to determine effects of body fat distribution while controlling for weight and other variables on the chance of conception.

SUBJECTS AND METHODS

From January 1986 to January 1988 all women attending a single fertility clinic for the first time (n=542) were asked to participate in the study. Three women refused to participate. The 39 women who did not start treatment before January 1989 were excluded from the analyses. Thus 500 women filled in a questionnaire and had anthropometric measurements taken at intake.

The self-administered questionnaire included questions on age, smoking habits, and other lifestyle characteristics. Regularity and length of the menstrual cycles, parity, degree of infertility of partner were ascertained by a doctor based on structured patient history.

Women were wearing only light indoor clothing without shoes when anthropometric measurements were taken. These included weight (kg), height (cm), waist circumference at the umbilical level and hip circumference at the level of the widest symphisis (cm). All measurements were taken by one doctor. Quetelet's index (kg/m2) was calculated as a measure of total fatness (body mass index) and waist to hip ratio as a measure of body fat distribution.

Women were referred to the clinic by a gynaecologist or other specialist (302, 61%), or by a general practitioner (101, 20%), or came to the clinic on their own initiative (85, 17%). (No information was available on 12 (2%)).

The main reasons for referral for artificial insemination were infertility (azoospermia) or subfertility (oligospermia) of the partner (152 (30%) and 236 (47%) respectively). Other reasons included sterilisation of partner (14, 3%), genetic abnormalities of partner (11, 2%), no partner (63, 13%) and other reasons (24, 5%). For the analysis women with azoospermic or sterilised partners and women with no partner were categorised in the subgroup with infertile partners. Most women had tried to become pregnant for two or three years, and most (412, 82%) had never been pregnant when they entered the study.
Before the first insemination all women were examined by one doctor. The mean menstrual cycle length was 28 days. Cycle length was classified as short (≤24 days) in 30 women, normal (25-35 days) in 431, and long (≥ 36 days) in 39. At the start of the treatment 368 women reported having regular cycles (less than four days difference on average). Forty seven women with very irregular and long cycles had ovulation induced by clomiphene. None of the other women received drugs to improve fecundity at the start of insemination treatment.
The youngest woman entering the study was 20 years old, and 10 were 40 or older. Smoking was reported by 260 women.

Inseminations and follow-up:
Intracervical inseminations were applied in subsequent menstrual cycles. Timing was based on previous cycle length, examination of the cervical mucus, and basal body temperature charts. Frozen semen was used from donors aged between 25 and 45 years with proved fertility (having fathered at least one child) and with sperm properties satisfying the World Health Organisation criteria.
Only first conceptions as a result of artificial insemination were used for the analysis. Insemination was defined as successful if the woman did not menstruate when expected and subsequently had a positive pregnancy test result. The follow-up period lasted until January 1989, and the largest number of cycles observed was 33.

Statistical methods:
The cumulative probability of conception by insemination cycle was calculated with Kaplan-Meier estimates for two waist-hip ratio groups.[9] The 41 women who did not report the result of their last insemination cycle (over the entire follw-up period) were recorded as not successful up to their last but one cycle. The anthropometric measures were missing for 11 women. Univariate proportional hazard regression analysis[10,11] was used to analyse the relation between probability of conception per cycle and each of the following variables separately: waist-hip ratio, body mass index, age, regularity and length of menstrual cycle, smoking, parity, degree of infertility partner.

Multivariate proportional hazard regression[10,11] was used to analyse the relation between waist-hip ratio and probability of conception per cycle while controlling for the above variables. Unadjusted and adjusted hazard ratios and 95% confidence intervals were calculated.
Both regression analyses were calculated from data on 448 women because of missing values in one of the exposure or confounding variables.

RESULTS

Insemination was successful within 12 cycles in 226 women, not successful in 274 women, including 38 women with an unknown result. Table 4.1 shows the distribution of the women according to waist-hip ratio, body mass index, and age, and the percentage that became pregnant in each category. The distribution of age, waist-hip ratio, and body mass index of the 38 women lost to follow up was not significantly different from that of women with known outcome ($p>0.05$). Abdominal fat preponderance (waist-hip ratio ≥ 0.80) was quite common (212 women). The percentage of women who became pregnant fell with increasing waist-hip ratio from 63% to 32%. Women with a body mass index under 20 kg/m2 were less likely to become pregnant than those with an index of 20-24.9 kg/m2. Only 22 women were obese (body mass index $\geq$ 30 kg/m2). Just four of these women became pregnant, suggesting a curvilinear relation between weight and fecundity. The percentage of pregnant women declined with older age and among the 62 who were 35 years or older only 17 (27%) became pregnant.

Table 4.1 Distribution of study population over categories of studied variables and percentages of pregnant women within 12 insemination cycles

Variable	No (%) of women (n=489)*	No (%) pregnant after 12 cycles
Waist-hip ratio:		
< 0.70#	32 (7)	20 (63)
0.70 - 0.75	99 (20)	50 (51)
0.75 - 0.80	146 (30)	69 (47)
0.80 - 0.85	138 (28)	57 (41)
≥ 0.85	74 (15)	24 (32)
Body mass index (kg/m^2):		
< 20.0	104 (21)	42 (40)
20.0 - 25.0#	303 (62)	145 (48)
25.0 - 30.0	60 (12)	29 (48)
≥ 30.0	22 (5)	4 (18)
Age (years):		
20 - 24#	67 (14)	34 (51)
25 - 29	229 (47)	113 (49)
30 - 34	131 (27)	56 (43)
≥ 35	62 (13)	17 (27)

* For 11 women no anthropometric measurements available.
\# Reference category. For waist-hip ratio and body mass index upper limits are exclusive.

The figure shows that the cumulative pregnancy rate by insemination cycle for women with waist-hip ratios <0.80 (pear-shape) was significantly higher than for women with ratios ≥0.80 (apple-shape) ($p = 0.008$). Because of the small number of women completing 24 cycles or more the suggestion in our data that both groups will eventually have the same pregnancy rate, must be interpreted with caution.

Figure 4.1 Cumulative pregnancy rate in 489 women having artificial insemination according to waist-hip ratio (277 women had ratio < 0.80 and 212 a ratio ≥ 0.80). Kaplan-Meier Logrank p = 0.0085

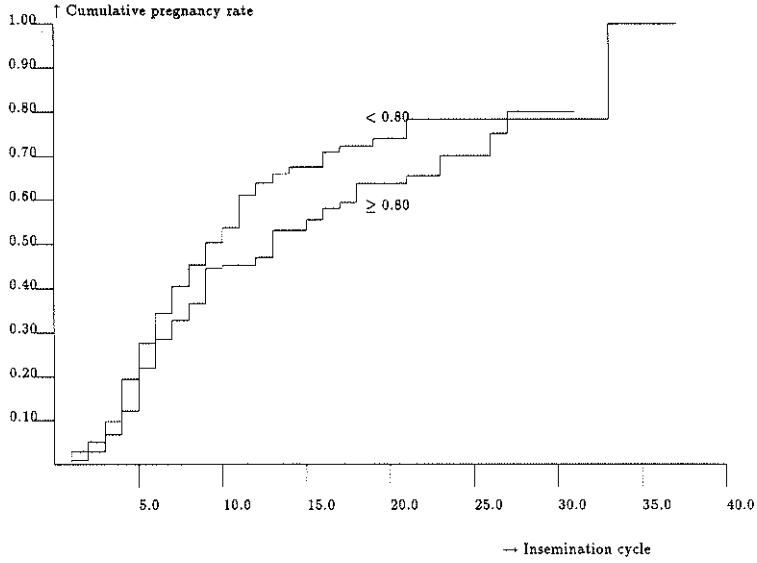

Table 4.2 shows the unadjusted hazard ratios and 95% confidence intervals for the effect of waist-hip ratio, body mass index, age, regularity and length of cycle, smoking, parity and reason for artificial insemination on probability of conception per cycle. Waist-hip ratio, age and body mass index were separately related to probability of conception per cycle. The multivariate proportional hazards regression in table 4.2 shows that the relation between waist-hip ratio and probability of conception per cycle, after controlling for all the other mentioned variables, remained essentially the same: waist-hip ratio yielded the highest independent significant contribution to probability of conception per cycle (hazard ratio 0.706, 95% confidence interval 0.562-0.887).

Table 4.2 Univariate and multivariate proportional hazard analysis of waist-hip ratio, age, body mass index, referral reason for artificial insemination, cycle length, cycle regularity, smoking, and parity on probability of conception per cycle over total follow up period*

	Univariate			Multivariate		
Variable	Hazard Ratio	95% CI	significance	Hazard Ratio	95% CI	significance
Waist-hip ratio (0.1)	0.704	0.568-0.871	p<0.001	0.706	0.562-0.887	p<0.0025
Age			p<0.0025			p<0.05
23	1.000			1.000		
27	0.977	0.763-1.251		0.970	0.750-1.253	
31	0.866	0.605-1.240		0.869	0.597-1.266	
37	0.603	0.382-0.952		0.638	0.396-1.027	
Body mass			p<0.05			p<0.10
index	0.854	0.675-1.073		0.837	0.662-1.058	
19.0	1.000			1.000		
22.3	0.901	0.749-1.084		0.939	0.775-1.139	
26.4	0.367	0.152-0.886		0.431	0.171-1.087	
33.1						
Referral reason for artificial insemination			n.s.			p<0.10
-Infertile partner	1.000			1.000		
-Subfert.partner	0.778	0.597-1.013		0.726	0.555-0.950	
-Other reasons	1.238	0.734-2.088		1.254	0.738-2.130	
Cycle length(1)	0.999	0.974-1.026	n.s.	1.003	0.974-1.033	n.s.
Cycle regularity	0.899	0.66 -1.23	n.s.	0.886	0.642-1.221	n.s.
Smoking	0.870	0.675-1.123	n.s.	0.914	0.704-1.187	n.s.
Parity	1.015	0.717-1.435	n.s.	0.811	0.566-1.162	n.s.

The relations were analysed by classes if the variable was categorical (cycle regularity, referral reason for insemination, smoking, parity), linear equation if the variable was continuous and significantly not quadratical (cycle length, waist-hip ratio), and quadratic equation (variable+variable^2) if addition of quadratic was significant (body mass index, age). Significance of variables was based on the effect of adding the parameters to the null model (univariate analysis) or the model with all other parameters (multivariate analysis).
For cycle regularity, smoking, and parity the hazard ratios express the difference of the second class with the first and for cycle length and waist-hip ratio the hazard ratios express an increase of 1 day or 0.1 unit respectively. For body mass index and age four values were chosen (medians of the classes in table 4.1). The hazard ratio and confidence intervals were calculated for these values, where the highest value was taken as "baseline". The result is analogous to the presentation of other variables when it is taken into account that many other values could have been chosen. Consequentially the significance of the parameters cannot be deduced from confidence intervals.
* N=448 women, due to missing values in one of the exposure or confounding variables.

DISCUSSION

This study shows that increasing waist-hip ratio is associated with fecundity in healthy women, indepentently of age, fatness, lenght and irregularity of menstrual cycle, parity, degree of infertility of partner, and smoking habits.
Both underweight and overweight women had a lower chance of becoming pregnant than women with normal weight, although this effect was greater for the obese than for the lean women. In addition, increasing age was associated with a reduced conception rate, which agrees with the results of our previous studies.[12,13]

Waist-hip ratio

Hartz et al showed that in about 12.000 American women aged 20-39 years participating in a slimming programme the degree of obesity and waist-hip ratio were independently and positively related to the prevalence of reported irregular menstruation, oligomenorrhoea (cycles longer than 36 days), and hirsutism.[3] Such a relation has been explained as the result of increased androgenicity.[5,6,14]
Based on information of cycle length and cycle irregularity in the period before insemination only 9% of the women in our study required clomiphene to regulate their cycle. Moreover, no obvious hirsutism was present in any of the women. Our study population therefore contained mainly healthy women of normal weight with apparently normal cycles, which makes comparison with the American study impossible.
Obese women or women with abnormal fat distribution with normal length of cycles (25-35 days) may have less regular cycles than women of normal weight and fat distribution. If this were true the decreased chance of conception could be explained by difficulties in timing of insemination, because timing may be less precise and therefore less successful in women with less regular cycles.
However, we found no relation between abnormal fat distribution or obesity and cycle regularity or whether ovulation had to be induced. Therefore, after correcting for all these potentially confounding factors, the highly significant relation between waist-hip ratio and the chance of conceiving, essentially remained the same (table 4.2). The reasons for our finding remain unclear. Insulin resistance (which is clearly associated with abnormal fat distribution or obesity)[15] at an early stage might increase the androgenic micro-environment of the follicle thus decreasing oocyte quality and the chance of conceiving *before* androgen levels are raised in serum and *before* cycles become irregular. Moreover, recent findings suggest that increased levels of luteinising hormone, which may also be associated with hyperinsulinaemia, might decrease the

spontaneous chance of conceiving and increase spontaneous abortion rates.[16] Unfortunately, no pre-treatment endocrine profiles were available in our study. Weight loss often restores normal cycle regularity[17] and sex hormone concentrations in obese women,[18] which indicates that obesity itself and not primary endocrine dysfunction is the cause of the reproductive problems. Further research is necessary to investigate mechanisms responsible for the low conception rate in normal weight women with an increased waist-hip ratio.

Other factors affecting fecundity

We also observed a tendency of a reduced chance of conception in women with a body mass index under 20 kg/m2. The association between leanness and fertility has been extensively explored by Frisch, who has suggested that menstruation and ovulatory cycles can only start above a certain threshold ratio of lean body mass to fat mass.[7] At body weights close to the required minimum women may still have anovulatory cycles, even if they are menstruating, because of a lack of peripherally produced oestrogen in adipose tissue.[7] The problem can be reversed by gaining weight. However, this explanation does not hold for our results because most of the lean women in our population did have regular cycles with biphasic basal body temperatures and did not require induction of ovulation. Moderate weight loss already seems to lead to subtle ovulation disorders and therefore to decreasing fecundity.

Changes in weight and waist-hip ratio during the follow up period could have led to misclassification of women. However, it is known that body mass index on average changes only 0.10 kg/m^2/per year.[19] Likewise no appreciable changes in waist-hip ratio occur with small fluctuations in weight.[20] Proportional hazard regression analysis over a 12 cycle follow up yielded essentially the same results as over the total follow up period.

Length and regularity of menstrual cycle were based on structured patient history at the start of the treatment. Prospective information on cycle variability during treatment would have led to potential selection bias. A minimum number of three cycles is needed to estimate variability, and women with low waist-hip ratios - that is more fertile women - would be more likely to be excluded from such an analysis, leading to an under-estimation of the relation between waist-hip ratio and fecundity.

Smoking has been associated with delayed conception.[21] In our study women who smoked had lower conception rates, though this did not reach significance. In the adjusted analysis subfertility of the partner became indicative for the fertility of the woman because of the fact that within this subgroup women with lower fertility are over-represented among those entering a donor insemination programme.[22]

CONCLUSIONS

In summary, in a prospective study of healthy women presenting for artificial insemination treatment we observed that increasing waist-hip ratio was negatively associated with the probability of conception per insemination cycle, before and after adjustment for age, fatness, cycle characteristics, smoking, parity, degree of infertility of partner.

Our results seem to agree with recent findings of a breast cancer study among post-menopausal women by Sellers et al.[23] They observed that a high waist-hip ratio, low parity, and greater age at first pregnancy were more important risk factors for breast cancer among women with a family history of the disease than among those without such a history. However, older age at first pregnancy and fewer children were not independent factors. They were unable to determine whether this risk profile reflected cultural inheritance or difficulty in becoming pregnant. Our results suggest that the second is true: women with high waist-hip ratio have difficulty in becoming pregnant.

As the prevalence of a high waist-hip ratio among healthy women is larger than the prevalence of obesity (15% and 5% respectively in our study) body fat distribution apparently has more impact on fertility than obesity.

Our findings are based on a cohort of women seeking insemination treatment because their partners had a fertility problem. Thus the population of women is primarily selected by the fertility status of the partner and not the women and probably is a fair reflection on the normal population of women.[8] Though our results need replication, this suggests that our results may be applicable to women attempting to conceive naturally.

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Chapter 5

LIFE-STYLE HABITS

MODERATE DRINKING HAS NO IMPACT ON FEMALE FECUNDITY

Boukje M. Zaadstra[1]
Caspar W.N. Looman[2]
Egbert R. te Velde[3]
J. Dik F. Habbema[2]
Jan Karbaat[4]

1 TNO Institute of Preventive Health Care, Child Health Division, Leiden, the Netherlands
2 Department of Public Health and Social Medicine, Erasmus University Medical School, Rotterdam, the Netherlands
3 Department of Obstetrics & Gynaecology, University Hospital, Utrecht, the Netherlands
4 Cryo Biological Laboratories Bijdorp, Barendrecht, the Netherlands.

5. LIFE-STYLE HABITS

SUMMARY

Little is known about the effect of alcohol intake on female fecundity (the ability to conceive). Women with high levels of alcohol consumption seem to have an elevated risk of primary subfecundity, however the effect of moderate alcohol intake is not known.
We prospectively studied the effect of moderate alcohol intake on female fecundity in a population of 500 women seeking artificial insemination treatment. Only first conceptions as a result of the artificial insemination were used for the analysis.
Women with moderate alcohol intake had a slightly higher, though not significant, probability of conception in comparison to non-drinkers.
Correcting for censoring and confounders (age and weight for length) the positive association between moderate alcohol intake and fecundity increased, though still not reaching significance levels (Hazard Ratio 1.20 95% C.I. (0.90-1.60); (p=0.20)). We conclude that moderate alcohol intake has no negative impact on female fecundity.

INTRODUCTION

Little is known about the effects of life-style habits as alcohol consumption, smoking, and coffee drinking on female fecundity (the ability to conceive). Olsen et al[1] mention in their retrospective study on smoking and drinking an elevated risk of primary subfecundity in women with high levels of alcohol consumption whereas moderate alcohol consumption does not seem to play a role in the development of subfecundity, but smoking does. Smoking has been associated with delayed conception.[2] The results of the Oxford Family Planning Association Contraceptive Study[3] also show decreased fertility among smokers, with a significant dose-response relationship. De Mouzon et al[4] studied prospectively the relation between smoking and fertility and found smoking to be related to decreased fertility when this factor was considered alone; when confounding factors were included in the analysis the relationship between smoking and fecundity disappeared. Recently Rosevaer et al[5] showed the nicotine metabolite cotenine to be related to impairment of fertilising capacity of oocytes in women undergoing in vitro fertilisation. There have been conflicting reports about the effect of caffeine intake on fecundity. Wilcox et al[6] reported that women who

drank more than the equivalent of one cup of coffee per day were half as likely to become pregnant per cycle, as women who drank less. A dose response effect was present. However, Riduan Joesoef et al[7] found caffeine intake not to be associated with infertility in 1818 infertile women and their primiparous controls. Most of the above mentioned studies are retrospective in design, either cohort or case-control, and were often undertaken in search of a relation between life-style habits and subsequent pregnancy outcome, and not for studying prospectively the relation between these life-style habits and fecundity.
Studies on the effect of alcohol consumption on female fertility are scarce, therefore we decided to primarily direct our attention to this life-style habit.
A population of women presenting for artificial donor-insemination (ADI) does provide an opportunity to study prospectively determinants of fecundity, while controlling for possible confounders.[8]
In the present study we prospectively studied 500 women aged 20-42 years presenting for ADI, to determine effects of life-style habits, in particular moderate alcohol drinking, on the probability of conception per cycle while controlling for relevant confounders.

SUBJECT AND METHODS

The study design has been described elsewhere[9], but for clarity we will repeat it briefly here. From january 1986 till january 1988 all women attending a single fertility clinic for the first time (n=542) were asked to participate in the study. The follow-up lasted until january 1989. 3 women refused to participate; 39 women who did not start with the actual treatment before january 1989 were excluded from the analysis. Thus a total of 500 women filled in a self-administered questionnaire including questions on age, alcohol drinking and smoking habits, drinking of coffee and other life-styles. Reproductive history and menstrual cycle characteristics were ascertained by a physician through a structured patient history. Before their first insemination all women underwent a medical examination by the same physician.
Women were either referred to the clinic by a gynaecologist or other specialist (61%), by a general practitioner (20%), or came on their own initiative (17%) (no information available in 2%). Main reasons for referral for artificial insemination were infertility (30%) or subfertility (47%) of the partner. Other reasons included sterilization of the partner(3%), no partner (13%), genetic abnormalities of partner (2%) or miscellaneous(5%).
The majority of women (82%) had never been pregnant when they entered the study.

The youngest woman was 20 years old, 10 women were 40 years or older. Mean age is 29.1 years old (deviance 4.4 years).

Exposure measurement

Alcohol drinking, as well as smoking and coffee drinking status were measured at intake.

For alcohol exposure measurement we combined the following questions on

a. drinking status: Do you occasionally drink alcoholic beverages? yes/no;
b. quantity: How many glasses on average per week (less than 10 glasses per week; between 10 to 25 glasses per week; between 25-50 glasses per week or more than 50 glasses per week). As only 1 woman reported to drink between 25-50 glasses per week and no woman reported in the category more than 50 glasses per week, we decided to divide the quantity of alcohol drinking into less versus equal or more than 10 glasses per week.
c. regularity: did you drink any alcoholic beverage during the last week before intake? as an indication for regularity of drinking.

Thus 4 categories were formed: 1. never drinkers; 2. irregular drinkers (less than 10 gl. per week but not last week); 3. regular light drinkers (less than 10 gl. and also last week) 4. regular drinkers (at least 10 gl. and drank that also last week).

Due to society's negative attitude towards women's drinking, underreporting of alcohol intake as well as underreporting of regularity of drinking is very likely to occur. Therefore we decided to divide the population also into non-alcohol drinkers (cat. 1) versus alcohol drinkers (cat. 2, 3 and 4 together). In so doing we also have the advantage of increasing the power of the study.

Smoking exposure is summarized by current smoking (yes/no), and coffee consumption by cups of coffee per day.

Alcohol drinking, smoking or coffee drinking status was not recorded on patient records, nor on follow-up cycle treatment records. Therefore this information was not readily available to the physician and could not influence treatment.

Influence of correlated variables

To assess the unbiased effect of alcohol intake we should add possible confounders to the model and inspect whether the effect parameters have changed. Next to alcohol intake, smoking and coffee drinking, we selected variables with possible effect on fecundity and possible correlation with alcohol intake, like age of the woman, body fat distribition (waist-hip ratio), body mass index (kg/m2), socio-economic status (the highest achieved educational level of the woman),

duration of menstrual cycle and parity (ever been pregnant before intake). Possible selection effects in the group of women applying for ADI because of subfertility of husband (very fecund women would already have become pregnant) will be handled by adding the referral reason for ADI (infertile or subfertile husband) as a possible confounder.

Missing variables

Forty-one out of 500 women did not report the result of the last insemination cycle. We recorded them as not pregnant after (exposure in) the last but one cycle. This means that all 500 women can be used for the hazard analysis. For the exposure and other variables we had some missing values. For alcohol intake 11 women did not give information. When the analysis would have been restricted to persons with complete information on all exposure and correlated variables, we would lose 79 women. This would be regretful when these variables would have no impact on fecundity. We will calculate several survival functions: univariate for alcoholintake, multivariate for alcoholintake plus potential confounders, multivariate for alcohol intake plus potential confounders plus correlated variables, and at last multivariate for alcohol intake plus confounders plus correlated variables plus other exposure variables together. For every subsequent survival analysis we will discard only women with missing values for the variables in the model at hand. This ensures that we use the maximum number of informative cases for each situation, but it also means that some more women have to be deleted because of missing values in each subsequent analysis.
To distinguish between selection and confounding effects we will first re-fit the last model for the population of the next analysis (i.e. differences are caused by the rejection of cases only) and then add new explanatory variables, this gives differences which are caused by confounding only.

Inseminations and follow-up

Intra-cervical inseminations were applied in subsequent menstrual cycles. Timing was based on previous cycle length, examination of the cervical mucus and basal body temperature charts. Frozen semen was used from donors between 25 and 45 years of age with a proven fertility (having fathered at least one child) and with sperm properties satisfying the W.H.O. criteria.

Only first conceptions as a result of the artificial insemination were used for the analysis. Insemination was defined as successful if no period appeared at the expected time and subsequently the pregnancy test became positive. The follow-up lasted until January 1989. The largest number of cycles observed was 33.

Methods of analysis

After some single cross tabulations, associations between alcohol intake, smoking and coffee drinking and possible confounders were calculated as Pearson correlation coefficients. The cumulative probability of conception by insemination cycle was calculated using Kaplan-Meier[10] estimates for drinkers and for non-drinkers; the difference between the 2 groups was assessed by Log-rank test. Univariate proportional hazard regression analysis[11] was used for analyzing the relation between probability of conception per cycle and each of drinking status, smoking status, and coffee intake for the total follow up period.
Multivariate proportional hazard regression[11] was used to analyze the relation between drinking status and probability of conception per cycle while controlling for age and Body Mass Index, also for the total follow-up period. The results were transformed into unadjusted and adjusted Hazard ratios and 95% confidence intervals. We have used the term hazard as shorthand for the more informative but longer term: conception rate per cycle.

RESULTS

Insemination was successful in 52% of the women and not succesfull in 48%, including 8% who did not report the result of the last insemination cycle. The relations with drinking, smoking and coffee drinking are shown in table 5.1.

Table 5.1 Distribution among women of alcohol drinking, smoking and coffee drinking, and the percentage of women that became pregnant in each category

Variable category	n	%	% pregnant
Drinking			
1. never	159	32	46
2. irregular	86	18	53
3. regular light	206	42	55
4. regular	38	8	58
	489	100	
missing	11		
	500		
Smoking			
1. no	234	47	56
2. yes	260	53	48
	494	100	
missing	6		
	500		
Coffee: cups/day			
1. none	55	11	56
2. < 5	237	49	49
3. 5-10	182	37	52
4. > 10	15	3	53
	489	100	
missing	11		
	500		

The percentage of women who became pregnant increased slightly per increasing drinking category. The mean number of cycles women needed to become pregnant or tried before stopping were not relevantly different between different categories of alcohol intake. The percentage of women who became pregnant was lower among smokers than among non-smokers. With increasing coffee drinking the percentage pregnant first decreased, followed by an increase.

To take into account the differences in time to conceive or time to withdrawal we used survival analysis. Table 5.2 shows the univariate Hazard ratios and 95% confidence intervals of alcohol drinking, smoking and coffee drinking woman on probability of conception per cycle over the treatment period. The effect of alcohol was absolutely not significant (p=0.85), but showed the same tendency towards a positive relationship as the simple cross tabulation of table 5.1. Dividing the population into non-alcohol drinkers versus alcohol drinkers increased the p value to p=0.40. Smoking was related to a lower probability of conception, coffee drinking didnot show a consistent pattern.

Table 5.2 Univariate proportional hazard analysis of alcohol drinking, smoking and coffee drinking on probability of conception per cycle for total follow-up period

Variable	Hazard Ratio	95% C.I.	p-value
Drinking (n=489)			p=0.85
1. never*	1.00		
2. irreg.	1.09	(0.75 - 1.57)	
3. reg.light	1.13	(0.84 - 1.52)	
4. regular	1.18	(0.73 - 1.90)	
A. no*	1.00		p=0.40
B. yes	1.12	(0.85 - 1.48)	
Smoking (n=494)			p=0.22
1. no*	1.00		
2. yes	0.86	(0.67 - 1.09)	
Coffee:cups/day (n=489)			p=0.51
1. none*	1.00		
2. < 5	0.82	(0.55 - 1.23)	
3. 5-10	0.93	(0.62 - 1.40)	
4. > 10	1.29	(0.59 - 2.81)	

* = reference group

Figure 5.1 Cumulative rate of pregnancy per insemination cycle for alcohol drinking (330) versus non-alcohol drinking women (159)

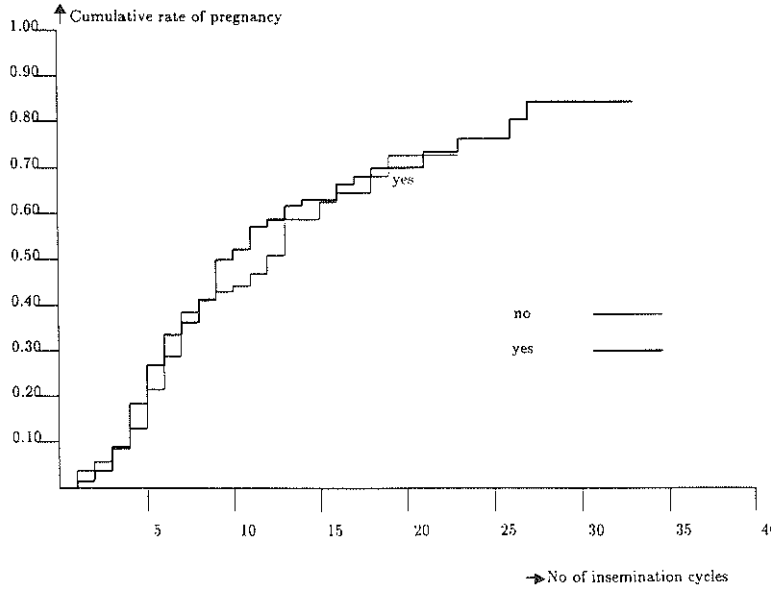

Figure 5.1 shows the cumulative conception rate by insemination cycle to be slightly higher for drinkers than for non-drinkers, though not reaching significance levels (logrank p=0.23). However the slightly positive direction of the association between alcohol drinking and conception probability could be explained by the influence of other variables. Therefore we selected the potential confounders: the variables that were correlated with alcohol intake and with fecundity rate. Only age and BMI were both correlated with alcohol intake (X^2: $p<0.001$ and p=0.089 resp.) and with fecundity rate (univariate proportional hazards: p=0.008 and p=0.009). The result of this multivariate proportional hazard analysis is shown in table 5.3a. The univariate analysis of alcohol intake on the dataset in table 5.2 however resulted in hazard ratio's (1.00 - 1.09 - 1.13 - 1.18), that is correction in the multivariate model for age and BMI increased the effect, though it is still not significant. Table 5.3b shows that the crude measurement scale for alcohol intake (no/yes) does not result in significant hazard ratio's either.

There are more variables in our dataset, like smoking and coffee drinking, but because these variables are not correlated with exposure (alcohol intake) and outcome (fecundity) simultaneously, we can not expect any confounding influence on the effect of alcohol intake. 137 women (27%) received ovulation induction with respect to the last insemination cycle. However, as there is no relation between alcohol level and ovulation induction, neither at intake, first or last cycle of treatment (p=0.40), there is no risk of confounding bias in the effect of alcohol from this source. Referral reason for insemination (infertility or subfertility of partner) was neither correlated with alcohol (p=0.60), nor with female fecundity (p=0.17). When we added these variables nevertheless to the multivariate model (i.e. SES, coffee drinking, smoking, body fat distribution, degree of infertility of husband, menstrual cycle characteristics, parity and age of menarche), it resulted in hazard ratio's of 1.00 - 1.23 - 1.10 - 1.02. The differences with table 5.3a however are caused mainly by selection effects: there are only 421 women with all variables present.

Table 5.3a Multivariate proportional hazard analysis of several levels of alcohol drinking, age and BMI on probability of conception per cycle for the total follow-up period

Variable	Hazard Ratio	95% C.I.	p-value
Drinking			p=0.64
1. never*	1.00		
2. irreg.	1.18	(0.807-1.714)	
3. reg. light	1.20	(0.885-1.640)	
4. regular	1.25	(0.771-2.033)	
age (yr)	0.96	(0.926-0.988)	p=0.005
BMI (kg/m^2)			p=0.008
BMI	1.67	(1.097-2.557)	
$(BMI)^2$	0.99	(0.980-0.998)	

* = reference group
n = 485 due to missing values

Table 5.3b Multivariate proportional hazard analysis of alcohol drinking (no/yes), age and BMI on probability of conception per cycle for total follow-up period.

Variable	Hazard Ratio	95% C.I.	p-value
Drinking			p=0.20
no *	1.00		
yes	1.20	(0.904-1.599)	
age (yr)	0.96	(0.926-0.988)	p=0.005
BMI (kg/m^2)			p=0.008
BMI	1.67	(1.097-2.559)	
$(BMI)^2$	0.99	(0.980-0.998)	

* = reference group
n = 485 due to missing values

DISCUSSION

The most interesting point in our study is that moderate alcohol intake does not lead to a decrease in probability of conception per cycle. On the contrary, if anything our data suggest a slight increase in probability of conception for drinkers in comparison to non-drinkers. At first we thought that the direction of this association could possibly be explained by censoring: a difference in the mean number of participating cycles among non-pregnant drinking women in comparison to non-pregnant never drinking women. However, this difference did not seem relevantly different between different categories of alcohol intake and, in a more adequate answer to this suggestion, the results of the univariate proportional hazard regression analysis also showed a slightly increased probability of conception per cycle in the drinkers (table 5.2). This result could be due to the influence of confounding variables. However, the results of the multivariate proportional hazards analysis (corrected for the effects of censoring and confounders) show that the association between drinking and probability of conception per cycle becomes stronger for all drinking categories, though still not reaching significance levels.

When we first found moderate alcohol intake to be positively associated with fecundity, we viewed it as a quirk in our data. However animal research seems to support the same observation. Mitchell and Kainen[12] determined the effects of alcohol on blastocyst implantation in the rat. They found that the time of implantation was advanced in alcohol treated rats. Stachecki et al[13] support this finding. Their results of studies in mice indicate that alcohol (ethanol) exposure can be both toxic and stimulatory to normal pre- and peri-implantation development, depending upon the stage of exposure and the dose used. Development appeared to be enhanced when early stage embryos were exposed to low concentrations of ethanol; at high concentrations ethanol had an inhibitory effect on the development of embryos to the blastocyst stage. Low dose ethanol treatment of early blastocysts caused precocious development of adhesiveness and subsequent outgrowth that is associated with implantation. Of course these results could be species specific and not applicable to humans. Our results are based on a prospective follow-up of women seeking donor-insemination, which provided the opportunity to study determinants of fecundity while controlling for confounders, which in an open population would be hardly possible.

We also looked if the sort of alcohol drink (predominantly beer, predominantly wine or predominantly (hard) liquor) made any difference on the probability of conception per cycle. There was no significant effect of any kind of alcohol, interestingly though beer showed a very slight negative effect (b=-0.067; hazard

of 0.935) on probability of conception per cycle, while wine (b=0.184; hazard of 1.20) and hard liquor (b=0.528; hazard of 1.69) were positively associated.
Increasing age was significantly and smoking almost significantly associated with a lower chance on conception, which is in agreement with previous studies[14,3]. Due to the curvilinear relation ship of BMI with fecundity, we squared BMI in the model. BMI had a significant influence, but our previous study[9] showed that this effect diminishes, when other variables, such as Waist to Hip Ratio (WHR), are included. Our results with regard to coffee consumption do not show a consistent pattern, which is also in agreement with the literature. Conflicting results have been found[6,7]. Other variables correlated with alcohol, such as SES didnot alter the results.
We categorized women according to their life-style habits (exposure status) at intake of the study. However, certain life-style habits may be modified during the period when the woman tries to become pregnant. To have some idea about the change in alcohol drinking behaviour over time, the level of alcohol consumption was asked twice; first at intake (reported here sofar) and again among those women who became pregnant, retrospectively after the end of pregnancy. There was some discrepancy: among women who conceived and who reported at intake to drink less than 10 glasses per week, 18% reported retrospectively (after pregnancy) not to have drunk at all within three months before conceiving. These were probably women who stopped drinking at the start of treatment. Next to this 6.7% changed to a different category of alcohol intake. The reason why we decided against inquiring to concurrent life-style habits during follow up, was to avoid any kind of the so called Hawthorne effect: that the mere measurement of behaviour may alter it. Murray et al[15] found that repeated questioning in the study group on smoking behaviour lowered itself the prevalence of smoking in the study group versus the control group. Also the doctors were not informed about the drinking, smoking nor coffee-drinking status of the women to avoid any selective treatment associated with fertility enhancing drugs, number of in-seminations etc.
One could argue that women who do not drink do so because of specific medical reasons f.i. using tranquilizers and other sorts of medicine which are not compatible with drinking. Therefore we analyzed the relationship between stress related variables like: feeling insecure about treatment,insomnia, being tense, headache, analgesics, tranquilizers and painful menstruation, by factoranalysis. However these variables didnot correlate strongly enough with each other (Principal Component Eigen Value 0.222) to imply a stress parameter. There was also no significant relation between drinking status and the use of sleeping pills (X^2:2.753 3df)nor between drinking status and the use of tranquilizers (X^2:2.026 3df).

In summary: in a cohort of healthy women of reproductive age seeking artificial donor insemination, we found that moderate alcohol intake has no negative impact on fecundity; that the association points to a positive direction, a finding confirmed in animal research. Conception probabilities of smokers were lower than non-smokers, though not reaching significance levels. The effect of coffee drinking on conception probability was inconsistent. This study confirmed increasing age to be significantly associated with decreasing probability of conception per cycle.

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Chapter 6

PREGNANCY OUTCOME

ARE CORRELATES OF FEMALE FECUNDITY ALSO ASSOCIATED WITH SUBSEQUENT PREGNANCY OUTCOME? RESULTS OF A COHORT STUDY

Boukje M. Zaadstra[1]
Caspar W.N. Looman[2]
S. Pauline Verloove-Vanhorick[1]
Egbert R. te Velde[3]
J. Dik F. Habbema[2]
Jan Karbaat[4]

1 TNO Institute of Preventive Health Care, Child Health Division, Leiden, the Netherlands
2 Department of Public Health and Social Medicine, Erasmus University Medical School, Rotterdam, the Netherlands
3 Department of Obstetrics & Gynaecology, University Hospital, Utrecht, the Netherlands
4 Cryo Biological Laboratories Bijdorp, Barendrecht, the Netherlands.

6. PREGNANCY OUTCOME

INTRODUCTION

The trend among women to delay pregnancy until they are 30 years or older has led to increased consumer demand for knowledge and assistance in preparation for pregnancy.[1] The identification of risk-factors affecting women's reproductive health together with those affecting the outcome of pregnancy (i.e. a healthy baby) deserves growing attention. Therefore we decided to study whether factors affecting female fecundity: maternal age,[2] biometry[3] (body fat distribution and weight) and life-styles[4] (drinking, smoking and coffee drinking) are also related to eventual pregnancy outcome.
A population of women presenting for artificial donor insemination provides a unique opportunity to study the effects of various exposures on reproductive and perinatal health.[5] In a cohort of 500 women (attempting to become pregnant), we studied in the 259 women who eventually conceived the outcome of their pregnancy.

MATERIAL AND METHODS

Materials

From January 1986 to January 1988 all women attending a single fertility clinic for the first time (n=542) were asked to participate in the study. Three women refused to participate. The 39 women who did not start treatment before January 1989 were excluded from the analysis. Thus 500 women filled in a self-administered questionnaire on age, smoking habits and other life-style characteristics and had biometric measurements taken at intake. Women were wearing only light indoor clothing without shoes when measurements were taken. These included weight (kg), height (cm), waist circumference at the umbilical level and hip circumference at the level of the widest symphysis (cm). All measurements were taken by one doctor. Quetelet Index (kg/m^2) was calculated as a measure of total fatness (Body mass index) and Waist to Hip Ratio as a measure of body fat distribution.
Maternal age was taken as the age in years at intake of the study. Alcohol drinking, as well as smoking and coffee drinking status was asked twice: prospectively *at intake* before treatment (to be indicative for exposure status *during treatment*) and again, among the women who conceived, retrospectively *after pregnancy* to assess exposure status *before and during pregnancy*. Of the 500 women starting treatment 259 women conceived within the follow-up time (only the first conceptions as a

result of insemination) and 241 did not, including 41 women who did not report the result of the last insemination cycle and could not be retrieved.

Exposure measurement at intake

For alcohol exposure we combined questions on drinking status, quantity and regularity to 4 categories: 1. never drinkers; 2. irregular drinkers (less than 10 glasses per week but not in the week before intake); 3. regular light drinkers (less than 10 glasses per week and also during the week before intake); 4. regular drinkers (10 glasses or more per week, every week).

Smoking exposure is summarized by current smoking (yes/no) and coffee consumption by cups of coffee per day (1. none; 2. < 5 per day; 3. 5-10 per day; 4. > 10 per day).

Alcohol drinking, smoking or coffee drinking status was not recorded on patient record, nor on follow-up cycle treatment records. Therefore this information was not readily available and could not influence treatment.

Exposure measurement after pregnancy

A self-administered questionnaire was sent October 1990 to 257 women who conceived, (2 women refused to participate in any follow-up) to retrospectively assess drinking, smoking and coffee drinking status before and during pregnancy, as well as to validate information about course, duration and outcome of the pregnancy, including information about the health of the child after birth into the present time. The retrospective assessment of alcohol drinking, smoking and coffee drinking led to 3 exposure levels per drinking, smoking or coffee drinking category.

Level 1: *abstainers:* (not within 3 months before pregnancy, nor during pregnancy)

Level 2: *stoppers:* (yes, within 3 months before pregnancy; no, during pregnancy)

Level 3: *users:* (yes, within 3 months before pregnancy; yes, during pregnancy)

As pregnancy is defined as a positive - conventional - pregnancy test after a non-occurring menses, the level 2 will predominantly include women who stopped *once* they knew they were pregnant, *after* conception had already occurred. Therefore level 2 measures the effect of drinking in early pregnancy as well. Level 3 measures the effect of drinking during early as well as the proceeding pregnancy together.

Due to the fact that women can change life-style habits in the period attempting pregnancy, the exposure measurements at intake do not necessarily coincide with retrospective exposure measurement during pregnancy.

Pregnancy outcome

The pregnancy outcome was divided into 4 main outcome variables.

1. *Fetal loss:* any fetal death occurring before 24 weeks among all conceptions;
2. *Congenital anomalies:* any congenital anomalies reported among live born singletons;
3. *Gestational age:* exact pregnancy duration in days from the first day of the last menstrual period among live born singletons;
4. *Fetal growth:* expressed as birthweight ratio (BWR: ratio of the observed birthweight to the expected mean birthweight, corrected for gestational age, sex and parity (0,+1), according to the charts by Kloosterman among live-born singletons.[6]

Great effort was taken to assess pregnancy outcome of the women non-responding to the questionnaire.

Perinatal mortality (stillborn of 24 weeks or more; liveborn, but death in first week of life) was not reported for any infant.

Methods

Univariate logistic analysis was used for analyzing the relation between maternal age, waist-hip ratio, Quetelet Index, drinking, smoking and coffee drinking status to probability of fetal loss, or congenital anomalies respectively. The results were calculated in un-adjusted Odds ratio and 95% confidence intervals. Linear regression was used for analyzing the relationship between the exposure variables and gestational age and birthweight ratio as outcome. F-ratio tests were used for testing the difference between exposure categories.

As only a few of the explanatory variables were significantly related to pregnancy outcome, we did not pursue multivariate analysis.

RESULTS

General Results

Out of 500 women, 259 conceived (a positive pregnancy test) of whom 2 refused to partake in any follow-up after conception. Pregnancy outcomes and the response rates per outcome category are shown in table 6.1. The results will now be presented per outcome variable; p values < 0.10 will be mentioned as indicative for an existing relationship.

Table 6.1 Pregnancy outcomes and Response rates per outcome category on questionnaire

Outcome of pregnancy	No (%) of outcomes n = 257			No (%) of responses n = 233		
- Spontaneous abortion	34			27		
- Extra uterine gravidity	3	38	(15)	3	31	(82)
- Stillbirth (22wk)	1			1		
- Child (singlet.)	213		(83)	196		(92)
- Child (twins)	6		(2)	6		(100)
- Total	257			233		
- Missing	2		(1)	24		(9)

Fetal loss

Of the 257 eligible conceptions 38 (14.8%) ended in fetal death before week 24. This is higher than the 11.6% mentioned by Wilcox for clinically recognized pregnancies.[7] However the percentage falls well within the range of the 95% C.I. (10.9% - 19.7%). As table 6.2 shows only age is highly significantly related to fetal loss. (O.R. 1.19 95% C.I. (1.08-1.29) p=0.0001).

The oldest age category ≥ 35 years shows relative to the youngest age category 20-24 years a nearly 20 times greater risk of fetal loss: O.R. 22.7 95% C.I. (3.88-132.79). Abdominal fat distribution experienced an higher percentage of fetal loss O.R. 1.41 95% C.I. (0.69-2.87); while it was peculiar that obese women experienced lower percentages. However both findings can be entirely attributed to chance; p-values were not significant. Alcohol, nor smoking was related to fetal loss; however coffee drinking was related to fetal loss (p = 0.09).

Table 6.2 Fetal loss n=38 N=257[1]

Distribution among pregnancies of Waist-Hip Ratio, Quetelet Index, age, alcohol drinking and coffee drinking; the percentage that became pregnant per category; unadjusted Odds Ratio and 95% Confidence and P-value per variable for fetal loss (any fetal death before week 24)

Variable	n/N	%	OR	95% C.I.	P value
WHR (250)					
< .70[r]	3/20	15	1.00	-	0.73
.70-.75	5/55	9	0.57	(0.12-2.63)	
.75-.80	11/75	15	0.97	(0.24-3.89)	
.80-.85	11/68	16	1.09	(0.27-4.37)	
≥ .85	6/32	19	1.31	(0.29-5.94)	
pear <.80[r]	19/150	13	1.00	-	0.34
apple ≥.80	17/100	17	1.41	(0.69-2.87)	
WHR cont.	36/250	14	1.16	(0.64-2.10)	0.62
Quetelet Index (253)					
< 20.0	9/49	18	1.32	(0.57-3.07)	0.56
20.0-25.0[r]	24/165	15	1.00	-	
25.0-30.0	4/35	11	0.76	(0.25-2.34)	
≥ 30.0	0/4	-	-	-	
Quet. cont.	37/253	15	0.92	(0.80-1.05)	0.18
Age (257)					
20-24[r]	2/55	4	1.00	-	0.001*
25-29	19/139	14	4.19	(0.96-18.26)	
30-34	11/50	22	7.47	(1.60-34.90)	
≥ 35	6/13	46	22.70	(3.88-132.79)	
Age cont.	38/257	15	1.19	(1.08-1.29)	0.0001*

continued

1 Totals may vary due to missing values

r reference group

* $p < 0.10$

Table 6.2 continued

Variable	n/N	%	OR	95% C.I.	P value
Alcohol (250)					
never[r]	11/71	15	1.00	-	0.99
irregular	7/45	16	1.01	(0.36-2.82)	
light	17/112	15	0.98	(0.43-2.23)	
regular	3/22	14	0.86	(0.22-3.41)	
abstainers[r] (233)	16/110	15	1.00	-	0.84
stoppers	10/78	13	0.86	(0.37-2.02)	
users	5/45	11	0.73	(0.25-2.14)	
Smoking (254)					
no[r]	19/130	15	1.00	-	0.87
yes	19/124	15	1.06	(0.53-2.11)	
abstainers[r] (232)	17/128	13	1.00	-	0.79
stoppers	6/36	17	1.31	(0.47-3.60)	
users	8/68	12	0.87	(0.53-2.14)	
Coffee drink. (244)					
never[r]	5/31	16	1.00	-	0.40
< 5 p.d.	19/115	17	1.03	(0.35-3.02)	
5-10 p.d.	13/95	14	0.82	(0.27-2.53)	
> 10 p.d.	0/8	-	-	-	
abstainers[r] (232)	2/30	7	1.00	-	0.09*
stoppers	1/25	4	0.58	(0.05-6.71)	
users	28/178	16	2.61	(0.59-11.59)	

1 Totals may vary due to missing values
r reference group
* $p < 0.10$

Congenital anomalies

Table 6.3 gives the listing of congenital malformation reported. They are evaluated according to the criteria used by the EUROCAT registry;[8,9] divided into major malformations (interferes with normal functioning), minor malformations (does not interfere with normal functioning) and no malformations. In total 5 major and 4 minor anomalies were registered out of the 192 responses to the retrospective questionnaire: 4.7% (95% C.I. 2.5%-8.8%). This figure compares well with the 4.0% congenital malformations found in a cohort of 2.092 infants followed by child health clinics in the first year of life (SMOCK study).[10] If we include malformations excluded by EUROCAT criteria, minor malformations increase to 10 leading to a total of 15 malformations: 7.8%, 95% C.1 (4.8%-12.6%). In the afore mentioned

SMOCK study total inclusion of all minor malformations, observed over 5 consecutive visits to the child health clinic in the first year of life, led to a prevalence of 27% congenital anomalies. Underreporting in our study is likely to be the case for minor malformations.

Table 6.3 Distribution of reported congenital anomalies among 213 eligible singletons

Major congenital anomalies		5
- cystic kidney	1	
- transposition of the great vessels	1	
- Beckwith-Wiedeman syndrome	1	
- hiatus hernia	1	
- stenosis of the urether	1	
Minor congenital anomalies		10
- coloboma of the left eye	1	
- predislocation of the hip	2	
- refraction anomaly of the eye	1	
- hemangioma*	1	
- nevus flammeus*	1	
- nevus pigmentosus*	1	
- single umbilical artery*	1	
- other anomalies extremities*	2	
Item answered in questionnaire: no anomalies		177
Item not answered in questionnaire		4
No response on questionnaire		17
Eligible singletons		213

* No congenital anomalies according to EUROCAT criteria

Except for age none of the exposure variables were related to congenital malformations (table 6.4). Increasing age led to an increasing chance on congenital malformations (p=0.002); O.R. 1.24 95% C.I. (1.08-1.42). (Note worthy is that the distribution of (major and minor) malformations according to EUROCAT criteria (see table 6.3) was significantly correlated with non-smoking status; i.e. smokers had a much lower chance on congenital malformations. However after extending the list to include more minor malformations, this relationship was not significant anymore. Since we know of no previous knowledge regarding this relationship, we take this to be a chance finding).

Table 6.4 Reported major and minor congenital anomalies. n=15, N=192[1]

Variable	n/N	%	OR	95% C.I.	P value
WHR (189)					
< .70[r]	1/16	6	1.00	-	0.90
.70-.75	4/45	9	1.46	(0.15-14.14)	
.75-.80	5/53	9	1.56	(0.17-14.42)	
.80-.85	3/52	6	0.92	(0.09-9.47)	
≥ .85	1/23	4	0.68	(10.04-11.63)	
pear <.80[r]	10/114	9	1.00	-	0.37
apple ≥.80	4/75	5	0.59	(0.18-1.94)	
WHR cont.	14/189	7	0.75	(0.31-1.84)	0.53
Quetelet Index (190)					
< 20.0	3/36	8	0.93	(0.25-3.55)	0.17
20.0-25.0[r]	11/124	9	1.00	-	
25.0-30.0	0/26	-	-	-	
≥ 30.0	0/4	-	-	-	
Quet. cont.	14/190	7	0.92	(0.75-1.14)	0.44
Age (192)					
20-24[r]	1/44	2	1.00	-	0.02*
25-29	6/107	6	2.56	(0.30-21.71)	
30-34	6/35	17	8.90	(1.02-77.29)	
≥ 35	2/6	33	21.50	(1.60-290.71)	
Age cont.	14/192	7	1.24	(1.08-1.42)	0.002*

continued

[1] Totals may vary due to missing values
[r] reference group
* $p < 0.10$

Table 6.4 continued

Variable	n/N	%	OR	95% C.I.	P value
Alcohol (186)					
never[r]	3/53	6	1.00	-	0.90
irregular	2/32	6	1.11	(0.18-7.02)	
light	6/83	7	1.30	(0.31-5.42)	
regular	2/18	11	2.08	(0.32-13.58)	
abstainers[r] (192)	6/92	8	1.00	-	0.17
stoppers	8/64	13	2.05	(0.67-6.22)	
users	1/36	3	0.41	(0.05-3.30)	
Smoking (190)					
no[r]	8/99	8	1.00	-	0.69
yes	6/91	7	0.80	(0.27-2.41)	
abstainers[r] (191)	9/107	8	1.00	-	0.67
stoppers	1/26	4	0.44	(0.05-3.51)	
users	5/58	9	1.03	(0.33-3.22)	
Coffee drink. (187)					
never[r]	1/20	5	1.00	-	0.33
< 5 p.d.	4/83	5	0.96	(0.10-9.03)	
5-10 p.d.	6/76	8	1.63	(0.19-14.26)	
> 10 p.d.	2/8	25	6.33	(0.49-82.01)	
abstainers[r] (192)	1/27	4	1.00	-	0.44
stoppers	3/22	14	4.10	(0.41-41.94)	
users	11/143	8	2.17	(0.28-17.02)	

1 Totals may vary due to missing values
r reference group
* $p < 0.10$

Gestational age

The exact duration of pregnancy could be calculated for 209 out of 213 live born singletons (98%). Deviations to shorter gestational age were larger than to higher gestational age. In order to achieve normally distributed residuals for the linear regression analysis, the distribution of gestational age was transformed by taking the exponent. Means were calculated according to the exponential distribution of this variable. Table 6.5 shows the mean gestational age in days per exposure category and the subsequent p-value. Increasing age led to a decrease in mean gestational age; however this relationship was not significant. Alcohol drinking was significantly related to shorter gestational age (p=0.03)), in the sense that women who drank before and around conception, experienced an almost 4 days on average

shorter duration of pregnancy than women who abstained all together. Smoking had borderline significance (p = 0.06) to shorter gestational age.

Table 6.5 Distribution of exposure variables for **mean** gestational age per exposure category. N=209[1] (Overall mean gestational age = 280.6 days; s.d. ± 11,5 days)

Variable	number	%	mean gest. age in **days**	P value (F-ratio test)
WHR (204)				
< .70[r]	16	8	279.4	0.45
.70-.75	48	24	280.4	
.75-.80	59	29	280.6	
.80-.85	55	27	282.4	
≥ .85	26	13	277.6	
pear <.80[r]	123	60	280.4	0.73
apple ≥.80	81	40	280.9	
WHR cont.	.78	± .06	n.a.	0.84
Quetelet Index (206)				
< 20.0	39	19	280.7	0.997
20.0-25.0[r]	132	64	280.5	
25.0-30.0	31	15	280.7	
≥ 30.0	4	2	280.9	
Quet. cont.	22.5	± 3.0	n.a.	0.96
Age (209)				
20-24[r]	51	24	281.2	0.48
25-29	115	55	281.2	
30-34	37	18	278.6	
≥ 35	6	3	276.4	
Age cont.	28.1	± 3.7	n.a.	0.55

continued

1 Totals may vary due to missing values
r reference group
* p < 0.10
n.a. not appropriate

Table 6.5 continued

Variable	number	%	mean gest. age in **days**	P value (F-ratio test)
Alcohol (202)				
never[r]	57	28	282.6	0.19
irregular	35	17	281.8	
light	91	45	279.4	
regular	19	9	277.9	
abstainers[r] (195)	92	47	282.9	0.03*
stoppers	65	33	279.3	
users	38	20	278.1	
Smoking (206)				
no[r]	107	52	281.9	0.06*
yes	99	48	279.1	
abstainers[r] (194)	108	56	281.7	0.12
stoppers	26	13	282.1	
users	60	31	278.4	
Coffee drink. (202)				
never[r]	23	11	282.6	0.52
< 5 p.d.	92	46	279.5	
5-10 p.d.	79	39	281.5	
> 10 p.d.	8	4	280.5	
abstainers[r] (195)	27	14	282.4	0.68
stoppers	22	11	280.5	
users	146	75	280.5	

1 Totals may vary due to missing values
r reference group
* $p < 0.10$
n.a. not appropriate

Fetal Growth

The Birth Weight Ratio (BWR) was used as an indicator for fetal growth. That is the ratio of the observed birthweight (BW) to the expected mean BW, corrected for gestational age, sex and parity (longer gestational age leads to higher BW, while boys and babies of multipara women tend to be heavier). This method to correct for gestational age is preferable to the commonly used method of linear regression of birthweight on gestational age. The last method is questionable because of the non linear relationship between mean birthweight and gestational age and because the relationship between the mean and the standard deviation of birthweight remains.[11]

The birthweight ratio however, has a mean independent of gestational age and a standard deviation independent of the mean. The overall BWR was .995.
Of all exposure variables drinking during pregnancy and smoking led to decreasing BWR with a dose-response relationship. The other variables did not lead to any significant relationship (table 6.6).

Table 6.6 Distribution of exposure variables for mean **birthweight ratio** (observed over the mean expected weight, corrected for gestational age, sex, parity per exposure category). N=200[1] (Mean BWR 0.995 s.d. ± 0.140)

Variable	number	%	mean BWR	P value (F-ratio test)
WHR (196)				
< .70[r]	16	8	0.997	0.82
.70-.75	46	24	0.985	
.75-.80	56	29	0.997	
.80-.85	53	27	1.016	
≥ .85	25	13	0.983	
pear <.80[r]	118	60	0.992	0.52
apple ≥.80	78	40	1.005	
WHR cont.	0.78	± 0.06	n.a.	0.996
Quetelet Index (198)				
< 20.0	38	19	0.972	0.71
20.0-25.0[r]	127	64	1.000	
25.0-30.0	29	15	1.006	
≥ 30.0	4	2	1.006	
Quet. cont.	22.12	± 2.99	n.a.	0.50
Age (200)				
20-24[r]	49	25	1.002	0.86
25-29	109	55	0.996	
30-34	36	18	0.978	
≥ 35	6	3	1.013	
Age cont.	28.14	± 3.75	n.a.	0.82

continued

1 Totals may vary due to missing values
r reference group
* $p < 0.10$
n.a. not appropriate

Table 6.6 continued

Variable	number	%	mean BWR	P value (F-ratio test)
Alcohol (195)				
never[r]	55	28	1.006	0.11
irregular	34	17	1.013	
light	87	45	0.994	
regular	19	10	0.924	
abstainers[r] (194)	91	47	1.008	0.05*
stoppers	65	33	1.004	
users	38	20	0.945	
Smoking (198)				
no[r]	102	52	1.014	0.05*
yes	96	48	0.975	
abstainers[r] (193)	107	55	1.009	0.19
stoppers	26	14	0.989	
users	60	31	0.969	
Coffee drink. (195)				
never[r]	22	11	1.014	0.60
< 5 p.d.	88	45	0.981	
5-10 p.d.	77	40	1.007	
> 10 p.d.	8	4	0.987	
abstainers[r] (194)	27	14	0.992	0.93
stoppers	22	11	1.004	
users	145	75	0.993	

1 Totals may vary due to missing values
r reference group
* $p < 0.10$
n.a. not appropriate

DISCUSSION

Within one and the same cohort of 500 women attempting pregnancy, the 259 women who conceived were followed to the outcome of the pregnancy. This unique design enables to study prospectively the effect of determinants on fecundity and pregnancy outcome.

Table 6.7 summarizes the effects of maternal age, biometry (fat distribution and fatness), life-style habits (alcohol drinking, smoking, and coffee drinking) on fecundity, fetal loss, congenital anomalies, gestational age and fetal growth.

Our study shows unequivocally that increasing maternal age leads to an increase in fetal loss, as well as congenital malformations. This is consistent with other research as well.[12,13] However the fact that increasing maternal age is also related to significant lower probability of conception, puts older women desiring to become pregnant at double jeopardy in comparison to younger women: it takes much longer to conceive and once pregnant it is much harder to maintain a pregnancy to term, and once maintained to term the chance on congenital malformation is greater. This is consistent with our research in another data-set.[2]

On the other hand it is re-assuring that neither fat-distribution (as expressed by waist-hip ratio) nor fatness (Quetelet index), being both of importance to the probability of conception[3] have any significant relation with any outcome (nor with fetal loss, nor congenital anomalies, nor gestational age, nor fetal growth). The confidence intervals are large, the lowest p value $p = 0.17$. It seems that these biometric parameters are of predictive value for fecundity only and not for maintaining a pregnancy thereafter. This is an important message to women desiring to become pregnant and asking for pre-conceptional advice and -care.

One could hypothesize that a factor affecting early reproductive loss, would lead to selective survival: only the best 'concepti' survive to term. However, the fact that congenital anomalies experience a sharp increase with the oldest age category, after these concepti also have gone through the risk period of fetal loss, suggests that the quality of the ovum is still at issue here.[14] Maternal age (within our age range of 20-40 years) hardly has any effect on gestational age or fetal growth.

Coffee drinking was only slightly related to fetal loss, but none of the other outcomes. The relationship of coffee drinking with fecundity is conflicting.[15,16]

Alcohol drinking and smoking however do deserve attention. In our other research we could not detect any negative impact of drinking on fecundity;[4] nor could we detect a relation with fetal loss as Armstrong did.[17] The effect of alcohol in our present study concentrates on late reproductive outcomes: shorter gestational age as well as lower BWR. The fact that the difference between abstainers and stoppers (app. 4 days), is greater than between stoppers and users (1 day) points to an effect of alcohol in the earliest phase of pregnancy; before women know they are pregnant.

This is also an important issue to proper pre-conceptional care. Women should be advised not to drink trying to get pregnant.
It is generally accepted that smoking is related to lower birthweight[18] even corrected for gestational age, sex, and parity. BWR of babies of smoking mothers were significantly lower than these of non-smoking mothers.
There is with regard to retrospective life-style exposure measurement always the possibility of selective recall related to the outcome at hand. Women might either overreport or underreport smoking and drinking habits; the first would lead to a bias away from the null value (and consequently exaggerated effects), the second situation would lead to bias toward the null; not finding an effect at all. However, the high response rate and the fact that the denominator (conceptions) is known, plus that effects of life-style habits measured at intake have the same direction, as the effects of life-style measured retrospectively is reassuring. Recall bias is very unlikely to have occurred.

The effects of the exposure variables were investigated in 257 women conceiving. To epidemiologic standards this is a fairly small population to detect any exposure outcome relationship; meaning the effect has to be very large to reach significance level (like age); otherwise a, real, but in our study small effect will not reach significance levels and can even point to the opposite direction. (This could be the case where we could not find a significant effect of smoking and alcohol on fetal loss). At issue here is the power of the study; for some relationships the size of our study might be too small to detect a relationship which is real. Therefore it is advisable to look if the direction of the relationship is consistent with prior knowledge or is biologically plausible.[19] In general this happens to be the case in our study.[20]
On the other hand constructing a summary outcome measure to increase the power of the study can lead to non-sense and loss of information. Analyzing relationships of the exposure variables with one summary outcome: (any adverse reproductive outcome versus non) is *not* advisable: it leads to disappearance of any significant relationship; even age was not related to outcome anymore.

Table 6.7 **Summary table**: exposure variables over fecundity and pregnancy outcomes (OR and means + P values)

Variable	Fecundity N=500 Hazard Ratio (p)	N=257 Fetal loss OR (p)	N=192 Cong.anom. OR (p)	N=209 Gest.age mean (p)	N=200 BWR mean (p)
WHR					
< .70[r]	1.00 (0.02)*	1.00 (0.73)	1.00 (0.90)	279.4 (0.45)	0.997 (0.82)
.70-.75	0.66	0.57	1.46	280.4	0.985
.75-.80	0.60	0.97	1.56	280.6	0.997
.80-.85	0.55	1.09	0.92	282.4	1.016
≥ .85	0.39	1.31	0.68	277.6	0.983
pear <.80[r]	1.00 (0.02)*	1.00 (0.34)	1.00 (0.37)	280.4 (0.73)	0.992 (0.52)
apple ≥.80	0.58	1.41	0.59	280.9	1.005
WHR cont. (0.1)	0.69 (<.001)*	1.16 (0.62)	0.75 (0.53)	n.a. (0.84)	n.a. (0.996)
Quetelet Index					
< 20.0	0.77 (0.006)*	1.32 (0.56)	0.93 (0.17)	280.7 (0.99)	0.972 (0.71)
20.0-25.0[r]	1.00	1.00	1.00	280.5	1.000
25.0-30.0	0.94	0.76	-	280.7	1.006
≥ 30.0	0.27	-	-	280.9	1.006
Quet. cont. (1.0)[1]	0.977 (0.20)	0.92 (0.18)	0.92 (0.44)	n.a. (0.96)	n.a. (0.50)
Age					
20-24[r]	1.00 (0.17)	1.00(0.001)*	1.00 (0.02)*	281.2 (0.48)	1.002 (0.86)
25-29	0.86	4.19	2.56	281.2	0.996
30-34	0.83	7.47	8.90	278.6	0.978
≥ 35	0.56	22.70	21.50	276.4	1.013
Age cont. (1.0)	0.965 (0.01)*	1.19(0.0001)*	1.24(0.002)*	n.a. (0.55)	n.a. (0.82)

continued

r reference group
1 (straight line not really adequate here to fecundity data: see ref. 3)
n.a. not appropriate

Table 6.7 continued

Variable	Fecundity N=500 Hazard Ratio (p)	N=257 Fetal loss OR (p)	N=192 Cong.anom. OR (p)	N=209 Gest.age mean (p)	N=200 BWR mean (p)
Alcohol					
never[r]	1.00 (0.85)	1.00 (0.99)	1.00 (0.90)	282.6 (0.19)	1.006 (0.11)
irregular	1.09	1.01	1.11	281.8	1.013
light	1.13	0.98	1.30	279.4	0.994
regular	1.18	0.86	2.08	277.9	0.924
abstainers[r]	n.a.	1.00 (0.84)	1.00 (0.17)	282.9(0.03)*	1.008 (0.05)*
stoppers		0.86	2.05	279.3	1.004
users		0.73	0.41	278.1	0.945
Smoking					
no[r]	1.00 (0.22)	1.00 (0.87)	1.00 (0.69)	281.9(0.06)*	1.014 (0.05)*
yes	0.86	1.06	0.80	279.1	0.975
abstainers[r]	n.a.	1.00 (0.79)	1.00 (0.67)	281.7 (0.12)	1.009 (0.19)
stoppers		1.31	0.44	282.1	0.989
users		0.87	1.03	278.4	0.969
Coffee drink.					
never[r]	1.00 (0.51)	1.00 (0.40)	1.00 (0.33)	282.6 (0.12)	1.014 (0.60)
< 5 p.d.	0.82	1.03	0.96	279.5	0.981
5-10 p.d.	0.93	0.82	1.63	281.5	1.007
> 10 p.d.	1.29	-	6.33	280.5	0.987
abstainers	n.a	1.00 (0.09)*	1.00 (0.44)	282.4 (0.68)	0.992 (0.93)
stoppers		0.58	4.10	280.5	1.004
users		2.61	2.17	280.5	0.993

* $p < 0.10$
r reference group
1 (straight line not really adequate here to fecundity data: see ref. 3)
n.a. not appropriate

In summary:

We studied the effects of biometric parameters, age and life-style habits on early and late pregnancy outcomes in a cohort of healthy women presenting for artificial donor insemination, because their partners had a fertility problem. Thus the population of women is primarily selected by the fertility status of the partner, and not selected through fertility status of the woman herself. The advantage of such a study-population is the possibility of prospectively following women from attempting pregnancy to pregnancy outcome and measuring exposure variables

prospectively as well as retrospectively. Next to that these women were highly motivated and will report life-style habits and other variables conscientiously. The mean BWR of liveborn singletons conceived by donor insemination was 0.995 meaning that the observed mean birthweight in our study population was the same as the mean expected birthweight (corrected for gestational age, sex and parity) of the Dutch reference population from the tables of Kloosterman.[6]
It is however known, that the absolute number of women conceiving through artificially insemination is lower than under natural circumstances.[21] However it are the *relationships* with the exposure variables and pregnancy outcomes which are at issue here. We can not think of any reason why these relationships would differ from a population of women conceiving under natural circumstances.

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Chapter 7

GENERAL DISCUSSION

GENERAL DISCUSSION

The cohort studies in this thesis were set up to gain insight whether certain risk-factors affect the fecundity as well as the subsequent outcome of pregnancy in the same women. The risk-factors were chosen because of their importance to women living in affluent societies. Planning has become an intrinsic part of an individual's live in western societies; and planning for a reproductive career is nowadays to women as important as planning for a professional career; let alone the problem of harmonizing these two. However, the reproductive career has its biological limitations: while over the last centuries the reproductive life span of women has expanded through an earlier menarche; the realization of reproduction has shifted towards later ages. This delayed childbearing is in itself not a new phenomenon, in fact it is a return to fashion of behaviour that characterized much of Western-Europe and parts of North America for perhaps several hundred years extending well into the 20th century. In the past, delayed childbearing was accomplished not by contraception but primarily through postponement of marriage and sexual intercourse.[1] Nowadays the planned use of contraception (even abortion) to control both timing and number of children is the norm.

Because many women delay childbearing, reproduction becomes compressed into the second half of the reproductive life-span (early thirties). With increasing age it takes longer to achieve a pregnancy and therefore pressure is amounting to shift the endpoints of the female reproductive life-span even beyond menopause. The technological possibilities, oocyte donation and freezing of embryos, are already there to fulfil this scenario.[2]
However, it is not by individual's choice alone that women decide to postpone childbearing; it is also the perception that societal constraints make such a postponement necessary. Women's behaviour should be understood as an outcome of the interaction between the actual situation in which they find themselves and their interpretations of it.[3] And when they find that a choice for a professional career cannot coincide with a choice for a reproductive career, than the only solution - if you want to have it both - is to have it one after another. And a smart woman will opt first to have her education and career already underway and then to have children. This is entirely reasonable because until recently even the lay press stressed the fact that women in their late thirties or early forties could become pregnant without problems, as long as the menstrual cycle pattern remained normal and regular.

It was generally known that women's fecundity declines with age, however confounding factors like their partners fertility and declining frequency of

intercourse with age could account for that fact as well. Therefore, the sole effect of maternal age and other determinants can best be studied in a population of women desiring to become pregnant by donor insemination, where quality of donorsemen and the number of inseminations are more or less standardized.[4] The complete time-axis can be monitored from attempting pregnancy to eventual pregnancy outcome. Because it is not only becoming pregnant, but also having a healthy baby that counts.
Our results show that the critical age, where female fecundity starts to decline is already at 31 years of age and next to that the capability to carry a pregnancy to term also declines around that age. We estimated that a 35 year old woman has half the per cycle probability of a 25 year old woman becoming pregnant with a healthy baby. This does not mean that half of all 35-year-old women are infertile, but it does mean that it will take much longer to obtain a pregnancy resulting in the birth of a normal, healthy child. This information should be known not only to mothers and the fathers to be; but also to policy makers in general. They should try to translate these medical findings into possibilities to diminish societal constraints for combining career and motherhood, upon which it will become attractive in the future for women to have children at a younger age than is currently the case.

The other determinants also focus on aspects of affluent societies: there is major concern with weight and diet, as well as with life-styles.
Obesity is a common condition in affluent societies. The prevalence of moderate obesity (25-30 kg/m^2) in dutch women between 37-43 year is estimated up to 30%. There is convincing evidence that obesity is associated with an increased risk for mortality and morbidity, particularly of cardiovascular and coronary heart disease and non-insulin dependent diabetes mellitus. The distribution of body fat plays a critical role in this context. An abundance of visceral fat (intra-abdominal fat: apple-shaped) is a stronger predictor of specific metabolic aberrations than total body fat.[5] If this is the case the effect of intra-abdominal fat on an intermediate outcome as fecundity (instead of chronic disease at later age) should be noticeable as well. This had not been studied before.
Our findings show that women with an abdominal type of fat distribution (apple-shape) have a significant lower chance of conceiving than women with a gluteal-femoral distribution (pear-shape), also when the cycle length and -regularity were taken into account. It is hypothesized that the lower conception rate is due to an increased insulin-resistance, possibly leading to a more androgenic environment of the ovary of these women. If so it would be worthwhile to see whether an unfavourable fat distribution and its consequences for fecundity - and in the long term for chronic diseases - could be changed by losing weight. Weight loss

might then become a more healthy alternative for infertility treatment than exogenous hormone treatment like ovulation induction. We plan to set up such an intervention study.

Life-style habits in affluent societies like alcohol drinking, smoking and coffee drinking have attracted much interest in relation to pregnancy outcome. Already in the 50's Simpson found that birthweight is decreased among babies born to mothers who smoked during pregnancy.[6] However, the relationship of life-style habits with fecundity cannot properly be addressed in a study population of pregnant women or women having delivered a baby. These retrospective studies, addressing time to pregnancy as an indicator for fecundity, take pregnancy, rather than attempt at pregnancy as the sampling unit. It does mean that highly fecund women are overrepresented and subfecund women will be underrepresented compared with the sampling in a prospective study of non-pregnant women.[7] This means that an exposure resulting in infertility, will consequently not show up in relation to pregnancy outcome and can therefore not be studied to its effect on reproductive health.
We studied the effect of life-style habits on fecundity as well as outcome of pregnancy within the same women. We realized that behaviour modification might happen over time, as well as that the timing and intensity of exposure might determine the type of outcome rather than its frequency.[8] Therefore the effects on reproductive health were analyzed over the total gestational time axis: fecundity, given attempts to pregnancy; fetal loss given all conceptions; and congenital malformations, gestational age and birthweight ratio given all live born singletons.
Our study of life-style habits confirmed results of earlier research, namely smoking lowers conception probability and decreases birthweight; the effect of coffee drinking is inconsistent on fecundity as well as on any outcome of pregnancy. It was not known whether moderate alcohol drinking had any effect on fecundity. Our study showed that moderate drinking had a positive, but non-significant, effect on fecundity; while moderate drinking during (early) pregnancy was associated with a significant shorter gestational age as well as a lower birthweight ratio (indicative for impaired fetal growth).

In summary:
Maternal age at older ages (over age 30) is of overwhelming importance to probability of conception, subsequent fetal loss and risk on congenital anomalies in live births.
Biometry, especially fat distribution, has a significant effect on probability of conception only, but not on outcome of pregnancy.

The effect of life-styles, alcohol drinking and smoking, are above all of importance to outcome of pregnancy: gestational age and fetal growth.

RECOMMENDATIONS

Risk reduction and health promotion in preparation for pregnancy must assume that intervention prior to conception is of enhanced value in comparison to intervention early in pregnancy. Our results show that maternal age and fat distribution are important factors determining female fecundity; maternal age is also of overwhelming importance to the risk of fetal loss and congenital anomalies; life-style habits are important factors in relation to birthweight and gestational age, especially the effects are noticeable when exposed in early pregnancy, around conception before women know they are pregnant.
Therefore our study leads to the following recommendations regarding preconceptional care.

1. From a medical point of view women should be advised to plan pregnancy at an early age, preferable before 30 years of age.
2. Policy makers should eliminate societal constraints and allow for structural possibilities to harmonize professional and reproductive careers of women (and men as well).
3. "Apple-shaped" women ought to know that it will take longer to conceive; an intervention study should be undertaken to investigate whether weight loss in these women improves conception rates.
4. Moderate alcohol drinking as well as cigarette smoking exposure in early pregnancy have mainly effect on the outcome of pregnancy; therefore women desiring pregnancy should be advised not to drink and smoke in order to enhance favourable pregnancy outcomes.

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SUMMARY

Chapter 1

Gives the rationale for the study to evaluate, whether maternal age, biometric parameters and life-style habits are determinants of female fecundity and subsequent outcome of pregnancy in cohorts of women attempting pregnancy.

Chapter 2

Assesses whether a population of donor inseminated women (ADI) provides an efficient alternative to an open population of women of reproductive age to study determinants on fecundity and outcome of pregnancy. Distribution of life-style habits in the donor inseminated population compared well with that within a general population survey in Rotterdam.

Chapter 3

The effect of maternal age on fecundity and outcome of pregnancy was retrospectively studied in a cohort of 751 nulliparous women, married to azoospermic husbands and never having received donor insemination before. The critical age (fall in fecundity) was estimated to start around 31 years of age; the probability of conceiving, as well as having a healthy baby declined after the age of 30. The combined chance of a woman aged 35, conceiving and having a healthy baby, is about half that of a woman aged 25.

Chapter 4

The effect of body-fat distribution on fecundity was prospectively studied in a cohort of 500 women under treatment for ADI. Waist to hip ratio circumference was used as an indicator for body-fat distribution; women with an abdominal fat preponderance (waist-to-hip ratio $\geq$ 0.80 apple-shape) had, corrected for all relevant confounders, a significant lower conception probability than women with fat preponderance in the gluteal-femoral region (waist-to-hip ratio $<$ 0.80 - pear-shape).

Chapter 5

The effect of life-style habits, specifically the effect of moderate alcohol intake on fecundity was prospectively studied in a cohort of 500 women under treatment for ADI. Women with moderate alcohol intake had a slightly higher, though not significant, chance to conceive in comparison to non-drinkers.

Chapter 6

To study whether age, biometry and life-style habits affect the outcome of pregnancy as well, within the cohort of 500 women attempting pregnancy through ADI, the 259 women who had conceived within the follow-up time, were retrospectively studied. Maternal age appears to be the most important determinant of fecundity, as well as of outcome of pregnancy (fetal loss and congenital anomalies).

Fat distribution is of significant importance to fecundity, but not to outcome of pregnancy. Life-style habits, in particular moderate alcohol intake, was not related to fecundity, but was significantly related to outcome of pregnancy; namely shorter gestational age and lower birthweight ratio than mothers who did not drink before or during pregnancy.

Chapter 7

The findings of the study are discussed within the context of societal constraints towards women, trying to combine a professional career with motherhood. Implications towards proper preconceptional care for women attempting pregnancy are discussed.

SAMENVATTING

Hoofdstuk 1

Geeft de aanleiding tot het onderzoek en de vraagstellingen: namelijk of moederlijke leeftijd, biometrische parameters en leefstijlgewoonten effect hebben op de kans op zwangerschap en het zwangerschapsresultaat bij cohorten vrouwen die zwanger willen worden.

Hoofdstuk 2

Toont aan dat een populatie vrouwen die behandeld worden met kunstmatige donor inseminatie (KID) een efficiënt alternatief is ten opzichte van het bestuderen van een open populatie vrouwen van vruchtbare leeftijd. De verdeling van leefstijlgewoonten in de KID populatie vrouwen week niet sterk af van die uit een algemeen populatie onderzoek naar leefstijlgewoontes in Rotterdam.

Hoofdstuk 3

Het effect van moederlijke leeftijd op conceptiekans en kans op een gezond kind werd retrospectief bestudeerd in een cohort van 751 nulligravidae, met infertiele partners en nooit eerder onder KID behandeling geweest.
De kritische leeftijd waarop kans op zwangerschap gaat dalen is rond het 31e levensjaar. Zowel de kans op conceptie, alsook de kans op een gezonde baby, dalen daarna snel. De gecombineerde kans van een 35-jarige vrouw op een zwangerschap met uiteindelijk een gezonde baby, is de helft van de gecombineerde kans van een 25-jarige vrouw.

Hoofdstuk 4

Het effect van lichaamsvetverdeling op zwangerschapskans werd prospectief bestudeerd in een cohort van 500 vrouwen onder KID behandeling. De verhouding middel: heup werd gebruikt als indicator voor lichaamsvetverdeling. Vrouwen met vet opgeslagen in de buikholte (≥ 0.80 zgn. 'appelvormig') hadden, gecorrigeerd voor alle relevante confounders, een significant lagere kans op zwangerschap, dan vrouwen met een vetverdeling rondom de heupen/dijen (< 0.80 zgn. 'peervormig').

Hoofdstuk 5

Het effect van leefstijlgewoonten, met name het effect van sociaal drinken op zwangerschapskans werd prospectief bestudeerd in een cohort van 500 vrouwen onder KID behandeling.

Vrouwen met sociaal drinkgedrag hadden een iets hogere, alhoewel niet significante, kans op zwangerschap in vergelijking met vrouwen die helemaal niet drinken.

Hoofdstuk 6

Om te weten of moederlijke leeftijd, biometrische parameters en leefstijlgewoontes naast effect op zwangerschapskans, ook effect hebben op het zwangerschapsresultaat, werden binnen het cohort van 500 vrouwen die zwanger wilden worden, retrospectief de 259 vrouwen bestudeerd, die binnen de onderzoekstijd zwanger waren geworden, op hun zwangerschapsresultaat.

Moederlijke leeftijd blijkt niet alleen de allerbelangrijkste determinant te zijn van zwangerschapskans, maar ook van het zwangerschapsresultaat; met name foetale sterfte als wel aangeboren afwijkingen.

Vetverdeling blijkt alleen van significante betekenis voor zwangerschapskans, maar niet voor zwangerschapsresultaat.

Leefstijlgewoontes, met name sociaal drinkgedrag, was significant gerelateerd met zwangerschapsresultaat (kortere gestatieduur, als wel lagere birthweight ratio), maar niet met zwangerschapskans.

Hoofdstuk 7

Tracht de bevindingen van deze studie te plaatsen binnen de maatschappelijke context en de belemmeringen voor vrouwen die trachten een baan en kinderen krijgen te combineren. Gevolgtrekkingen voor een goede pre-conceptionele zorg bij vrouwen met zwangerschapswens worden besproken.

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CURRICULUM VITAE

24-11-1947	Geboren te Gorinchem uit Friese ouders
1960-1965	Christelijke Hogere Burger School 'Oude Hoven' te Gorinchem. Eindexamen HBS-A
1965-1966	American Field Service Scholarship: Studentenuitwisseling naar Kansas City, Verenigde Staten
1966-1974	Doctoraal examen Sociaal-Culturele Wetenschappen, Rijksuniversiteit Utrecht. Hoofdvak: Theoretische Sociologie Bijvakken: Medische Sociologie, Ziekenhuiswetenschappen en Demografie Tijdens studie: kandidaatsassistent (1 jr) en doctoraal assistent (2 jr) afd. Theoretische Sociologie bij Prof.dr. P. Thoenes Doctoraal onderzoekstage (6 mnd) in een Family Planning kliniek in Paramaribo, Suriname Tevens stagebegeleider (2 jr) M.O. Pedagogiek studenten Utrecht en gedurende weekends verpleegassistente (2 jr) (Johanniter Orde), afd. Interne Geneeskunde, destijds Antonius Ziekenhuis te Utrecht Actief in diverse studentencomitees
1974	Docent Medische Sociologie, Sociale Academie, Den Bosch
1975-1980	Wetenschappelijk medewerker Medische Faculteit Erasmus Universiteit te Rotterdam bij Instituut Maatschappelijke Gezondheidszorg (voorm. Hoofd Prof.dr. L. Burema) en Instituut Epidemiologie (voorm. Hoofd Prof.dr. H.A. Valkenburg)
1980-1982	Recipiënt Querido Stipendium Praeventiefonds en Fulbright-Hays Grant voor studie in de Verenigde Staten 1980-1981: Harvard University, School of Public Health and Massachusetts Institute of Technology, Sloan School of Management: leidend tot een Master Degree in Public Health 1981-1982: Harvard School of Public Health and Harvard Medical School: leidend tot Master of Science Degree in Epidemiology

1982	Visiting Research Worker, University of Oxford U.K., Department of Community Medicine and General Practice. Hoofd Prof.dr. Martin Vessey
1983-1985	Docent Epidemiologie en Statistiek. Post Academiale Opleidingen Stichting Sociale Gezondheidszorg Utrecht (voorm. Hoofd Drs. H. Leliefeld)
1985-heden	Staf-epidemioloog, onderzoekslijn Perinatale Epidemiologie afdeling Jeugd en Gezondheid (Hoofd Prof.dr. S.P. Verloove-Vanhorick), Nederlands Instituut voor Praeventieve Gezondheidszorg TNO, Leiden
1989-heden	Gastvrijheidscontract Instituut Maatschappelijke Gezondheidszorg, Medische Faculteit, Erasmus Universiteit Rotterdam in verband met bewerking van onderhavig dissertatie onderzoek

Woonachtig te Linschoten. Moeder van Adriaan (1982) en Willemijn (1986).