Structured Data Collection in Clinical Andrology

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The research described in this thesis was performed at the Andrology Outpatient Clinic & Laboratory, University Hospital Dijkzigt-Rotterdam, and the Institutes of Medical Informatics and Endocrinology & Reproduction, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, The Netherlands.

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Gestructureerde Dataverzameling in de Klinische Andrologie

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List of abbreviations

ANOVA	analysis of variance
ARIS	andrology research information system
ART	assisted reproduction technology
ASCII	American Standard Code for Information Interchange
AUC	area under the curve
CDU	colour Doppler ultrasonography
CFTR	cystic fybrosis transmembrane conductance regulator
CPR	computer-based patient record
CV	clinical varicocele
ELISA	enzyme-linked immunosorbent assay
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
hCG	human chorionic gonadotropin
HIS	hospital information system
hMG	human menopausal gonadotropin
ICSI	intracytoplasmic sperm injection
IUI	intra-uterine insemination
IVF	in vitro fertilisation
LH	luteinising hormone
MAR	mixed antiglobulin reaction
MESA	microsurgical epididymal sperm aspiration
ORCA	Open Record for Care
PMR	paper-based medical record
RIA	radioimmunoassay
ROC	receiver operating characteristic
SD	standard deviation
SDE	structured data entry
SEM	standard error of the mean
SHBG	sex hormone binding globulin
SQL	standard query language
SV	subclinical varicocele
TESE	testicular sperm extraction
TML	testicular microlithiasis
TV	testicular volume
WHO	World Health Organisation

Chapter 1

General Introduction

Infertility

Infertility has been defined by the World Health Organisation (WHO) as: *"lack of conception after at least 12 months of unprotected intercourse"* [1]. Whereas this definition concerns the inability of the couple to conceive, we use the terms male and female infertility for the individual partners of the infertile couple. Only a small proportion of these infertile patients is sterile due to, for example, azoospermia and anejaculation in the male, or anovulation and bilateral Fallopian tube occlusion in the female partner. The infertile population consists of patients with sterility, reduced fertility (subfertility), and fertile patients with belated pregnancy. Assuming a normal pregnancy rate of 20% per cycle [2], the cumulative pregnancy rate is 91% in one year, illustrating that onset of a pregnancy may take more than a year, even when both partners have an optimal reproductive health. It is important to discern that also for infertile couples the pregnancy rate without treatment may be considerable. Given a 5% pregnancy rate per cycle for infertile couples, the cumulative pregnancy rate per year is 46% (Fig. 1). The term fecundability refers to the conception rate per menstrual cycle, and it is therefore more correct to speak of a lower fecundity than of low fertility in couples with a prolonged time to pregnancy.

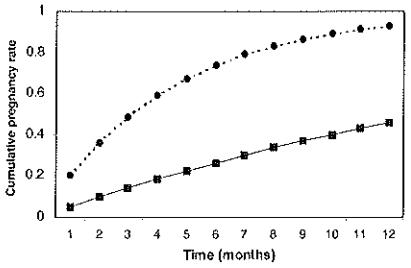

Fig. 1.
Cumulative pregnancy rate for infertile (squares) and fertile couples (circles), assuming a 5 and 20% pregnancy rate per cycle, respectively.

Estimations of the prevalence of infertility are difficult to make, are based on different approaches and criteria, and show a large variation [3-5]. The reported prevalence ranges from 5 to 35%. The pattern of infertility shows a substantial geographical variation, with the highest prevalence in African countries [6-9]. For industrialised countries, the prevalence of infertility in couples actively pursuing parenthood is generally estimated at 10-20% [4,10-17]. Although infertility is not a life-threatening condition, its social and psychological impact on the partners, and their relationship, can be considerable. The later age at which couples start trying to conceive in the developed countries is resulting in more involuntary childlessness, due to the lower pregnancy rate with older age of the female partner, and the higher probability of an adverse pregnancy outcome (e.g., spontaneous abortion) [18]. Moreover, the number of couples seeking professional help for infertility is growing, due to the awareness that assisted reproduction technology (ART) may provide an effective treatment [19]. With the increasing expenses of infertility medicine, there is pressure to strive for a more effective and economic healthcare [20]. This should be supported by expanding knowledge on the causation and treatment of infertility, based on sound clinical research.

The couple's inability to conceive may have either a male or a female cause, or a combination of both (Fig. 2). The coincidence of both a male and female factor in couples consulting a physician for infertility is more frequent than expected on the basis of the prevalence of male and female infertility [21,22]. An explanation for this phenomenon is that optimal reproductive functions in one partner compensate for reduced fertility in the other partner [23,24]. In this light, it is important to thoroughly examine both partners of an infertile couple [25].

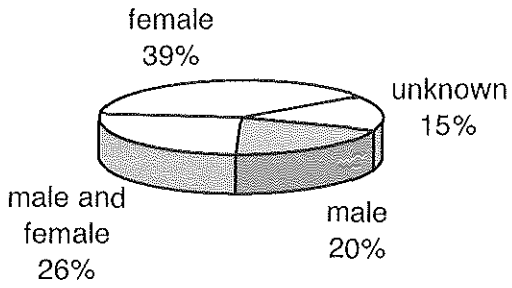

Fig. 2.
The prevalence of male and female causes for infertility (based on WHO, 1987 [9]).

The public interest in and awareness of infertility has increased since several studies seemed to provide some evidence for a decrease in semen quality over the past 30 to 50 years [26-31]. There is substantial evidence that the incidence of testicular cancer has increased in the last decades [32,33]. Also, the incidence of testicular maldescent and hypospadias appears to be increasing [34-39]. Exposure to environmental endocrine disrupters during foetal life has been postulated as a common cause for this increase in disorders of the male reproductive system [40,41]. The factual aetiology of male infertility is, however, largely unknown. The growing number of identified genetic abnormalities associated with male infertility may provide a clue to the aetiology of male infertility [42].

Andrology

The discipline of andrology concentrates on male reproductive health and dysfunction, encompassing the topics male infertility, sexology, erectile dysfunction, senescence, and contraception. Andrology is a relatively young discipline in the medical sciences, and is formed by the multidisciplinary co-operation and overlap of primarily endocrinology, dermatology, gynaecology, and urology. One of the objectives outlined by the European Academy of Andrology is: *"to raise the scientific standards of andrology by basic research, to develop better standards, and to develop diagnostic procedures and therapies based on scientific rather than empirical standards"* [43]. The investigations reported in the current thesis are an effort to contribute to these goals.

Evidence-based andrology

"Evidence-based medicine is the conscientious, explicit and judicious use of current best available evidence in making decisions about the care of individual patients", and *"the practice of evidence-based medicine means integrating individual clinical expertise with the best available external clinical evidence from systematic research"* [44]. Although the number of randomised controlled trials in the field of andrology has grown substantially in the last two decades [45], this discipline is still young, and a backlog in research is being worked through. A systematic review of research data in andrology by O'Donovan *et al.* revealed that the *"best available evidence"* was of poor quality [45].

The Cochrane Collaboration is an international organisation that aims to help people make well informed decisions about healthcare by preparing, maintaining and ensuring the accessibility of systematic reviews of the effects of healthcare interventions, in conformance with the principles of evidence-based medicine [46,47]. So far, four reviews on the treatment of male infertility have been published by this organisation. All four studies concerned men with idiopathic infertility, defined as infertility for which the primary cause is unidentified. These men were treated with either anti-oestrogens, bromocriptine, androgens, or kinin enhancing drugs, versus a placebo or 'no treatment' group [48-51]. The authors concluded that *"The number and quality of randomised studies in male infertility are insufficient to allow firm conclusions in most cases"*. The study populations were very heterogeneous, composed of subgroups with different unidentified causes, with only oligozoospermia as common symptom. Moreover, it has to be realised that the Cochrane reviewers (Vandekerckhove *et al.*) did not assess the methodology of semen analysis. Studies on external quality control have shown wide variations in sperm concentration, motility, and morphology in the same semen sample [52-54]. It is obvious that these variations will affect descriptive diagnoses, such as oligozoospermia.

Standardisation

The clinical examination of the infertile male is not uniform. Diagnoses may arise from different diagnostic criteria; moreover, different diagnostic procedures are used which are also subject to varying precision and interpretation. Patient management and data collection may differ from patient to patient, physician to physician, and from centre to centre. The World Health Organisation's 'Special Programme on Research, Development and Research Training in Human Reproduction' has recognised this flaw, and a special 'Task Force on the Diagnosis and Treatment of Infertility' was established in 1978. This task force defined a standard protocol for the investigation of the infertile couple, which was tested on a population of 9,000 couples in

25 countries. The standard evaluation results in a set of well-defined diagnoses. The 'WHO Manual for the Standardized Evaluation and Diagnosis of the Infertile Couple' provides a reference standard for infertility medicine [1]. Simultaneously, the 'WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction' was compiled [55]. Together, these manuals should provide an essential and effective set of standard protocols leading to appropriate diagnoses and optimal therapy. Adoption of these standards in infertility centres would lead to clearly defined study populations that can be pooled and compared. The use of the WHO manual for semen analysis is widespread, as is reflected in the frequent references to the manual in the 'Materials and Methods' sections of publications concerning sperm quality. Use of the WHO manual for a standardised evaluation of infertility is, however, not common practice. In the belief that standardisation would result in a valuable source of data for clinical research, and ultimately improved andrological care, we have adopted these standards.

Andrological treatments

The WHO diagnostic classification of male infertility is aimed at identifying the underlying causes, and at reaching a diagnosis that enables optimal treatment planning. Classification of male infertility on the basis of sperm parameters only, does not reveal the underlying aetiology. Evaluation of a treatment on a heterogeneous group of infertile men characterised by 'abnormal semen' is less likely to find an effect than on a selected population with a well-defined diagnosis as a basis for treatment. Unfortunately, even with a thorough diagnostic work-up, the cause of infertility cannot be detected in a considerable number of infertile men. In the WHO study on 9,000 patients, idiopathic semen abnormalities accounted for 50% of the cases of infertility [21]. This high figure underlines the need for further research on the pathogenesis of male infertility, and the development of appropriate diagnostic procedures and treatments.

For those patients with an identifiable cause for their infertility, various treatments are used. A major deficit of the present repertoire of treatments is that most of them have not been subjected to a thorough evaluation. Evaluation of treatments of male infertility is difficult. The ultimate outcome measure is pregnancy, which means that also female factors (ovulation disorders, tubal occlusion) as well as the interaction between partners (coitus frequency, timing of intercourse around ovulation, duration of abstinence) can be considered as serious confounders. Moreover, pregnancy rates are low in case of male infertility. This implies that large groups of patients have to be studied to evaluate treatments. For example, to confirm an increased cumulative pregnancy rate from 10 to 15% within one year in a randomised

controlled trial, 200 pregnancies in 1,600 couples would be required [56]. These numbers of patients are difficult to recruit in a single centre, and standardised multicentre research may provide a solution. Therefore, a standardised patient assessment and structured reporting is especially important in andrology.

Biochemical and morphological evaluation of semen quality is another outcome measure, which is not obscured by female factors. However, semen analysis must be assessed meticulously, complying with strict conditions, since many biases and confounders are involved [57].

The computer-based patient record

The common paper-based medical record (PMR) may suffice for care of the individual patient, by a single physician. Medical care, however, is becoming more complex, involving several specialised care providers. This complexity, together with a growing need for healthcare data, may benefit from the immense evolution of information and computer technology. The promises of computer technology for reporting of patient data, medical audit and administrative use have been recognised decades ago, and led to the introduction of medical information systems.

Strengths of the PMR are portability, easy browsing, and a common structure among medical disciplines, with which physicians are familiar since the time of their professional training. A challenge for computer-based patient record (CPR) developers is therefore to combine the strengths of the PMR with the potential of computer support. Obvious advantages of the CPR over the PMR are legibility and availability to more than one healthcare worker at different locations through networked computers. Further potential advantages are the ability to arrange or sort data in different configurations without having to duplicate the data, the ability to search data on a particular patient or group of patients, and the ability to analyse the data. Advanced systems provide automatic reminders, decision support, critiquing, prognostic models, sharing of records, telemedicine, multimedia (e.g., video sequences, radiological images), etc. [58].

A primary concern when dealing with medical data is that the data must be unambiguous and accurate. Data may easily be misinterpreted when they are used outside the context of individual patient care, in which they were recorded. Limitations of the data (e.g., implicit relationships, ambiguity, and inaccuracy) have to be fully understood before the data are used for clinical research. These pitfalls may be avoided by appropriate structuring of data. For data to be useful for research, data should not be recorded as 'free text', but rather in a formalised manner, referred to as structured data entry (SDE). SDE has proven to collect more complete and less ambiguous data compared with the paper equivalent [59]. ORCA (Open Record for

Care), developed by Van Ginneken *et al.* [60], is an advanced CPR model, using SDE based on knowledge about the domain.

Andrology in Rotterdam

The Andrology outpatient clinic of the University Hospital Dijkzigt-Rotterdam was established in 1989, and has from the beginning used a standard protocol for the evaluation of the infertile male. This protocol already contained all items required according to the WHO manual, which was first published in 1993. Appendix A shows the items collected in our clinic and laboratory. It includes a thorough examination of semen, according to WHO guidelines. Most data are stored in dedicated components of the Hospital Information System (semen analysis, examination of the genitalia), part is stored in generic components (serum hormones, radiology), and a few items are recorded in the PMR only . The dedicated components use standard forms for SDE, which comprise a guideline and a checklist for the examination of the infertile male. This approach stimulates completing the data, and a uniform approach among physicians, but is not flexible (adapting the data set requires changes to the underlying database and data-entry forms, and recording of incidental findings is not supported). A common approach is feasible for the highly specialised field of andrology. At the start of the study described in this thesis, the structure of the separate systems was not suitable for integration and statistical analysis of the data.

Aim of this thesis

Since the evaluation of diagnostic procedures and treatments in andrology requires large numbers of patients, standardisation is of particular importance. The aim of this thesis is to study the value of a thorough and standardised evaluation of male infertility, and to evaluate andrological diagnostics and treatments. This investigation is based on a large collection of patient data that was obtained in a standardised fashion, and collected in a structured manner during routine patient care during the last decade (1989 – 1999).

The principal research objectives are:

- to investigate the feasibility of an information system for flexible access to integrated and structured andrological patient data
- to survey andrological care by:
  - assessing the prevalence of abnormalities and diagnoses
  - charting of treatment options for this population
  - evaluation of diagnostic procedures and treatments

- to assess the value of a standardised approach for both clinical research and patient care.

Outline of this thesis

This general introduction is followed by chapters that address the research objectives. Chapter 2 reports on the development of a research information system for andrology. The problems with existing andrological data sources are discussed, and the feasibility of transparently integrating existing data and prospective data collection in ORCA is studied. This latter research information system has been employed for the subsequent research questions. In Chapter 3, the significance of standardised evaluation of male infertility is addressed and the general characteristics of the andrological population are assessed. The prevalence of abnormalities and diagnoses according to the standard WHO evaluation are studied, treatment options are determined, and the value of additional diagnostic procedures is addressed. The following three chapters discuss in more detail two specific diagnostic modalities and a therapeutic procedure. Chapter 4 deals with the potential diagnostic value of using serum inhibin B measurement as a new endocrine marker of spermatogenesis. Chapter 5 describes the diagnostic value of routine examination of the scrotal contents with colour Doppler ultrasonography in male infertility. The effect of treating a varicocele – varicose veins in the scrotum – on sperm quality is assessed in Chapter 6. Chapters 3 to 6 can be seen as an evaluation of the value of standardised and structured data collection. Chapter 7 contains the general discussion of the current research, and ends with the conclusions of this thesis. Finally, an overall summary is presented.

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Chapter 2

Restructuring Routinely Collected Patient Data: ORCA Applied to Andrology

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Abstract

Hospital information systems do not always cover all required detail per specialty. This may lead to scattering of data over disparate systems and the paper record. The ORCA (Open Record for Care) CPR offers a generic structure for record sharing, and record keeping tailored to specific needs. We studied whether a semantic integration of existing and new data was possible, using the ORCA structure. Existing andrology data, originating from separate sources, were utilised for this purpose. During normalisation, validation and explication steps, latent problems in the source data were exposed and removed, followed by a merge with new data items. By conversion of source data to ORCA, a unique representation of medical concepts in the database was attained, facilitating retrieval of univocal data for multiple purposes. We conclude that the expansion to the andrology domain, including transparent integration of existing data, provides support for the generality of ORCA.

1. Introduction

The motivation to apply electronic data storage in medicine is based on the obvious drawbacks of the paper-based medical record (PMR), including illegible handwriting, poor organisation of documents, missing data and ambiguous data. Furthermore, the PMR is available at only one location at any time [1,2].

With the introduction of hospital information systems, the legibility and hospital-wide accessibility of data have been improved. These systems were initially developed for storage of more general patient data (e.g., administration data, clinical chemistry and medication), and are not always tailored to the specific needs of medical specialists. This has resulted in the complementary use of dedicated systems. As a result of these developments, data can be scattered over several sources and systems, which may lead to redundancy and inconsistencies.

These problems will be outlined in general, and in more detail for the Andrology outpatient clinic of the University Hospital Dijkzigt-Rotterdam. Andrology is the branch of medicine that deals with male reproductive health and dysfunction. Patient care and clinical research on diagnostics, prognostics, and treatment of male infertility, require accurate storage of patient data and flexible access to reliable data. During recent years, this department has been storing most patient data in generic systems of the hospital information system (HIS), in dedicated HIS components, and in local PC databases. Some disadvantages, experienced by the andrologists, were that these data could not be retrieved through one user interface, and that data structures do not lend themselves to exploitation for research purposes. Integration of the multi-source data is one prerequisite to cope with these problems, but it is not the solution in all respects, as will be clarified in this paper.

Current developments in computer-based patient records (CPRs) focus on advanced features, including strategies for data entry and retrieval, multiple views [3-10], and transparent integration of diverse information sources on different platforms [11-13]. In essence, the conceptual model underlying the CPR database determines the functionality of the resulting applications for data entry, consultation, and retrieval [14,15]. At our department, Van Ginneken *et al.* have developed a powerful CPR with a graphical user interface [16,17], which has recently been named ORCA (Open Record for CAre). It provides a general core model for sharing of records by physicians, and it allows tailoring of data entry to the specific needs per specialty. ORCA was initially designed to offer a well-structured CPR for prospective use, with the objective to improve completeness and reduce ambiguity. The introduction of a new CPR is, however, complicated by the fact that existing data in predecessor systems are not easily incorporated. The real challenge is not to offer a view on existing data tables, but to achieve a uniform and fully semantic integration of existing and new data sets.

We describe the problems with routinely collected data in the initial situation in andrology, followed by an explanation of the foundations and structure of the ORCA model. We will outline the steps that have to be taken to explicate the meaning of multiple-source data to an intelligible level. This data set, suitable for univocal interpretation, was expanded with attributes to be gathered prospectively. The resulting semantic merge was modelled according to the ORCA model. The discussion will focus on the strengths and weaknesses of ORCA for the restructuring of routinely collected data.

1.1 Source Data and Problems

Most of the data pertaining to andrology patients is contained in generic components of the HIS, in andrology specific HIS tables and in dedicated PC databases. Data obtained by physicians is entered on-line, results of andrology laboratory tests (e.g., semen analyses) are entered by laboratory personnel. The source data was retrieved from the (sub)systems as individual extraction files in plain ASCII format. The data sources we could retrieve were (1) history, previous history and physical examination, (2) semen analyses, (3) data on intra-uterine inseminations, (4) therapies, diagnoses and follow-up, (5) clinical chemistry results, (6) radiology diagnoses, and (7) surgery reports.

Data analysis in the initial situation was complicated by the scattering of data over several sources, and partly inconsistent and rigid database structures. We also encountered errors in code lists and validity constraints. Most imperfections were not recognised during data entry, and became apparent when data were used for research.

The problems described below, are the main ones encountered in the present case. They may also represent general disadvantages when dealing with multi-source data. Some will be illustrated with examples from our experience:

1 **Problems related to incomplete normalisation**

a. Integrity problems:

- Entity integrity: no unique identifier for records, e.g., more than one ID per patient, or unknown ID.
- Referential integrity; e.g., changes in the values of key attributes are not inherited by related tables.
- Redundancy: duplicate records and fields. Identical medical concepts can exist more than once, in several sources or within one source. Lack of cross-referencing causes inconsistency.

b. Ambiguity: the indistinct relation between database items and medical concepts. Fields can contain a combination of medical concepts. A correct interpretation of these attributes is impossible without explicit knowledge of such constructions. For instance, the 'Mumps' field in the past history section holds both whether or not the patient has suffered mumps, and the age at which a patient has had mumps. Codes 1 to 97 represent the age when mumps occurred, 0 = 'no mumps', 98 = 'unknown age' and 99 = 'mumps unknown'. Such constructions can lead to misinterpretation of data, and make queries more complex than necessary.

c. Implicit relationships: the meaning of attribute codes or values can be different, dependent on other attributes. Proper interpretation of such a value or code requires retrieval of the medical context from the contents of other data fields.

d. Repeating attributes: The storage of more than one medication was supported by a repeating set of attributes within one record, restricting the maximum number of medications to the number of repetitions.

2 **Problems related to the scattering of sources:**

a. Integration problems: data analysis and decision support are complicated when data reside in more than one source.

b. Versions: different versions of a system are in use. For example, for look-up of semen analysis results, two tables have to be consulted.

c. Formats; e.g., the date in the order dd-mm-yy and mm-dd-yy in different systems.

3 **Other imperfections:**

a. Incorrectness: e.g., no validity checks, several codes for one condition, improper default values.

b. Incomplete representation of time: data items are not always explicitly marked with time-stamps. For example history, medication and diagnosis are stored in one source file. Although these sets of data may have been gathered and entered at different dates, they are all stored in one record with one date.

c. Correction by substitution: Correction of false findings or changing a field-value in these systems is achieved by replacing the old value with a new. In this manner, correction of erroneous data, or an adjustment of insights, means a loss of existing data.

These problems may lead to misinterpretation of data, especially when data are used outside the context of data entry as in clinical research.

1.2 Goals

We predetermined the following goals:

To realise a new situation with flexible access to existing patient data for clinical research and patient care. This goal was to be reached by integration and validation of patient data from disparate sources in one structure that explicates medical semantics as far as possible.

To study whether data structures, both the existing and those to be used prospectively, can be represented in the ORCA structure, to achieve a fully integrated CPR.

2. Materials and Methods

2.1 The ORCA-CPR Model

The Department of Medical Informatics, Erasmus University Rotterdam, has developed the well-structured ORCA CPR [16-18]. ORCA has been specifically designed for use in specialised care, which encompasses many different medical domains. The main aims of ORCA were (1) to have a record that would encourage the recording of well-structured and more complete data, suitable for decision support and data analysis, (2) to offer flexible access to the record contents via a variety of views, and (3) to allow sharing of records among co-treating physicians. The latter presents a challenge since the variability among specialists requires that the CPR can be tailored to specific needs, whereas sharing of records demands a general structure.

The ORCA model supports the creation of specialised subrecords that differ in content, but share the same data structure. It is this common data structure that gives ORCA its transparency, i.e., a uniform interface for the collection and consultation of data, irrespective of the medical domain. The core of this data structure is shown in Fig. 1. It is based on the idea that the record is a report over time of medical events, pertaining to a given patient. A 'Patient Event' is a set of data that can be considered to have been discovered at one moment, to be valid from one moment, and to have originated from one source. Examples of such events are: the patient visit, a laboratory report, an X-ray report, etc. The 'CPR Event' is only created as an entity to accommodate the situation where data, pertaining to one patient event, are entered in different sessions. A patient event may consist of one or more components, called 'Actions'. Examples of actions are: history, physical examination, a drug prescription, and a laboratory test result. Expanding ORCA with new domains may involve the creation of subtypes of actions with attributes, specific for the domains involved.

For data sharing and analysis, non-redundancy is crucial. Using only relational tables as subtypes of actions bears the danger of creating numerous tables where overlapping items are represented by different attributes. Ideally, each medical concept has one unique representation throughout ORCA. Therefore, there are two

models for storing the actual data: (1) a relational model for data that are domain-independent, and (2) a controlled vocabulary for data that are domain-dependent. The laboratory test result, the drug prescription, and the diagnosis are typically domain-independent in the sense that the attributes by which they are described are always the same: each drug prescription is characterised by the name of the drug, the dosage, the route of administration, and the frequency of intake. Data pertaining to history and physical examination are highly domain-dependent: specialists differ in topic and in detail when describing their findings. Recording of such data is supported by a knowledge-driven interface that creates instantiated trees of medical concepts as patient data. For example, each blood pressure measurement will be stored as an instantiation of the unique concept 'blood pressure', irrespective of the specialty or domain involved.

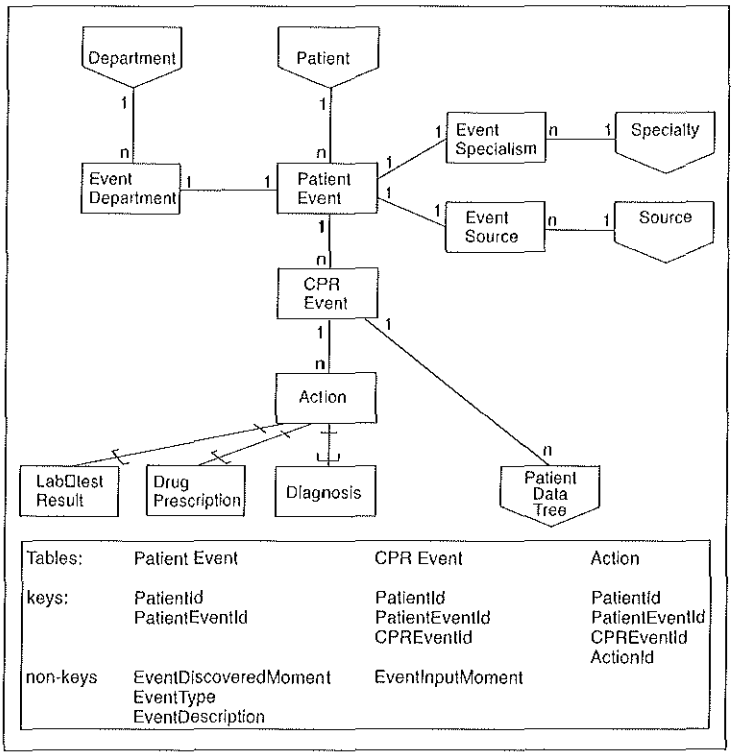

Fig 1.
The ORCA core model with actions to represent domain-independent data and "Patient Data Tree" to represent the domain-dependent data. Events are linked with department, specialty and source (author), to support the departmental, specialty, and author view in the patient profile.

The 'patient profile' in the ORCA interface provides a view on the contents of the CPR. This view can be customised, but includes by default the current diagnoses, a list of events (most recent ones in view), current medication, and allergies and sensitivities. The ORCA model supports a departmental, specialty, author, and overall view on the data pertaining to the selected patient. The patient profile gives direct access to the more detailed sections of the record. Depending on the type of actions involved, ORCA will present the appropriate interface for data entry or consultation. Hence, all data that are represented in ORCA are directly accessible in a fully integrated fashion.

Time-stamping is an important aspect in ORCA, since it allows recording of evolving insight without violating the requirement of a medical record to be permanent: it is not legally allowed to overwrite existing data. Accurate representation of time is essential for reconstruction of the chronology of events [19]. Time-oriented representation of data is critical to make good use of clinical data [20], since longitudinal clinical databases represent a valuable resource for medical audit and decision making [21]. As can be seen in the core data model, the events have attributes for the recording of transaction time (EventInputMoment) and valid time (EventDiscoveredMoment). Where applicable, an additional time-stamp is included at the action level: for example the SampleMoment in laboratory test results. In other words, ORCA is based on a temporal data model, enabling both recording of 'valid time' and 'transaction time' [22,23].

The specialised andrology subrecord has been named ARIS (Andrology Research Information System). We will use the name ORCA-ARIS where ORCA tailored to the andrology domain is meant.

2.2 Database Design

For the design of the Andrology database, an information analysis was performed. The information needs were deduced from the existing computerised data collections and the requirements that were inventoried from the andrology staff. To elucidate the value, meaning and validity of attributes, the knowledge of domain experts was essential. Data structures were restructured according to the conventions for logical data models [24,25]. The original andrology data was normalised up to the Boyce-Codd normal form in accord with the following rules:

1. Repeating attributes within a record must be reduced to single attributes in one or more separate tables: e.g., initially, a fixed number of medications was included as a recurring group of identical attributes in a source file.
2. Implicit one-to-many relationships within tables, (i.e., repeating groups), have to be made explicit.

3. Non-key attributes must be fully dependent on the primary key. For instance the *partnerId* in the initial relation *HISTORY (patientId, partnerId, etc., historydate, duration_of_infertility, coitus_frequency, etc.)* was dependent on the key *patiendId*, but not on the key *historydate*. The *partnerId* will be split off and represented in the relation *Couple (patientId, partnerId, etc.)*.
4. All relationships between tables have to be via the key attributes: e.g., fields that are split off will relate to the remaining group by inheritance of a common set of key attributes.
5. Relations should not contain transitive dependencies. Non-key fields that depend on other non-key fields are segregated.

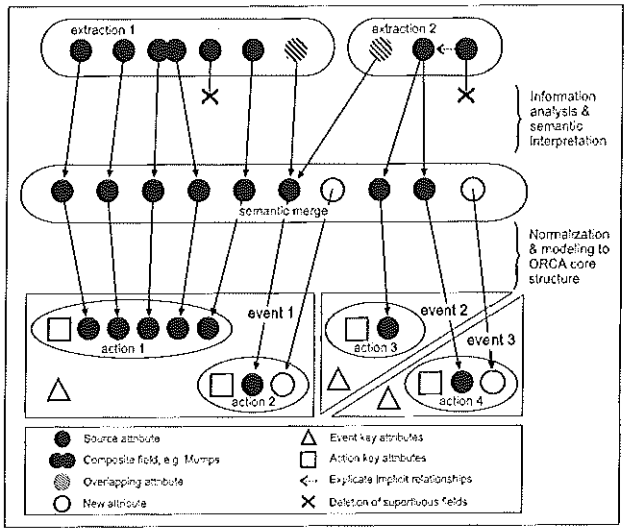

Fig 2.
The semantic integration of source data and new data, and the subsequent representation in the ORCA core structure.

Not all problems with the original data were solved by normalisation. Some conversion steps required semantic interpretation (depicted schematically in Fig. 2). Semantically overlapping fields were removed after domain experts indicated the most accurate ones. Composite fields were split up to separate fields. Implicit relationships were explicated. For instance, a semen analysis can be performed for different reasons, with a different method: to diagnose infertility, or during preparation for artificial insemination. The interpretation of the value of an attribute, in this case *sperm_concentration*, depends on another attribute, *semen_analysis_type*. Creating separate data sets for each medical context in which *sperm_concentration* is measured (i.e., *semen_analysis_type*), will make the medical semantics explicit.

The integration and restructuring of andrology data in a properly designed relational database solves some of the problems with the initial sources. Our objective was not only to make a clean sweep of the existing data sources, but also to see whether the data could be represented in the core structure of ORCA. Since we worked with existing data, events and actions had to be defined retrospectively. Patient events were identified using the definition stated in section 2.1: 'a set of data discovered at one moment, valid from one moment, and originating from one source'. Examples of events for the andrology data are the patient visit and a radiology report. Two other examples of events, 'semen analysis' and 'insemination preparation', were derived from one extraction file, where assignment of the actual data to one of these events depended on the *semen_analysis_type*. This is represented by 'event 2' and 'event 3' in Fig. 2, that are the result of the conversion of 'extraction 2'. Within an event, one or more actions (i.e., medical entities) were defined with the aid of domain experts on clinical daily practice. Examples are andrological previous history, physical examination, and drug prescription.

2.3 Conversion and Merging

Values of attributes that represent the ORCA core structure, shown in Fig. 1, were deduced from existing data where possible, and otherwise generated by the database. This resulted in the following operations for:

- PatientId: a unique key was generated by the database, and a mapping to the original HIS-PatientId was created.
- PatientEventId: this key is generated by the database for each new EventDiscoveredMoment.
- EventDiscoveredMoment. This time-stamp was:
  1. Directly derived from a corresponding attribute in the original data set.
  2. Partially derived from the source files. For example, only the date is stored in the source files, without the time of day. In this case, the time of day in ORCA-ARIS was chosen arbitrarily at 00.00 hours.
  3. Actions were stored without explicit time-stamp in one record. Because it is plausible that the point of time is equal for the separate actions, the date connected with the source file was used for the event.
- CPREventId: this Id was generated by the database. To accommodate entering of data regarding one Patient Event in different sessions, the CPR Event has a one-to-many relationship with the Patient Event. Since these sessions could not be reconstructed from already collected andrology data, the CPR Event and Patient Event in ORCA-ARIS have a one-to-one relationship.

- EventInputMoment: because this time-stamp reflects data entry in ORCA-ARIS, we assigned the transaction time at conversion.
- ActionId: generated by the database.
- EventType: for example 'Consultation'.
- EventDescription: not applicable.

The conversion was performed by a conversion script written in C, with embedded SQL. In preparation for this script, all existing values for an attribute were recovered. The script was based on these values to ensure that all original data were converted to meaningful data in the ARIS database. Values in fields were checked for correctness, multiple but equivalent codes were converted to a unique code, and composite fields were separated. Data that could not be converted to correct data in ORCA-ARIS were reported back to the Andrology outpatient clinic for inspection and correction.

The conversion script consists of functions to read the ASCII source files and write them to the ARIS database: (1) reading a record and identifying the different attributes, (2) checking the correctness of elements, (3) conversion to new attributes and values, (4) creating attributes and values for events, actions and time-stamps, and (5) importing the data into the ARIS database. We are currently developing the controlled vocabulary to enhance structured data entry in ORCA-ARIS. Data items pertaining to history and physical examination will then be represented in tree-structures, to preserve semantic uniformity.

2.4 The Hermes Workstation

ORCA-ARIS is part of the Hermes client-server environment. The Hermes workstation is designed to support access to and analysis of data from different sources [12,13].

We are currently moving from the HERMES-UNIX to the Windows NT/Windows '95 platform with an MS-SQL server and Delphi applications. Our software is currently transcribed, and adjusted to the latest insights.

3. Results

Since the results ensue logically from the methods, an elaborate discussion of the results would mainly reflect the Materials and Methods Section. We confine ourselves here to reporting on the feasibility of the remodelling and conversion of andrology data to the ORCA-based model.

For all data, the corresponding actions and events could be discerned. To be able to assign exact values to the time-stamps, presuppositions were made for some events, as described earlier.
There were no structures in the original data sets that could not be converted to meaningful data in ORCA-ARIS. All overlapping fields were removed. Invalid codes that could not be brought back to the intended value were converted to 'unknown' in ORCA-ARIS. Since the meaning of these items was also unknown in the initial sources, this is not a loss of information. Some of the 'old' data is not represented in the new situation because this was a choice by the Andrology staff. These were attributes in the initial sources that were not used for patient care.
The ORCA-ARIS database consists of 43 relations and 298 attributes, as opposed to the original nine files containing 175 items. A large part of the increase in the number of attributes is caused by the generation of new key fields, events, actions and time-stamps. The database currently contains data on 7,000 patients.

4. Discussion

Several authors have stated that large clinical databases are a valuable source for research, although retrieval can be cumbersome. We recognise the limitations they describe, involving organisation and validity of data, the need for database experts to gain access to the data, the investment needed to retrieve the desired data [26,27], and the *"imperfect capture of clinician thought processes"* [28]. These limitations must be clearly understood to permit clinical research with patient data [29,30].

The initial problems with separate sources and incomplete normalisation have caused ambiguity, incorrectness and redundancy in the andrology data. Data that are stored in a certain clinical context may be correctly interpreted when consulted in the same context. When, however, these data are retrieved for other purposes, such as medical research, there is a risk of misinterpretation. Performing research with these sources requires intense conferring between researchers, physicians, and data managers [31,32]. Knowledge on the clinical procedures and database structures is essential to judge correctness, meaning and certainty of database items. The explicit meaning of terms (i.e., knowledge on the semantics) was not captured by the data model, but resided in only one or two domain experts, i.e., a medical and a database expert. The loss of an expert may result in the inability to perform correct data retrievals and valid clinical research. By capturing semantics in ORCA, this knowledge is explicitly represented, and will permanently reside in the database. This should result in less misinterpretation of data and decreased miscommunication between physician and data manager.

By making a clean sweep of the existing data sources, equivocalities that could have frustrated data interpretation or analysis were brought to light. Many examples of invalid retrieval or misinterpretation could be given here; the problems stated in Section 1.1 also serve as examples. We will cite one problem here: the mumps attribute, an example of ambiguity in the source data, illustrates the potential misinterpretation of data. A researcher might want to know the incidence of mumps infections during puberty or adulthood (e.g., mumps at > 12 years of age), since this can result in testicular infection and impaired sperm quality. By selecting men with a value >12 for the mumps attribute, not only the intended men are selected, but also those with the values 98 and 99, coding for 'patient has suffered mumps at unknown age' and 'unknown whether patient has had a mumps infection'.

This research question illustrates that a simple one-to-one transfer of the attributes in separate sources, to attributes in one relational data model, will not explicate the meaning of the data. A thorough assessment of the meaning of attributes and attribute contents is essential to elucidate potential problems with patient data. Based on this assessment and the ORCA model, the new ORCA-ARIS data structure was built. Conversion of original data to this normalised structure was achieved by a semantic merge. Implicit relationships were explicated, so that a one-to-one relationship between medical concepts and their representation in the database was attained.

A lesson we learned from this study is that existing, retrospectively gathered data can be integrated in the ORCA model, for which conversion to actions and events is essential. For the andrology data, actions and events could be discerned with the help of a medical specialist in this domain. The EventDiscoveredMoment time-stamp could be deduced from the data, although not always to the exact time of day. In those cases we assigned 00.00 hours as the time of day. This may result in an incorrect representation of the chronology of events within one day, which is identical to the initial situation. Since in daily practice the exact time-stamps were obviously not needed, we do not expect this to be a significant problem. A strength of using ORCA for restructuring of data is that it does not produce a revised stand-alone record: a general structure is utilised for sharing data among co-treating physicians, whereas also the necessary amount of detail per specialty can be supported.

The transparent integration has resulted in a uniform view on both existing data and data to be gathered prospectively. For logistical reasons, the data entry facility of ORCA is not yet in use in a routine setting. To fully exploit the ORCA potential, data entry should also be through the ORCA-ARIS interface, since this will stimulate and effectuate structured, complete, and accurate data collection.

5. Conclusions

A uniform and fully semantic integration of existing and new andrology data was achieved with the ORCA core model. Latent imperfections in the source data such as validity problems, ambiguity and inconsistencies were exposed and removed. The quality of data in ORCA-ARIS has been improved by this approach. The one-to-one relationship between medical meaning and database representation reduces misinterpretation of data and miscommunication between physician and data manager in a research setting.

An important conclusion from this exercise is that the ORCA model, as of yet applied for prospective CPRs for internal medicine and a cardiology outpatient clinic, was sufficiently general and powerful for expansion to the domain of andrology, including a uniform and semantic integration of existing data. These experiences provide support for the generality of the ORCA model, which is intended to be suitable for other medical domains as well.

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Chapter 3

The Significance of Standardised Evaluation of Male Infertility

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Abstract

To achieve improvement in healthcare, standardisation of data collection and evaluation of diagnostic methods and treatment is necessary. The aim of the present study was to investigate the value of routine collection of a uniform dataset for standardisation and clinical research in an andrology clinic. Data were collected in a structured computer-based patient record, promoting complete and comprehensive data, and stimulating a uniform patient approach among physicians.

Data on the standardised examination of 1,549 infertile men were evaluated. Population characteristics and basal associations are reported. A cause for infertility was identified in 52% of the men on the basis of the standard evaluation defined by the WHO. This proportion was increased to 66% by additional scrotal colour Doppler ultrasonography, and identification of men with subnormal serum gonadotrophins with normal testosterone levels. For more than 30% of the patients, an effective andrological treatment was available. The thorough diagnostic evaluation identifies subgroups that require further study on aetiology, diagnosis, and treatment.

Structured collection of patient data stimulated a uniform patient approach among physicians, enabling appropriate inventorisation of patient characteristics. This approach also greatly facilitated the evaluation of diagnostic and therapeutic modalities. An accurate diagnostic evaluation is mandatory for appropriate therapy and further research in andrology.

Introduction

Quality of andrological care may be improved by systematic use of standardised diagnostic methods and treatments. This approach allows to collect reliable and complete data for andrological research. Although, ideally, research is performed in a prospective design, routinely collected patient data are a potential source for clinical research. This demands, however, an infrastructure that promotes collection of structured data.

Computers are valuable tools for recording, organising and analysing medical data. An increasing number of centres use systems for electronic data collection. However, a frequent drawback of current systems is a limited scope, resulting in scattering of data over disparate systems, redundancy, and ambiguity. Data on a single patient may be readily retrieved from these sources, but the structure of the data is often unsuitable for research on groups of patients. As a result, data retrieval is laborious and prone to error.

A computer-based patient record (CPR) has been developed (named ORCA; Open Record for Care) [1], which provides a general model for all patient data from different specialties and departments. ORCA was used to integrate existing andrology data into one transparent univocal structure [2].

The aim of this study was to evaluate the value of systematic use of a well-structured CPR for andrological research.

Materials and Methods

Data sources

Andrology-specific patient data collected during history taking, physical examination and semen analyses were retrieved as extractions from dedicated computer systems. From 1990 to 1995, semen analyses on 3,000 patients were gathered, as well as histories, physical examinations and serum hormone values on 1,800 patients. Other data were retrieved from general hospital information systems: clinical chemistry (e.g., FSH, LH, testosterone), radiology (scrotal colour Doppler ultrasonography, venography of the testicular veins), pathology (Johnsen score in testicular biopsies), and surgery (e.g. varicocele ligation, testicular biopsy, vaso-vasostomy).

The contents of the separate andrology data sources were integrated according to the ORCA structure. During the data conversion process, redundant and invalid data were removed, and ambiguity was solved by creating 1:1 relations between medical concepts and database items [2]. Data on patient groups were retrieved from the ORCA database using the standard query language (SQL).

Clinical data

A complete clinical work-up was available for 1,549 patients. Andrological patient work-up was performed according to the WHO standardised approach [3].

Semen analyses were performed on at least two semen samples according to the WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction [4]. Since it is unknown which of the two analyses best characterises the individual's semen, we calculated the mean values of two analyses.

Hormone assays: Serum FSH and LH were determined using Amerlite FSH and LH assays (Orange Clinical Diagnostics, Amersham, UK); normal ranges are 2-5 and 1.5-8 IU/l, respectively. Total serum testosterone was measured by radioimmunoassay, as described earlier [5]; normal range 10-30 nmol/l. These normal ranges are based on a group of healthy male blood donors [personal communication]. Sex hormone-binding globulin (SHBG) was determined using the immunofluorometric assay provided by Diagnostic Products Corporation (Los Angeles, CA, USA).

Scrotal ultrasonography was performed with a high-frequency duplex echotransducer (≥ 7.5 MHz) equipped with colour flow imaging. The criterion for varicocele was a vein diameter in the pampiniform plexus ≥ 3 mm [6]. Retrograde blood flow in these vessels was used as a supporting sign [7].

Varicocele treatment consisted of high ligation of testicular veins using the Palomo approach [8].

Diagnostic classification

The WHO has defined a standardised approach for the investigation of infertility, which includes extended guidelines and decision rules on the diagnostic classification of patients [3]. This diagnostic classification was applied to our andrology population. Two items of the formalised WHO checklist were not collected in a coded form, but as free text. Namely, 'systemic causes' (i.e., systemic diseases, high fever, drug or alcohol abuse, environmental or occupational factors) and 'history of medication with possible adverse effect on fertility' were not available in a structured format.

Statistical analysis

Correlations were studied with Spearman's correlation procedure. Differences between patient groups were tested with one-way ANOVA, and with Fisher's least-significant difference method for pairwise comparisons. Skewly distributed variables were transformed logarithmically. Two-sided *P*-values less than 0.05 were considered significant. Statistical analysis was performed with SPSS 7.5 for Windows software.

Results

Descriptive data

General characteristics of the 1,549 andrology patients are given in Table 1. Sperm parameters were subnormal in 98% of the patients. Due to azoospermia in 12% of the patients, the 10th percentile is zero for sperm parameters. Blood hormone levels were within the normal range in 81, 44, and 88% for FSH, LH, and testosterone, respectively. Male infertility was primary in 81% of the cases, and 19% of the men had achieved a pregnancy before. Infertile men were referred to the andrology outpatient clinic by gynaecologists (49%), general practitioners (32%), urologists (15%) and various other specialties (4%).

Table 1 General characteristics of the study population.

Variable	Median	10th Percentile	90th Percentile
Age (years)	32.4	26.2	41.5
Duration of infertility (years)	2.5	1.3	6.4
Total testicular volume (ml)	30.0	19.0	40.0
Ejaculate volume (ml)	3.5	1.6	6.0
Sperm concentration (10^6/ml)	6.5	0.0	45.0
Progressive motility (%)	14.0	0.0	45.0
Sperm count (10^6/ejaculate)	22.5	0.0	162.5
FSH (IU/l)	4.0	1.4	11.7
LH (IU/l)	2.6	1.2	5.6
Testosterone (nmol/l)	17.6	10.9	27.1

WHO diagnoses

A single diagnosis was assigned per patient following the WHO diagnostic classification. The distribution of diagnoses is shown in Table 2. The group of patients with the diagnosis *Causal factor* (35.6%) may had more than one causal factor (e.g., both a varicocele and a history of accessory gland infection). In 14 azoospermic men with both normal testicular volume and serum FSH level, no testicular biopsy was assessed. Although possibly having obstructive azoospermia, this latter group was categorised as having idiopathic azoospermia, since a normal testis biopsy score is a requirement for classification as obstructive azoospermia [3]. Besides the WHO diagnoses, we defined the additional diagnosis 'subnormal FSH and LH levels, with normal testosterone level' (prevalence 4.9%), which was not primarily considered as hypogonadotropic hypogonadism. Table 3 summarises the therapy options for these diagnoses.

Table 2 Distribution of diagnoses among 1,549 infertile men, according to the WHO classification.

	Number of patients	% of total
No demonstrable cause	7	0.5
Sexual / ejaculatory dysfunction	72	4.6
Immunological cause	170	11.0
Seminal plasma abnormalities	3	0.2
Causal factor[a]	602	38.8
clinical varicocele	*220*	*14.2*
inguinal hernia	*91*	*5.9*
vasectomy	*44*	*2.8*
history of cryptorchidism	*139*	*9.0*
CBAVD	*6*	*0.4*
acquired testicular factor	*63*	*4.1*
accessory gland infection	*82*	*5.3*
hypogonadotropic hypogonadism	*53*	*3.4*
low FSH and LH, normal testosterone[b]	*76*	*4.9*
Idiopathic oligozoospermia	427	27.6
Idiopathic asthenozoospermia	166	10.7
Idiopathic teratozoospermia	20	1.3
Obstructive azoospermia	17	1.1
Idiopathic azoospermia	65	4.2

[a]: causal factor in the presence of abnormal semen quality

[b]: this diagnosis is not a standard WHO diagnosis

Table 3. Frequency of diagnoses with a therapy option other than IVF or ICSI.

Diagnosis	Frequency (%)	Therapy
Hypogonadotropic hypogonadism	3.4	GnRH, gonadotrophins
Obstructive azoospermia	1.1	Surgical correction
Immunological infertility	11.0	Sperm washing
Sexual or ejaculatory dysfunction	4.6	Counselling; α-adrenergic agonist, cholinergic antagonist, urinary sperm insemination for retrograde ejaculation; insemination or surgery for epi-/hypospadias
Testicular tumour	0.5[a]	Cryopreservation, hemicastration in case of unilateral tumour
Varicocele	30.4[a]	Embolisation, surgical ligation
Infections	5.3	Antibiotic drugs
Subnormal FSH and LH with normal testosterone	5.0	GnRH, gonadotrophins, anti-oestrogens, minimised exposure to oestrogenic toxicants

[a]: The frequency of varicoceles and tumours was extrapolated from a subgroup of 1,130 men examined with colour Doppler ultrasonography

Scrotal ultrasonography

Scrotal ultrasound was added to the routine diagnostic work-up during the observation period, and was performed in 1,130 of the 1,549 men. The prevalence of abnormal findings on scrotal colour Doppler ultrasonography is given in Table 4. In about 40% of the men, at least one scrotal abnormality associated with infertility was found; 66% of these abnormalities was not discovered by palpation. In 28% of men diagnosed with idiopathic semen abnormality, a scrotal anomaly was found on ultrasonography.

Table 4. Findings on scrotal color Doppler ultrasonography in 1,130 infertile men, and the number of findings that were also palpable.

	Cases (n)	%	palpable
Varicocele	343	30.4	137/343
Testicular tumor	6	0.5	1/6
Testicular cyst	4	0.4	0/4
Testicular microlithiasis	9	0.8	0/9
Epididymal cyst	83	7.3	16/83

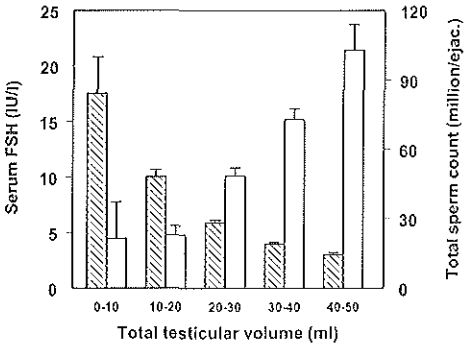

Fig 1.
Mean serum FSH (striped bars) and total sperm count (open bars) displayed against the total testicular volume (r = -0.44 and r = 0.32, respectively; $P < 0.001$). Error bars represent standard errors.

Basal correlations

Figure 1 shows the FSH levels and sperm counts, grouped on the basis of total testicular volume. The correlation between testicular volume and sperm count (r = 0.32), and the inverse correlation between testicular volume and FSH levels (r = -0.44) were significant (P<0.001). Figure 2 shows the significant inverse correlation between FSH and sperm count (r = -0.36, P<0.001), whereas Fig. 3 shows the positive correlation between FSH and LH (r = 0.46, P<0.001). The serum testosterone level was negatively correlated with age (r = -0.10, P = 0.009), whereas the serum SHBG level was positively correlated with age (r = 0.06, P = 0.03; Fig. 4).

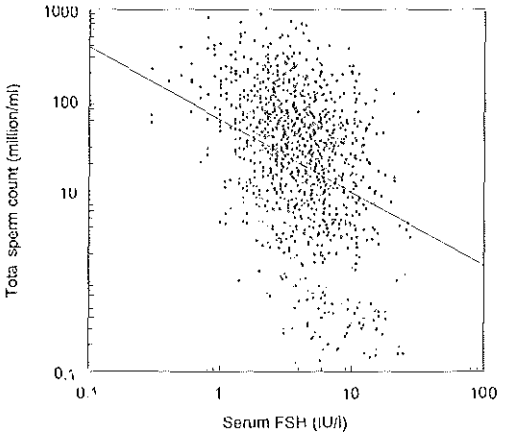

Fig. 2.
Total sperm count plotted against serum FSH of 1,549 infertile men ($r = -0.36$, $P < 0.001$).

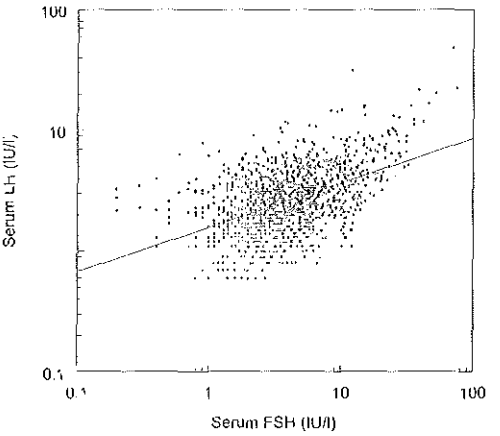

Fig. 3.
Serum FSH plotted against serum LH of 1,549 infertile men ($r = 0.46$, $P < 0.001$).

FSH, LH, and testicular volume in subgroups

The serum FSH concentration was different for subgroups of subfertile men ($P < 0.001$; Fig. 5). Serum FSH levels were significantly higher in men with varicocele, history of cryptorchidism, idiopathic oligozoospermia, and idiopathic azoospermia, compared with men with a normal sperm concentration ($P < 0.001$). Similarly, serum LH levels were significantly higher and total testicular volume was significantly lower in these subgroups compared with the group with normal sperm concentration ($P \leq 0.001$).

Effect of varicocele ligation on treatment policy

In The Netherlands, the criterion for ICSI in cases of andrological infertility is a total motile count below 1 million motile spermatozoa per ejaculate [9]. Of 139 men who were treated for varicocele, 39 fulfilled this criterion before varicocelectomy; in 44% of this group, the total motile count increased to above 1 million after varicocele ligation.

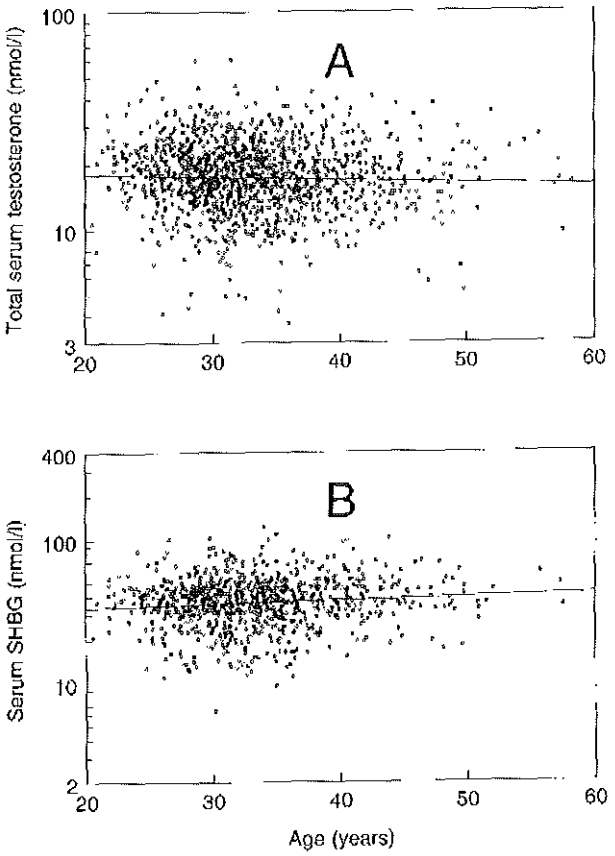

Fig. 4.
Total serum testosterone (panel A) plotted against age ($r = -0.10$, $P = 0.009$) and the serum SHBG levels (panel B) plotted against age ($r = 0.06$, $P = 0.03$).

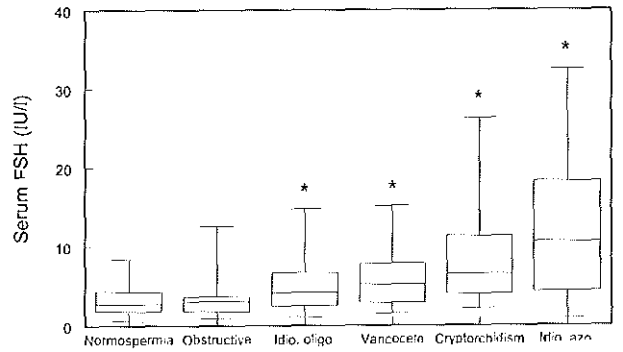

Fig. 5.
Boxplot of serum FSH in subgroups of subfertile men (idio. oligo.: idiopathic oligozoospermia; idio. azo.: idiopathic azoospermia; obstructive: obstructive azoospermia;
*, a significantly higher mean FSH level compared with the normal sperm count group, $P < 0.001$).

Discussion

Standardisation of the work-up of andrology patients is a indispensable tools for improvement of the quality of patient care. In the present study, the systematic collection of data in electronic sources promoted a standardised patient approach. By restructuring and integration of separate data sources in our CPR, a large amount of unambiguous data has become available for clinical research. In a setting where the paper medical record (PMR) is the only method of capturing data, retrospective research requires the impractical reviewing of PMRs. Valuable data may be missed, because data is not collected in a uniform and structured form, and data may be illegible. Using a well-structured CPR, availability, accessibility, and analysis of the contents will be more efficient than with the PMR [10].

The scientific value of the structured database is demonstrated by the reported population characteristics and basal correlations. Comparison of the distribution of WHO diagnoses with other centres is difficult, since the study populations differ due to e.g. geographical differences [11] and specific characteristics of the fertility centres. For example, Nieschlag [12] suggested that the high frequency of hypogonadism (8.9%) in his andrology population may be an overrepresentation caused by the specialisation of their institute. Compared with a large WHO study [13], the most conspicuous difference was that only 0.5% of our study population had *no demonstrable cause* (normal semen quality, normal sexual and ejaculatory function) compared with 54% in the WHO study. The design of the WHO study differed from ours, in that infertile couples were evaluated, in 39% of which only a female factor was detected, and in 15% no male or female cause could be diagnosed. Of the patients in the current study, 96% had impaired sperm concentration or motility, according to WHO criteria. Sperm morphology has also been determined, but differentiation between a normal and abnormal percentage of normal sperm is complicated by the lack of a biologically relevant lower normal percentage of normal forms [14]. In our centre, most of the patients were referred by gynaecologists, urologists and general practitioners after an initial semen analysis had indicated a male factor for the couple's infertility. The prevalence of oligozoospermia in these initial semen analyses at our semen laboratory is 34% for cases that are not subsequently referred, compared with 74% in patients referred to our andrology clinic. The referral of selected patients with a male factor may explain the overall higher frequency of identified causes in our centre. Compared with our study, only the incidence of 'infectious factors' was higher in the WHO study, which may in part be explained by the higher prevalence of infectious factors in African and Latin-American centres, constituting 25% of the patients in the latter study [11].

An inventory was made of treatment options in our male infertile population. We focussed on first-line treatment of impaired semen production, deposit and function, whereas the assisted reproduction technologies IVF and ICSI were excluded from this inventory. Obviously, assisted reproduction technology (ART) can be an effective second-line treatment when a first-line treatment has failed, or is not appropriate. Especially the known and unknown genetic risks of ART [15] should promote the use and development of rational treatments of male infertility. Effective treatment of impaired sperm production and function was available for some of our patients. Treatment may be effective for hypogonadism [16] and ejaculatory disorders [17]. Chronic systemic diseases (e.g., diabetes mellitus, Crohn's disease) often exert a deleterious effect on fertility, and fecundity may benefit from adequate control of the primary disease. Chronic use of medication with a negative effect on sexual function or semen quality may be replaced with alternative drugs. In cases of testicular malignancy, cryopreservation preceding treatment of the malignancy is indicated. Moreover, after unilateral orchidectomy in men that pursue pregnancy, the remaining testis should be shielded from radiotherapy, and exposure to chemotherapy should be limited [18,19]. Early detection and treatment of testicular tumours is important in this respect. Treatment of obstructive azoospermia may be effective, depending on the nature and location of the obstruction [20]. A patency rate of 86% has been reported after microsurgical reversal of vasectomy [21]. Counselling may alleviate sexual disorders [22].

Apart from the therapies with proven effectiveness, the efficacy of therapies for several other diagnoses is inconclusive. We identified unexplained subnormal serum FSH and LH levels in 5% of these patients. Whereas gonadotrophins and anti-oestrogens as empirical treatment for normogonadotropic infertile men were not effective in relatively small study groups [23,24], there seems to be a rationale for these treatments in the selected subgroup with low-level gonadotrophins. If these low gonadotrophin levels and low sperm counts are the result of exposure to environmental factors with oestrogenic activity [25], treatment with anti-oestrogens may be an interesting topic for future research. Treatment of immunological male infertility with corticosteroids has not proven effective [26,27] and may provoke side-effects [26,28]. Immunological infertility may be treated with sperm washing and intra-uterine insemination [29,30]. Based on the subgroup of 1,130 men investigated with scrotal ultrasound, the prevalence of varicocele was 30.4% in our clinic. Improvement of semen parameters following varicocele treatment is a consistent finding in most studies [31-33], including randomised controlled studies [34,35]. Therefore, an improved spontaneous pregnancy rate is expected. However, the randomised controlled trials were inconclusive with respect to pregnancy rates. The relevance of varicocele

repair is illustrated by the present observation that in 44% of men with less than 1 million motile spermatozoa per ejaculate, improvement to above this threshold was achieved. Infection of the urogenital tract, as present in 5% of our patients, is another diagnosis for which the benefit of treatment is still inconclusive, due to a lack of randomised controlled studies using modern antibiotics.

In 44% of our study population, unexplained impaired semen quality was found with the standard WHO diagnostic work-up. This figure is reduced to 34% if the prevalence of varicoceles and testicular tumors detected with scrotal sonography is extrapolated for the entire study group. An accurate diagnostic work-up may elucidate where further research on development of diagnostic procedures and satisfactory therapy is needed. In a collaborative study, genetic abnormalities associated with male infertility (i.e., CFTR gene mutations, Y chromosome microdeletions, and other chromosomal abnormalities) in 26% of 58 men with less than 106 motile spermatozoa per ml ejaculate [36]. Others reported a similar prevalence of genetic abnormalities [37]. Although treatment of these abnormalities may not be possible, genetic counselling before ICSI is indicated.

The significance of a proper diagnostic work-up is not only to ensure that an appropriate therapy is deployed for infertility. Part of the diagnoses may require therapy to prevent development or progression of other disorders (e.g., testicular malignancy, osteoporosis secondary to hypogonadism), or further deterioration of male fertility (e.g., epididymitis or obstruction as a result of a *Chlamydia trachomatis* infection).

The large standardised data collection enabled to evaluate numerous andrological research questions. We confirmed the positive correlation between testicular volume and sperm count, FSH and LH; and the negative correlation between testicular volume and FSH, and FSH and sperm count. We also showed a significantly higher serum FSH level in men with varicocele, history of cryptorchidism, and idiopathic semen abnormality, compared with men with a normal sperm count. These observations are in line with what would be expected based on the literature [13]. Especially evaluation of correlations that are diluted - by pooling of infertile men with different disorders, confounding factors, and variation - may require a large study population. The correlations between age on the one hand, and serum testosterone and SHBG on the other were weak, and could not have been found in a small heterogeneous group of infertile men. Similar correlations were shown previously in a group of healthy men, with a more appropriate age range for studying these associations [38].

The current results demonstrate the value of routine data collection based on a uniform andrology dataset. CPRs can aid in the structured collection of these data, and form an efficient and reliable source of data for clinical research and other purposes (e.g., quality control, management information, case finding). The accurate evaluation of infertile males can lead to well-defined diagnoses. A proper diagnostic work-up is essential for optimal therapy. Finally, we emphasise the importance of standardisation among fertility centres, which may enable meaningful comparison and pooling of data for multicentre research to further improve the quality of andrological care.

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Chapter 4

Serum Inhibin B as a Marker of Spermatogenesis

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Abstract

Inhibin B is produced by Sertoli cells, provides negative feedback on FSH secretion, and may prove to be an important marker for the functioning of seminiferous tubules. The purpose of the present study was to examine the relationship between the spermatogenic function of the testis of subfertile men and the serum concentrations of inhibin B and FSH. These parameters were estimated in a group of 218 subfertile men.

Serum inhibin B levels were closely correlated with the serum FSH levels ($r = -0.78$, $P<0.001$), confirming the role of inhibin B as feedback signal for FSH production.

The spermatogenic function of the testis was evaluated by determining testicular volume and total sperm count. Inhibin B levels were significantly correlated with the total sperm count and testicular volume ($r = 0.54$ and $r = 0.63$, respectively; $P<0.001$).

Testicular biopsies were obtained in 22 of these men. Inhibin B was significantly correlated with the biopsy score ($r = 0.76$, $P<0.001$). Receiver operating characteristic analysis revealed a diagnostic accuracy of 95% for differentiating competent from impaired spermatogenesis for inhibin B, whereas for FSH a value of 80% was found.

We conclude that inhibin B is the best of the available endocrine markers of spermatogenesis in subfertile men.

Introduction

FSH is currently regarded as the most important endocrine parameter in the evaluation of male infertility [1]. Its secretion can be suppressed by the testicular hormone inhibin, which is produced in Sertoli cells and may, therefore, be a serum marker for Sertoli cell function. Attempts to confirm this role of inhibin originally yielded contradictory results. Results of heterologous inhibin assays demonstrated that serum inhibin levels were stimulated with exogenous FSH and decreased after treatment with GnRH antagonists, radiotherapy-induced testicular damage, and testosterone [2-6]. In contrast, a negative correlation between inhibin and FSH levels could not be shown, and inhibin levels in fertile controls and subfertile men with testicular disorders were not different [7].

This discrepancy can now be explained on the basis of the aspecificity of the inhibin assay that was used. Inhibin is a dimer of an α- and a β-subunit. Depending on the type of β subunit, (β_A or β_B), inhibin A or inhibin B is formed.

The antibodies used in the heterologous inhibin radioimmunoassay (RIA) were directed against the α-subunit, and they detected both dimeric inhibin and biologically inactive monomeric α-subunits [8]. Since new specific sandwich assays for inhibin A, inhibin B, and uncombined α-subunits have been developed, studies have been undertaken to investigate the role of inhibins in male and female endocrinology.

One major finding is that inhibin B is the physiologically important form of inhibin in the male, serum inhibin A levels being undetectable [9]. The finding that castration results in undetectable inhibin B levels indicates that circulating inhibin B is produced by the testes [10]. Furthermore, recent papers have reported a strong negative correlation between FSH and inhibin B in fertile and subfertile men [9,11-14].

Little information is available on the correlation of inhibin B with the severity of spermatogenic defects in subfertile men. So far, lower inhibin B levels were reported in a limited number of subfertile men, compared with fertile controls [10]. More recently, inhibin B was found to be correlated with the sperm concentration in a study of 349 normal men [12] and in a mixed group of 65 men with normal and impaired spermatogenesis [13].

The aim of this study was to further investigate the clinical value of inhibin B estimations in subfertile men and to correlate inhibin B levels with clinical history, testicular volume, testicular biopsy score, and sperm characteristics. Subsequently, we analysed the additional value of inhibin B, compared with that of FSH, with special emphasis on the differentiation between normal and impaired spermatogenesis.

Subjects and Methods

Patients

The study comprised 218 consecutive patients that were referred to our andrology outpatient clinic with fertility problems (age, 21-57 years). In the period September 1996 until October 1997, 235 new patients were enrolled, of which 17 were excluded from further study on the basis of medication (n = 7; androgens or anti-estrogens), unilateral castration (n = 4), hypogonadotropic hypogonadism (n = 3), systemic disease (n = 1; renal failure), or chromosome translocation (n = 2).

Infertility of the couple was defined as a duration of infertility of more than 1 year. Patients were subjected to a thorough clinical evaluation according to the WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple [15]. Patients were diagnosed with normospermia (n = 49; ≥20 million sperm / ml), idiopathic moderate oligozoospermia (n = 69; 5-20 million sperm / ml), idiopathic severe oligozoospermia (n = 58; >0 and <5 million sperm / ml), idiopathic azoospermia (n = 15), obstructive azoospermia (n = 6, normal FSH, normal testicular size, and Johnsen score > 8; in 3 of these men, congenital absence of the vas deferens was diagnosed with ultrasonography), history of cryptorchidism (n = 17, accompanied by oligozoospermia), or Klinefelter's syndrome (n = 4).

Hormone analyses

Serum samples were stored for a period of 1 day to maximal 5 weeks at –20°C before analysis. Inhibin B was measured using kits purchased from Serotec Limited, Oxford UK [16]. The within-assay coefficient of variance (CV) was less than 9%, and the between-assay CV was less than 15%. The lowest detectable inhibin B concentration was 5 pg / ml (based on the mean value of the blanks + 2 SD). A value of 2.5 pg / ml (the mean of the undetectable range) was assigned to test results below 5 pg / ml. Serum FSH and LH were determined with the Amerlite FSH and LH assays (Orange-Clinical Diagnostics, Amersham, UK). Within-assay and between-assay CVs are less than 3, less than 8% and less than 5, less than 15% for FSH and LH, respectively. Total serum testosterone was determined by RIA as described earlier [17] (within- and between-assay CV: less than 6 and less than 9%). Using the above assays, mean (SD) FSH, LH and testosterone levels in a group of normal men were 2.5 (1.3) IU / l, 3.6 (1.9) IU / l, and 17.6 (6.8) nmol / l [18]. Per patient, all hormone concentrations were analysed in the same blood sample.

Semen analysis

Semen analyses were carried out according to the WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction [19]. Per patient, the results of the two semen analyses that were performed closest in time to the hormone analyses were selected. Per patient, the average sperm count was calculated. The median time difference (semen analysis date - blood sampling date) was 22 days (10th and 90th percentiles: -23, 51.4 days). Semen samples were obtained and assessed in 205 of the 218 patients.

Testicular evaluation

Testicular volume was estimated with the Prader orchidometer. Bilateral biopsy specimens were available on 22 of the 218 patients. Testicular biopsies were performed to discriminate impaired spermatogenesis from excurrent duct obstruction as a cause for azoospermia or severe oligozoospermia. Criteria for testicular biopsy were: azoospermia accompanied by a normal FSH level, or less than 5 million sperm / ml ejaculate. Biopsy specimens were scored using the method described by Johnsen [20], as modified by Aafjes *et al.* [21]. Seminiferous tubule cross-sections were rated with a score from 1 to 10, based on the most advanced stage of spermatogenesis observed. The mean score of at least 50 tubules was calculated per biopsy, both for the left and right testis. Tubules scored 10 for complete and abundant spermatogenesis with at least 5 condensed spermatids; 8 when all stages of spermatogenesis were present, but less than 5 condensed spermatids were seen; 7 when no condensed spermatids, but at least 5 round spermatids were present; 6 when no condensed spermatids, and less than 5 round spermatids present; 5 when no spermatids, but 5 or more spermatocytes present; 4 when no spermatids and less than 5 spermatocytes were present; 3 only spermatogonia present; 2 for Sertoli cells only; and 1 for no cells in the tubular section. It was previously shown that spontaneous pregnancy is possible when a biopsy score of ≥ 8 is present, but highly unlikely below a Johnsen score of 8 [21].

Statistical analysis

The FSH, LH, testosterone, and sperm count variables were transformed logarithmically to achieve a normal distribution. Correlations were calculated with Pearson's method. Differences between patient groups were tested with one-way ANOVA, followed by Fisher's least-significant difference method for pairwise comparisons.

The performance of inhibin B or FSH estimations in discriminating between normal and impaired spermatogenesis (Johnsen score ≥ 8 or <8) was described by receiver operating characteristic (ROC) statistics. ROC curves were drawn by plotting

the sensitivity against the false positive rate (1-specificity) for varying cut-off levels of inhibin B and FSH. A non-discriminating test would follow the diagonal line of the figure, whereas a 100% accurate so-called gold standard test would coincide with the upper left corner of the box. By comparing the areas under the curve (AUCs) for inhibin B and FSH, the diagnostic values of both hormones were compared [22]. AUCs were estimated with the Wilcoxon statistic [23].

Independent variables predictive of the biopsy score were identified with linear multiple regression analysis. Two-sided *P* values less than 0.05 were considered significant. Statistical analyses were carried out with the SPSS 7.5 for Windows statistical software package.

Results

Inhibin B was detectable in all but 6 men, with a mean concentration of 144.2 ± 4.9 (SEM) pg / ml. Subdivided by diagnosis, the mean (SEM) serum inhibin B concentrations were 244.0 (31.6) for obstructive azoospermia, 181.9 (9.1) for normospermia, 166.1 (7.3) for moderate oligozoospermia, 128.4 (8.8) for severe oligozoospermia, 52.0 (14.4) for idiopathic azoospermia, 7.3 (2.5) for Klinefelter's syndrome, and 118.1 (18.3) pg / ml for patients with a history of cryptorchidism (Fig. 1). Compared with the group with normospermia, the mean serum inhibin B levels were significantly lower in the groups with severe oligozoospermia, idiopathic azoospermia, Klinefelter's syndrome, and a history of cryptorchidism. The mean inhibin B level in patients with obstructive azoospermia, in which spermatogenesis may be normal, was significantly higher, when compared with the other groups.

Table 1. Correlations between Inhibin B, FSH, LH, testosterone (T), testicular volume (TV, sum of left and right testis), mean bilateral Johnsen score, and sperm count are *in italic*, and number of patients (*P* value) are *boldface*.

	Inhibin B	**FSH**	**LH**	**T**	**TV**	**Johnsen score**	**Sperm count**
Inhibin B		*-0.78*[a]	*-0.41*[a]	*0.20*[a]	*0.63*[a]	*0.76*[a]	*0.54*[a]
FSH	218 (<0.001)		*0.55*[a]	*-0.17*[a]	*-0.56*[a]	*-0.64*[a]	*-0.55*[a]
LH	218 (<0.001)	218 (<0.001)		*0.01*	*-0.34*[a]	*-0.35*	*-0.32*[a]
T	208 (0.004)	208 (0.02)	208 (0.97)		*0.18*[a]	*0.38*	*0.13*
TV	166 (<0.001)	166 (<0.001)	166 (<0.001)	164 (0.02)		*0.34*	*0.38*[a]
Johnsen score	22 (<0.001)	22 (0.001)	22 (0.11)	21 (0.11)	20 (0.14)		*0.53*[a]
Sperm count	205 (<0.001)	205 (<0.001)	205 (<0.001)	202 (0.07)	156 (<0.001)	17 (0.03)	

[a]: Correlation coefficients are statistically significant.

Table 1 shows the correlations of inhibin B with parameters related to spermato-

genesis. The inhibin B levels were correlated with the total sperm count ($r = 0.54$, $P<0.001$; Fig. 2); patients with obstructive azoospermia were excluded from this correlation and other correlations with the sperm count. The inhibin B levels were also significantly correlated with the total bilateral testicular volume (Fig. 3), the Johnsen score (Fig. 4), and negatively with serum FSH (Fig. 5) and LH levels. The correlation of inhibin B with LH was not significant if it was adjusted for the FSH level ($r = 0.03$, $P = 0.63$), with which LH is closely correlated.

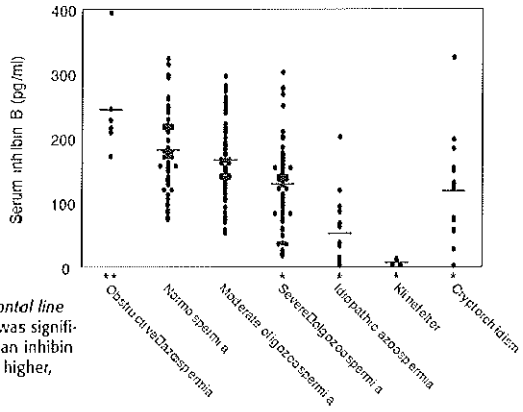

Fig. 1
Serum inhibin B levels in subgroups of subfertile men. The *horizontal line* per group indicates the mean level. (*, the mean inhibin B level was significantly lower, compared with normospermia, $P < 0.05$; **, the mean inhibin B level in patients with obstructive azoospermia was significantly higher, compared with other groups, $P \leq 0.01$).

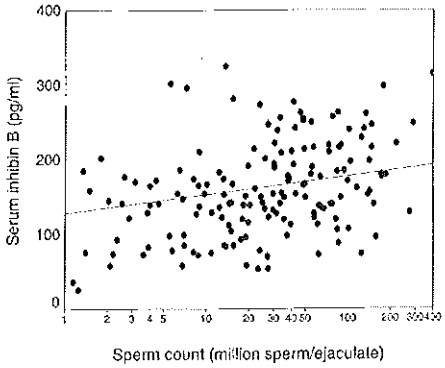

Fig. 2.
Serum inhibin B plotted against the total sperm count in 205 subfertile males ($r = 0.54$, $P < 0.001$).

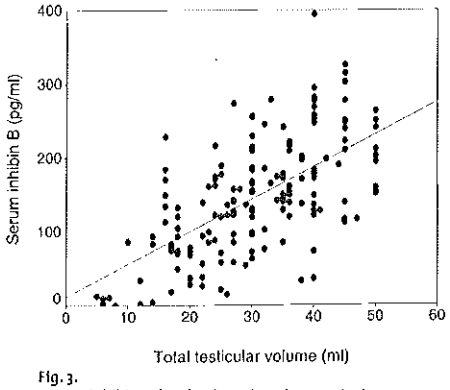

Fig. 3.
Serum inhibin B levels plotted against testicular volume of 166 subfertile males ($r = 0.63$, $P < 0.001$).

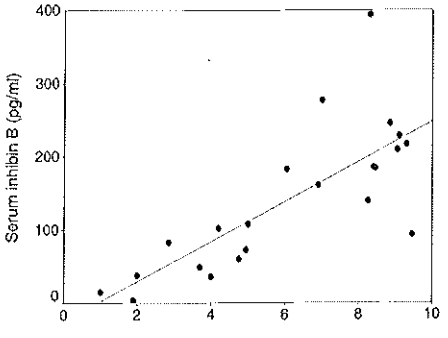

Fig. 4.
Serum inhibin B levels plotted against the mean bilateral Johnsen scores of 22 subfertile males ($r = 0.76$, $P < 0.001$).

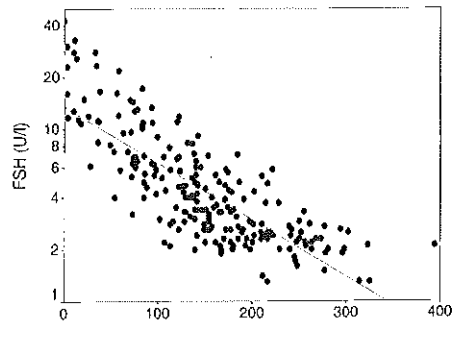

Fig. 5.
Serum FSH concentration plotted against serum inhibin B in 218 subfertile males ($r = -0.78$, $P < 0.001$).

The accuracy for differentiating adequate (Johnsen score ≥ 8) from impaired spermatogenesis (Johnsen score <8), on the basis of inhibin B and FSH levels, was estimated from the area under the ROC curve (Fig. 6). The AUC was 0.95 (SE: 0.07) for inhibin B and 0.80 (SE: 0.12) for FSH. The areas under the ROC curves for inhibin B and FSH were statistically different ($P = 0.04$). Depending on the desired sensitivity or specificity of inhibin B or FSH, a cut-off level can be deduced from the ROC curves. When the point on the curve closest to the upper left corner of the box corresponding to 100% sensitivity and 0% false positives (100% specificity) was selected, this resulted in cut-off levels of inhibin B less than 139 pg / ml and FSH more than 4.9 IU / l to identify patients with impaired spermatogenesis. The sensitivity and specificity corresponding to these cut-offs were 83 and 90% for inhibin B, and 75 and 80% for FSH, respectively.

In addition, linear multiple regression was performed to study whether hormonal variables could account for the variation in the mean bilateral Johnsen score. The Johnsen score was predicted from FSH and inhibin B, the variables correlated with it in Table 1. Sperm count was not entered as independent variable, because an unrevealed (partial) obstruction may bias the bi psy score vs. sperm count correlation. The regression procedure indicated inhibin B as the best predictor of the Johnsen score, explaining 58% of the variance. In the resulting regression equation [Johnsen score = 4.78 + (0.017 x inhibin B) + (0.05 x FSH)], the coefficient for inhibin B was statistically significant (P = 0.007), whereas FSH did not improve the regression model (P = 0.24).

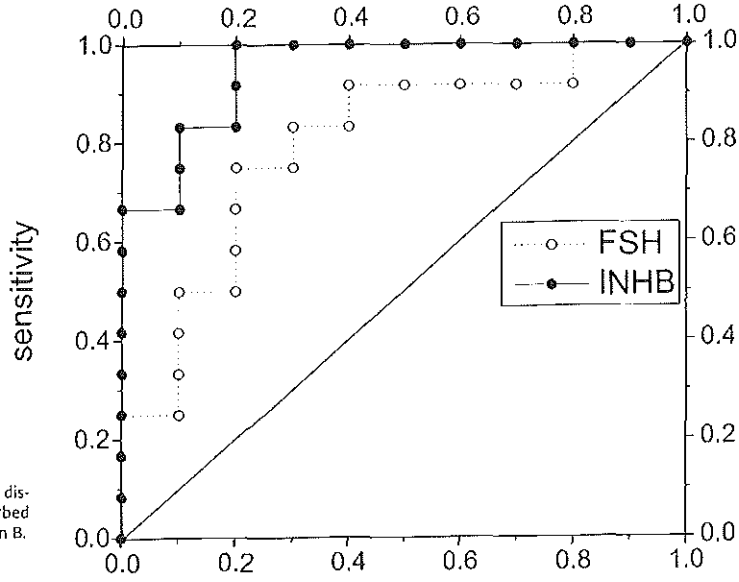

Fig. 6.
ROCs of inhibin B and FSH for discriminating normal and disturbed spermatogenesis. INHB, Inhibin B.

Discussion

In this study, we demonstrate a significant correlation between sperm concentration, sperm count, and testicular volume on the one hand, and serum inhibin B levels on the other. These results provide strong evidence that inhibin B is an important marker of the competence of Sertoli cells and spermatogenesis in the human, which is in accordance with the few reports on inhibin B and quality of spermatogenesis up to now [9,10,12,13]. In the first two studies, a lower inhibin B concentration was noted in small groups of men with azoospermia, testicular disorders, and infertility, as compared with fertile controls. More recently, results of two larger study populations became available, showing a significant positive correlation of inhibin B with sperm concentration in 349 normal men [12], and with sperm concentration and testicular volume in 65 men with normal and impaired spermatogenesis [13].

We now provide further evidence for the value of inhibin B as a marker of spermatogenesis by the novel finding of a statistically significant positive correlation with the most accurate assessment of spermatogenesis in our setting, the testicular biopsy score. We compared the accuracy of FSH and inhibin B levels to distinguish between patients with competent and impaired spermatogenesis, based on the Johnsen score. The area under the ROC curve, corresponding to the accuracy of the diagnostic method, was significantly larger for inhibin B. Multiple linear regression analysis also revealed that FSH had no significant additional predictive value for the Johnsen score above inhibin B.

The choice for a cut-off level for inhibin B or FSH to discriminate competent from impaired spermatogenesis depends on the priority of high sensitivity or specificity. We arbitrarily chose the cut-off level closest to the upper left corner of the box. The resulting cut-off levels for inhibin B (<139 pg / ml) and FSH (>4.9 IU / l) were surprisingly close to the cut-off levels for these hormones based on control populations. A lower normal limit for inhibin B has not been defined, but it was 140.6 pg / ml (95% confidence interval, 140.6-225.7) for a group of 18 semen donors [9]. We use 5.1 IU / l as the upper normal limit for FSH based on the mean plus 2 SD {2.5+(2*1.3)} in a population of normal men [18].

The present data show significant differences in mean inhibin B levels between diagnostic subgroups. The inhibin B levels were significantly lower in patients with a spermatogenic defect, as compared with the group with normospermia. Patients with obstruction as the sole identified cause for azoospermia had normal inhibin B levels, which were significantly higher, compared with other subgroups. With the aspecific heterologous assay for inhibin, no differences in inhibin levels between comparable subgroups were found [7].

A further advantage of inhibin B measurement is that it reflects the function of the total testicular tissue, whereas a biopsy may not be representative for the entire testis. Multiple biopsies, which are nowadays performed for testicular sperm extraction, often show a large variation in the completeness of spermatogenesis [24]. This heterogeneity of spermatogenesis is even more conspicuous in patients with impaired spermatogenesis, where sections with complete spermatogenesis may be found among others with germinal cell aplasia, referred to as focal spermatogenesis [25]. It has to be established whether inhibin B levels can demonstrate the presence of focal spermatogenesis and, in this way, could reduce the need for invasive testicular biopsies. It is not unlikely that, in many cases, the area of spermatogenesis is too small to substantially increase serum inhibin B levels.

FSH was regarded the most important endocrine marker for testicular function until now [26]. The diagnostic value of inhibin B for spermatogenetic disorders seems to be better. This may be explained by the fact that inhibin is a direct product of the seminiferous tubules, and that its secretion is stimulated by the presence of advanced stages of spermatogenesis [27]. In contrast, FSH levels are also affected by GnRH, estradiol, and testosterone.

In conclusion, we have confirmed the role of inhibin B in FSH regulation, and we have found a strong correlation of inhibin B levels with spermatogenesis. Our results provide further and novel evidence that inhibin B is the best of the known endocrine markers for spermatogenesis. Inhibin B estimation may prove an alternative for testicular biopsy in the differentiation between normal and impaired spermatogenesis.

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Chapter 5

Is Routine Scrotal Ultrasound in Infertile Men Advantageous?

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Abstract

Purpose: To determine the value of routine scrotal ultrasonography in the evaluation of male infertility.

Subjects and Methods: Scrotal colour Doppler ultrasonography reports of 1,372 infertile men were reviewed to assess the prevalence of scrotal abnormalities. Ultrasound findings were compared with clinical findings.

Results: The prevalence of scrotal abnormalities was 38%. A testicular tumour was found in 0.5%, varicocele in 29.7%, testicular cyst in 0.7%, testicular microlithiasis in 0.9%, epididymal cyst in 7.6% and hydrocele in 3.2% of the cases. Overall, 67% of the sonography findings were not discovered by palpation. Only 1 out of 7 testicular tumours was suspected upon palpation. Sixty percent of the varicoceles were not found upon physical examination. The rate of testicular tumours (1/200) was higher than reported for the general population (1/20,000).

Conclusions: Routine scrotal ultrasound provides valuable information in the diagnostic evaluation of infertile men. Substantially more pathology is detected compared with clinical palpation. Particularly the high prevalence of testicular malignancies underlines the clinical relevance of routine scrotal ultrasonography in infertile men.

Introduction

Examination of the scrotal contents may reveal pathology affecting male fertility. Varicocele is the most common abnormal finding in infertile men with subnormal semen. In general, screening for varicocele is performed by palpation. Although the varicocele can be diagnosed as a palpable distension of the spermatic cord, a poor accuracy of palpation has been reported [1]. Therefore, we use colour Doppler ultrasonography (CDU) as alternative for varicocele palpation in infertile males. Ultrasonography combined with colour Doppler examination is the method of choice for imaging of scrotal organs [2] and allows a more objective and precise assessment of varicoceles [3].

Concomitant with screening of the scrotal contents for varicocele, CDU can reveal other abnormalities that are not identified by history taking and physical examination. The prevalence of scrotal abnormalities in infertile males, in particular testicular and epididymal anomalies, has not been studied extensively up to now, since scrotal CDU is generally not employed as a routine procedure in the work-up of the infertile man. In recent years, a higher incidence of testicular tumours in infertile men as compared with the normal population has been reported. This suggests that male infertility is associated with an increased risk of testicular malignancy [4]. Routine scrotal CDU in the high risk group of male infertility may reveal testicular tumours that are not detected by physical examination.

The aim of the present study was to investigate the value of routine scrotal ultrasonography in infertile men, and to compare ultrasound findings with physical examination.

Subjects and Methods

The study comprised 1,372 infertile men (age 20-58 years) who were referred to our andrology outpatient clinic in the period 1990 to 1996. Infertility was defined as the inability to achieve a pregnancy within 1 year. Patients were referred after an initial semen analysis had revealed abnormal semen quality. Patients were subjected to a thorough evaluation according to the 'WHO manual for the standardized investigation of the infertile couple' [5]. This evaluation included estimation of testicular volume with the Prader orchidometer, varicocele palpation, assessment of location, size and consistency of testes and epididymides. Ninety-nine percent of the patients had abnormal semen in 2 analyses, according to WHO guidelines [6].

CDU of the scrotal contents was executed on a routine basis with a high-frequency duplex echotransducer (7.5 MHz and higher), equipped with colour flow imaging. The presence of a varicocele was diagnosed on the basis of a venous diameter of 3 mm or more with increasing diameter during Valsalva manoeuvre or when chan-

ging from supine to upright position [7]. Increased venous retrograde flow in the pampiniform plexus in upright position, or during Valsalva manoeuvre, was used as a supporting sign [8].

Although scrotal ultrasound was originally performed to detect varicoceles, other abnormalities were also reported. Retrospectively, the reports were searched for scrotal anomalies which were classified as varicocele, testicular cysts, testicular microlithiasis (TML; characterised by diffuse punctate non-shadowing hyperechoic foci in the testicular parenchyma), testicular tumours, epididymal cysts, and hydrocele. Scrotal palpation and CDU were performed independently by several investigators.

Results

With scrotal CDU, one or more abnormalities were observed in 38% (521/1,372) of the infertile men. Table 1 shows the prevalence of the separate findings.

Suspicion of a tumour was raised in 7 patients, on the basis of a circular inhomogeneous area. The diagnosis of a tumour was confirmed by surgery in all cases. The tumours were classified by pathologists as a Leydig cell tumour in 5 cases and as seminoma in 2 cases. Only in one of these patients the testicular tumour was suspected on the basis of palpation of a testis with hard consistency and a volume larger than contralateral (25 *vs.* 12 ml.). The diameter of this spherical tumour was 3 centimetres. The 6 non-palpable tumours had a maximum diameter of 0.5, 0.5, 0.8, 1.0, 1.5 and 2.5 centimetres, respectively. The latter tumour had a soft consistency, and palpation of the testis was complicated by a high scrotal location. This patient had a history of unilateral cryptorchidism of the affected testis. Of other men with a non-palpable testicular tumour, one had a history of epididymo-orchitis, and one a history of bilateral cryptorchidism.

Table 1. Findings upon scrotal colour Doppler ultrasonography in 1,372 infertile men

	Cases (N)	%
Varicocele*	407	29.7
Testicular cyst	9	0.7
Testicular tumour	7	0.5
Testicular microlithiasis	12	0.9
Epididymal cyst	104	7.6
Hydrocele	44	3.2

*: Bilateral varicocele in 11% (N=45/407)

Varicocele was the most frequent finding observed during scrotal CDU examination. The results of palpation and CDU as method to detect a varicocele are compared in Table 2. The sensitivity of palpation to detect a varicocele was 40%, specificity was 91% and the positive predictive value was 66%, as compared with CDU.

Table 2. Palpation compared with colour Doppler ultrasonography in diagnosing varicocele

		Palpation		
		+	-	
Colour	+	163	240	403
Doppler	-	85	873	958
		248	1,113	1,361

Four of 12 patients with TML had a history of cryptorchidism, and another patient had a history of a large-cell calcifying Sertoli cell tumour in the removed contralateral testis. Testes were retractile in one patient with TML. Four of the 44 hydroceles detected with CDU were palpable. In 15 hydroceles, a history of a testicular trauma (N=3), a history of urogenital surgery (N=10; inguinal hernia, varicocele repair, orchidectomy, vasectomy, vaso-vasostomy or orchidopexy), and a history of epididymitis (N=2) had been revealed. In 1 out of 9 cases, the testicular cyst was palpable. Epididymal cysts were palpable in 22 of the 104 cases. Four patients with epididymal cysts had complaints of pain in the scrotum, 3 had undergone vaso-vasostomy, and 8 had a history of a urogenital infection.

Discussion

The prevalence of 38% scrotal abnormalities found during scrotal CDU in our infertile population was unexpectedly high. Only in 33% of patients with ultrasonographical abnormalities, palpation of the scrotal contents had revealed abnormalities. Examination of the scrotal contents with CDU was primarily performed for detection of a varicocele. The prevalence of varicocele was 29.7%. These varicoceles were generally unilateral on the left side, and bilateral in 11% of the cases. Although the CDU reports were not standardised for other scrotal findings, hydroceles were reported in 3.2%, epididymal cysts in 7.6%, testicular cysts in 0.7%, testicular tumours in 0.5%, and TML in 0.9% of the patients. Due to the retrospective design of our study, a fertile control group was not available. Two other studies using routine scrotal sonography in large groups of infertile men reported a prevalence of 50 and 57% scrotal abnormalities in 1,048 and 200 cases, respectively [9,10]. The somewhat higher prevalence in these studies may be explained by the additional classification of 'enlarged epididymis', 'testicular inhomogeneity', 'abnormal testis location' and 'testi-

cular hypoechogenicity'. Inhomogeneity of the testicular tissue has been associated with a reduced testicular biopsy score and sperm count [11]. Another advantage of scrotal CDU is the more accurate estimation of testicular volume compared with clinical measurements [12].

A sensitivity of ultrasound to detect scrotal abnormalities of 98.5% has been reported, with a diagnostic accuracy of 77-100%, depending on the type of abnormality [2]. The addition of colour Doppler technology to ultrasonography has amplified its diagnostic accuracy [2].

In 7 patients, a suspicion of a tumour was raised by CDU and confirmed by surgery and pathology. Only one tumour was suspected by palpation of the testis. The prevalence of testicular tumours in our infertile population (1/200) is in agreement with the high rate of testicular tumours in infertile patients reported by others [9,13,14]. Testicular cancer has been associated both with cryptorchidism and infertility, and a common aetiology has been suggested [4,15]. Although we have not performed scrotal CDU in a control group of fertile males, the rate in our infertile population (1/200) is higher than reported in the general European population (1/20,000) [16]. On the basis of this high frequency, sonographic screening for testicular tumours in infertile men may be warranted.

Early detection of testicular tumours is especially important in men pursuing paternity, so that sperm cryopreservation or assisted reproduction can be performed before spermatogenesis is further affected by the tumour or its treatment. Early treatment may prevent a more aggressive treatment, thereby limiting damage to spermatogenesis from radiation and chemotherapy in the contralateral testis [4].

The prevalence of TML was 12 out of 1,372 patients. This phenomenon has been associated with Klinefelter's syndrome, infertility, a history of cryptorchidism, and testicular neoplasm [17]. In one of our patients with TML, a Sertoli cell tumour had been diagnosed in the contralateral testis, which was removed earlier. Four out of 12 TML cases had a history of cryptorchidism. In one patient with TML the testes were retractile. In cases of TML, repeated testicular ultrasound to identify tumour development may be indicated.

In 9 patients a testicular cysts was diagnosed, which was suspected by palpation in only one patient. Intratesticular cysts require a further differentiation, to exclude the possibility of a teratoma [18].

The incidence of epididymal cysts was 7.6% (104/1,372). Of the cysts, 21% was also reported as a palpable thickening of the epididymis. The clinical significance of epididymal cysts is unclear. The epididymal cysts were not equivalent to epididymal obstruction, since azoospermia was observed in only 2 out of 17 patients with bilateral epididymal cysts.

Varicocele in 407 of 1,372 men (29.7%) was the most frequent finding. We report a low sensitivity of palpation (40%), which emphasises that many varicoceles in the infertile population are undetected. The accuracy of palpation is disputed since a low inter-observer agreement [1], sensitivity, and specificity have been reported [3,8,19]. CDU is a more accurate and objective screening method [3], and allows grading on the basis of venous diameter or reflux characteristics.

Conclusions

Colour Doppler ultrasonography is a valuable method for diagnosing scrotal abnormalities in the infertile population. Scrotal ultrasonography frequently detected scrotal abnormalities that were non-palpable. Especially the relatively high prevalence of testicular neoplasms stresses the importance of routine scrotal ultrasound in infertile men.

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Chapter 6

Improvement of Sperm Count and Motility after Ligation of Varicoceles Detected with Colour Doppler Ultrasonography

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Abstract

The debate on the efficacy of varicocele ligation for improvement of semen parameters and pregnancy rates is ongoing. In addition, no consensus exists as to the benefit of treatment of subclinical varicoceles. The aim of this study was to investigate, retrospectively, the effect of high ligation of both subclinical and clinical varicoceles on sperm count and motility. The value of several factors from history taking and physical examination for the prediction of successful varicocelectomy was analysed. A total of 139 patients, operated on for a unilateral varicocele on the left side, were studied. Varicoceles were subclinical in 73 patients, based on colour Doppler ultrasonography, and 66 varicoceles were clinical, based on palpation in addition to ultrasonography. Comparison of semen parameters before and after surgery revealed a significant improvement. The median sperm count increased from 10.0 to 14.7, and 18.2 to 28.6 million / ejaculate, in patients with subclinical and clinical varicoceles, respectively ($P < 0.001$). The percentage improvement in median sperm count in subclinical varicoceles was not statistically different from the improvement in clinical varicoceles. Mean progressive motility improved significantly after ligation ($P < 0.001$). The improvement in motility in subclinical varicoceles, from 16 to 23%, was significantly larger than the 24 to 27% improvement in clinical varicoceles. The increase of sperm count was related positively with testicular volume before surgery ($P < 0.05$). The increase in sperm motility was significantly lower in patients with a history of cryptorchidism ($n = 22$, $P < 0.05$).

The present data show that ligation of varicoceles detected with Doppler ultrasonography, whether palpable or not, results in an increase of sperm concentration and motility.

Introduction

Varicocele is the most frequently identified male factor in couples consulting with fertility problems. Varicocele has been associated with adverse effects on sperm concentration, motility and morphology, testis size and histology, blood hormone levels, and pregnancy rates [1]. Most studies on the effect of varicocelectomy have reported improvement in male fertility, but the degree of improvement varies substantially. Mordel *et al.* [2] reviewed fifty reports, in which improvement of semen parameters and pregnancy rates after spermatic vein ligation varied from 0 to 92% (mean 57%), and 0 to 63% (mean 36%), respectively. The results of two prospective, randomised controlled studies also reported different outcomes of varicocele occlusion in terms of alteration in sperm quality and pregnancy rate [3,4].

Possible reasons for the differences in outcome of varicocelectomy between studies are differences in the composition of patient groups (e.g. duration of infertility, age, the size of the varicocele and preoperative semen characteristics [5]).

The size of the varicocele may influence the outcome of varicocele ligation. Marsman & Schats[6] reviewed the literature on the controversial concept that the subclinical varicocele (SV) is detrimental to spermatogenesis, and that SV ligation improves semen quality. Like the more generally accepted treatment of the clinical varicocele (CV), the reported effects of SV ligation on sperm characteristics and pregnancy rates show a substantial variation, and it is unclear whether SV and CV patients benefit similarly from varicocele treatment.

Moreover, no consensus exists as to the method of choice for diagnosing varicoceles. Palpation can be performed routinely, but a low specificity and sensitivity have been reported [7]. Since palpation is not accurate, other modalities are utilised to identify CV and SV (e.g. thermography, venography, colour Doppler ultrasonography, Doppler stethoscope).

In the current paper, we present the effect of the Palomo procedure on sperm concentration and motility in 139 patients with a varicocele detected using colour Doppler ultrasonography, that was either palpable or not. The cohort of SV ($n = 73$) is one of the largest reported so far. Factors that may predict successful varicocelectomy were analysed.

Patients and Methods

Patients

A group of 139 patients who underwent retroperitoneal high varicocele ligation [8] were included in this study. Varicoceles were graded as clinical if the distension of the pampiniform plexus was visible or palpable (with or without Valsalva manoeuvre), with the patient in the upright posture, and were confirmed by colour Doppler ultrasonography (CDU). Varicoceles were graded subclinical whenever palpation was negative, but CDU was positive. Since varicocele is defined as venous reflux in the pampiniform plexus, usually caused by incompetent valves and resulting in dilatation of the veins, the varicocele can be diagnosed both on the basis of reflux and venous diameter. We used CDU (high-frequency duplex echotransducer ≥ 5 MHz) to determine venous diameter (ultrasound) and direction and velocity of bloodflow (Doppler sonography). Ultrasonography was considered positive when the diameter of veins was 3 mm or more with increasing diameter during Valsalva manoeuvre or when changing from supine to erect posture [9]. Doppler ultrasound was considered positive when increased venous retrograde flow in the pampiniform plexus was detected in erect posture, or during Valsalva manoeuvre [10]. Varicocele ligation was performed using the Palomo approach [8]. Inclusion criteria for surgery were infertility (with a duration of more than 1 year), presence of a varicocele and subnormal sperm parameters (< 50% progressive motility, < 20 million sperm/ml, or < 40 million sperm/ejaculate). Azoospermic patients with varicocele were not treated. The age, duration of infertility, testicular volume prior to surgery (Prader orchidometer), type of infertility (primary or secondary) and history of cryptorchidism and accessory gland infection [11] for the study population are given in Table 1.

Table 1. General characteristics of the study population. Values are means ± SD or percentages

	Subclinical varicoceles (n = 73)	**Clinical varicoceles (n = 66)**
Age at surgery (years)	32.7 (4.6)	32.6 (4.8)
Years of infertility	3.8 (2.5)	2.9[a] (1.6)
Total testicular volume (mL)	28.4 (7.2)	30.0 (8.6)
Primary infertility (%)	89	86
Cryptorchidism (%)	23	8[a]
Accessory gland infection (%)	5	5
Sperm antibodies (%)	5	6

[a], $P<0.05$, clinical versus subclinical varicocele

Semen analyses were performed according to WHO guidelines, and comprised volume, sperm concentration and percentage progressive motility [12]. The total sperm count (sperm concentration x ejaculate volume) was used as outcome variable instead of sperm concentration, to correct for differences in ejaculate volumes between and within patients. Sperm antibodies were detected with the direct mixed antiglobulin (MAR) reaction test (SpermMar IgG Test, Ferti Pro, N.U., Beernen, Belgium). MAR binding of 40% or more of motile spermatozoa was regarded as positive. Semen samples were obtained after a 2-5 day abstinence period. All semen analyses in the 2 years before varicocele ligation, and in the period from 70 days to 2 years after ligation were assessed. The mean number of assessed semen analyses was 2.3 (range 1-7) before and 2.4 (range 1-8) after ligation.

Data management and analysis

Practically all patient data of the Andrology outpatient clinic are stored electronically in subsystems of the hospital information system. To exploit the potential of these separate data collections, an Andrology Research Information System (ARIS) has been developed, based on the ORCA (Open Record for Care) electronic patient record, that validates and integrates these data sources, facilitating clinical research on patient data [13].

For patients who fulfilled inclusion criteria, data were retrieved from ARIS by a query on: date of birth, primary/secondary infertility, duration of infertility, history of cryptorchidism, history of accessory gland infection, semen analyses, date of the varicocele ligation, testicular volume, and the result of scrotal CDU.

The effect of surgery on total sperm count was assessed with a linear regression model with random coefficients (SAS program Proc Mixed). In the model, the effect of surgery was represented by a surgery indicator variable being zero for semen analyses before the operation, and being one for semen analyses after the operation. To account for dependency introduced by the fact that each patient had two or more measurements, model intercept and the regression coefficient of surgery were assumed to be random and possibly correlated (the correlation representing the association between pre-surgery sperm count or motility level and the surgery effect). To obtain a normally distributed dependent variable, sperm count was logarithmically transformed. Since absolute increases in means on the logarithmic scale correspond to relative increases in the median on the original scale, the results for sperm count are expressed using medians. To investigate whether a factor (e.g., CV *vs.* SV) modified the surgery effect, the factor and its interaction with the surgery indicator variable were added to the model.

The effect of surgery on motility was assessed analogously. Because no normalising transformation could be found, sperm motility was used untransformed in the regression analysis. To account for the non-normal distribution of motility, the standard errors of the regression coefficients were estimated robustly, i.e. without using the normality and homoscedasticity assumption [14].

Differences in means between groups were tested with the independent-samples *t*-test, and differences in percentages with the $\chi 2$ method. Means are presented with standard errors. Two-sided *P* values < 0.05 were considered significant, and statistical analyses were carried out using the SAS System 6.12 for Windows statistical software package.

Results

Effect of varicocelectomy on the total sperm count

Treatment of the varicocele resulted in a statistically significant increase in the median total sperm count ($P < 0.001$, Table 2). The positive relative effect of surgery on the number of spermatozoa in the ejaculate was not significantly different for CV and SV. The median sperm count and mean progressive motility were higher in CV than in SV ($P < 0.05$). There was a statistically significant interaction between total testicular volume and the effect of surgery on the total sperm count, irrespective of the varicocele size ($P < 0.001$); this amounted to a 2.5% higher sperm count for each extra 1 mL total testicular volume. Subjects with history of cryptorchidism had a lower initial sperm count (4.6 million/ejaculate) which was ≈ 30% of that found in other patients (17.2 million/ejaculate; $P < 0.001$), but the relative increase in total sperm count was not statistically different compared with other varicocele patients. Duration of infertility, age at surgery, primary/secondary infertility, accessory gland infection and antibody-coated spermatozoa had no association with improvement in sperm count. In 28% of the cases, no improvement in total sperm count was observed. The sperm count before varicocele surgery was not correlated with the improvement of sperm count postsurgery ($r = -0.21$, $P = 0.11$).

Effect of the varicocelectomy on progressive sperm motility

Varicocele treatment significantly increased the progressive sperm motility ($P < 0.001$, Table 2). In SV, the improvement in sperm motility was larger than in CV ($P < 0.05$). Basal sperm motility was lower in SV *vs.* CV ($P < 0.05$). Duration of infertility, age at surgery, primary/secondary infertility, accessory gland infection, bilateral testicular volume and antibody-coated spermatozoa were not correlated with the improvement in sperm motility. Patients with a history of cryptorchidism had a significantly lower progressive sperm motility prior to surgery (7%) when compared with other varicoceles (23%), and the effect of surgery on motility was negligible. In 31% of cases, no improvement in progressive motility was observed. The percentage of progressive motility before surgery was not correlated with the magnitude of the improvement of motility following varicocelectomy ($r = -0.20$, $P > 0.1$).

Table 2. Mean semen parameters (95% confidence interval) before and after varicocele ligation in all patients and clinical / subclinical varicocele subgroups

	n	Median total sperm count (10^6/ejacul.)[a]		Mean sperm motility (%)[a]	
		before	after	before	after
All	139	13.9 (11.0-17.7)	21.2 (16.6-27.0)	21 (18-23)	25 (22-28)
Subclinical	73	10.0 (7.8 - 14.2)	14.7 (10.2-21.0)	16 (13-19)	23[b] (19-27)
Clinical[c]	66	18.2 (12.8-24.8)	28.6 (20.7-39.4)	24 (20-28)	27 (22-30)

[a] $P < 0.001$, before versus after varicocele ligation in all patients

[b] $P < 0.05$, greater motility improvement in subclinical versus clinical varicocele

[c] $P < 0.05$, higher basal total sperm count and progressive motility in clinical varicocele versus subclinical varicocele.

Discussion

Our finding that varicocelectomy improves sperm counts and motility is in agreement with the majority of reports. Mordel *et al.*[2] reviewed 38 varicocele studies that reported the percentage of patients with improvement in sperm parameters (range 0-92%). Overall, an improvement in semen parameters was seen in 57% of the total of 4,654 patients in these studies, calculated as weighed mean. In only three of these studies was no increase of sperm characteristics reported. However, most of the reviewed studies did not include untreated control groups. In two more recent randomised controlled studies, no alteration of sperm parameters was observed in the control groups during 1 year of follow-up, whereas the total sperm count improved significantly in treated patients [3,4]. Improvement in sperm motility is not a consistent result of varicocele surgery, and was only statistically significant in one of these two studies [3].

There is a large variation in the magnitude of the effects of varicocelectomy on semen parameters between studies (reviewed by Schlesinger *et al.*[15]). This variation has been attributed to differences in size of the varicocele, baseline semen quality, duration of infertility, testicular volume and the reliability of diagnostic and therapeutic methods, among other possibilities [1].

We found a positive correlation between initial testicular volume and the improvement in sperm count, whereas a history of cryptorchidism gave a significantly smaller increase of sperm motility. From the regression equation, a nullifying effect of small testis size on improvement of sperm count was calculated at a total bilateral volume of smaller than 12 mL, which was present in only one patient of our study population (with testicles of 5 mL on both sides). A smaller testicular volume may indicate more progressive or additional testicular pathology, which does not respond to surgery. This may also explain the lack of improvement in sperm motility following varicocele treatment in the subpopulation with a history of cryptorchidism. Age of the man, duration of infertility and sperm count and motility before surgery were no significant indicators for the benefit of varicocelectomy in our population.

A reason that has been postulated for differences in treatment outcome, is variance in the size of the varicocele between study populations. Both in SV and CV, testicular atrophy has been observed [16]. Several authors reported equal semen improvements in SV *vs.* CV following surgery, or reported a slightly higher improvement in SV [10,17-20]. A greater increase in semen quality in CV compared with SV was noted by Tinga *et al.*[21], Bsat and Masabni[22] and Jarow *et al.*[23]. Our results are in line with the argument that ligation of both SV and CV is effective in terms of improvement in spermatogenesis. We found significant improvement of the sperm count, irrespective of the grade of the varicocele, and a significantly larger increase in progressive sperm motility in SV.

A comparison of SV and CV is difficult as the CV diagnosis is based on palpation, which is less objective and more prone to errors than is ultrasonography, for example. The accuracy of detection of varicoceles by physical examination has been shown to be correlated with the experience and expertise of the physician [24]. The reported false positive rate of palpation compared with venography varies from 24 to 67% [7,10,25,26], and was only 5% in one study [27]. Since palpation is not a very accurate screening method, we suggest to routine performance of CDU, which may find additional pathology (e.g. spermatocele, testicular tumours; Nashan *et al.* [28]) and can measure testicular volume accurately [29]. Instead of grading the varicocele as SV or CV (I-III), grading could be based on vein diameters or reflux measured with CDU. The diagnosis varicocele on the bases of CDU had been compared with venography by others. A good sensitivity and specificity of 90-98% for CDU was found [7,10,30]. Venography, however, may also produce false results [31,32].

In conclusion, we studied a large group of SV detected by routine colour Doppler ultrasonography, and a group of CV. Both were treated effectively in terms of semen improvement. We reason that as long as treatment of CV is accepted as an effective treatment of subfertility, the treatment of SV in infertile men with subnormal semen parameters is equally legitimate. Colour Doppler ultrasonography seems to be a good method for screening for varicoceles in infertile men, that can be treated effectively.

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Chapter 7

General Discussion and Conclusions

Research objectives

This research was initiated to improve our insight in the frequency of causal factors underlying male infertility, and in the value of diagnostic and therapeutic procedures. As described in Chapter 1, evaluation of andrological treatments requires large patient populations, which can only be achieved by prolonged single centre studies or multicentre research. Therefore, a standardised patient assessment and structured reporting is especially important in the field of andrology. We studied the value of structured data collection during a thorough and standard routine assessment of male infertility for the evaluation of andrological care. Such data collection could result in valuable data for clinical research and other purposes, on condition that the validity of the data is warranted. This chapter is drawn up of sections discussing the achievements per research objective. The objectives were:

- to investigate the feasibility of an information system for flexible access to integrated and structured andrological patient data

- to evaluate andrological care by:
  - exploring andrological patient data
  - charting of treatment options for our population of infertile men
  - evaluation of diagnostic procedures and treatments

- to assess the value of a standardised approach for both clinical research and patient care.

Feasibility of a research information system for andrological data

In various centres, a wealth of clinical data is generated concomitant with the use of information systems for routine collection of patient data. In several publications, a varied utilisation of electronic repositories of patient data has been suggested [1-4]. Advanced information systems may provide additional functionality. For instance, modern systems are used for critiquing, decision support, automatic reminders, post-marketing surveillance of drugs, quality assurance, medical audit, etc. The high expectations of electronic data collection may, however, be disturbed by limitations of the data and data systems. Problems with the data, such as difficulty to access and integrate data, existence of ambiguous data, and therefore time-consuming data handling, were reported by us (Chapter 2) and others [5,6]. Causes for these imperfections were technical restraints and unsuitable data structures.

A computer-based patient record (CPR) is primarily intended for data entry and data access to support individual patient care. The use of the record for epidemiological or clinical research imposed other requirements on the structure of the CPR. It is a challenge to develop a CPR that is suitable for this purpose, but which is still intuitive, simple and fast in the support of patient care. The 'Committee on Improving the Patient Record' of the Institute of Medicine, National Academy of Medicine, USA [1] has addressed the value of CPRs and has, at the same time, provided requirements for the development of modern CPRs.

The ORCA CPR has been developed as an advanced model for integrated data collection, providing a general core structure to permit exchanging and sharing of data, at the same time offering sufficient detail for different specialties [7]. Chapter 2 described the implementation of an ORCA subrecord for andrology. Although a large amount of clinical data on the patients of the Andrology outpatient clinic (University Hospital Dijkzigt-Rotterdam) was available initially, lack of simple access and an inappropriate data structure have hampered clinical research. The steps for the transfer of the existing data into ORCA were (1) the design of a normalised database, covering existing and additional data items, and allowing explicit representation of formerly implicit data; (2) correction of contents where possible; (3) conversion of the separate sources to one new structure.

It appeared in our study that all data available from various sources could be integrated by the ORCA model. The ORCA model was suitable for transparent integration of both existing data and data to be collected prospectively. The one-to-one relationship between medical meaning and database representation in the ORCA model reduced the risk of data misinterpretation. The problems with the original andrological data sources have been abstracted to general impediments for data conversion and integration. For our situation, the problems have been overcome successfully. To fully exploit the potential of an advanced CPR, data entry should be directly through its user interface. The inventory of problems and appropriate solutions can guide the transition of existing data to ORCA in other medical domains. Hopefully, CPR developers may accommodate a data structure that prevents the problems that we encountered.

Exploring andrological data

At the start of this project, no clear picture was available concerning the frequency of abnormalities associated with infertility in the population of infertile men seen at the Andrology outpatient clinic, although patient data had been collected in a structured manner for several years. The semantic integration of andrological data in ORCA made the data suitable for analysis. The extent of the data enabled numerous

patient searches and selections.

As a first evaluation, the andrological data were explored in a more general manner (Chapter 3). Three diagnostic and therapeutic modalities were evaluated separately in more detail (Chapters 4, 5 and 6), as discussed below. The potential of exploring the data is illustrated by the assessment of the prevalence of abnormalities and diagnoses, study of associations between clinical findings, and comparison of subgroups. An example is the distinct serum FSH levels in diagnostic subgroups of infertile men (Chapter 3, Fig. 5) implying different aetiologies, and underscoring the proposition that male infertility cannot be described by semen analysis alone. Exploration of andrological data gives insight in the population, and feedback on routine clinical practice. In addition, findings described in the andrological literature can be subjected to a critical revaluation with institutional data.

The new data structure facilitated criteria-based selection of data for the assignment of diagnoses according to World Health Organisation (WHO) classification rules. With the standard evaluation defined by the WHO, causes for the semen abnormalities were identified in 52% of our patients. With ancillary diagnostic methods – scrotal ultrasonography and serum hormone assays - this percentage increased to 66%. The proportion of men with no identified cause is, however, still high, and should be further reduced. This reduction may be achieved by advanced semen testing [8] and genetic screening. The number of identified genetic anomalies associated with infertility is rapidly increasing [9]. In our clinic, the three most common genetic abnormalities associated with male infertility (i.e., mutations in the cystic fybrosis transmembrane conductance regulator gene, Y chromosome microdeletions, and other chromosomal abnormalities) were present in 26% of 58 men with severe oligozoospermia or azoospermia [10].

Routinely collected data have become available for multiple purposes. Apart from the evaluation of andrological data described in this thesis, data are used for case finding and selection of patients for clinical studies. In the preparation of research protocols, consultation of existing data may provide an estimate of the expected difference between groups, treatments, or performance of diagnostic methods. Retrospective data can also indicate the number of inclusions to be expected per time period. Both are important to study the feasibility of prospective studies by means of a power analysis.

Treatment options for this population

The standardised evaluation developed by the WHO is aimed at arriving at diagnoses that support the most appropriate treatment choice. We could identify a cause for infertility in 66% of our patients. A condition for which a rational androlo-

gical treatment is available was diagnosed in 30% of the males (Chapter 3). IVF and ICSI were not included as andrological treatments, as will be described below. For other diagnoses, treatments have been described, but results are contradictory and inconclusive. In 34% of the men, infertility was idiopathic. The Cochrane Collaboration has laid bare a lack of good evidence for empirical treatment of idiopathic infertility, and concluded: *"The number and quality of randomised studies in male infertility are insufficient to allow firm conclusions"* [11]. Apparently, some treatments are valuable for subgroups of men with idiopathic semen abnormalities. The reasons for the differences between responsive and non-responsive patients are poorly understood. The clue to better therapy may lie in better understanding of basic pathophysiological principles, permitting causal interpretation of clinical findings.

The use of anti-oestrogens for treatment of idiopathic male infertility has not proven effective in a meta-analysis by the Cochrane Collaboration, although the beneficial effect was bordering on significance [12]. Feedback of androgens at the hypothalamic and pituitary level is partially mediated by oestrogens, derived from androgens. The rationale for treatment of male infertility with anti-oestrogens is based on counteracting the normal negative feedback on the hypothalamus-pituitary-testis axis by oestrogens. The resulting increase in FSH and LH secretion, and subsequently testosterone, may stimulate spermatogenesis [13]. The studies in the meta-analysis used anti-oestrogens in patients without obvious signs of increased oestrogenic fedback. We have found that 5% of our patients with idiopathic infertility according to the WHO diagnostic classification has combined subnormal serum concentrations of FSH and LH. These symptoms may indicate suppression at the hypothalamic and pituitary level by oestrogenic activity. These patients were not classified as having an 'endocrine cause', since their serum testosterone level was not evidently suppressed. It might well be that this subgroup would show a beneficial effect of treatment with anti-oestrogens, for trying to improve their fertility. Another example of a subgroup which may benefit from treatment is the group of idiopathic infertile men with mild hypospermatogenesis based on testicular biopsies, in which exogenous FSH stimulated spermatogenesis, whereas men with maturation arrest did not respond to this treatment [14].

An obstacle in evaluating andrological treatments is the multifactorial nature of male infertility, where treatments are in general directed at only one cause. Apart from aberrant classical semen parameters, other aspects of natural conception, such as timing of coitus, and the ability of sperm to traverse the cervical mucus, undergo the acrosome reaction, and bind and fuse with the oocyte, may be suboptimal. Evidently, female factors also play a role. Evaluation of assisted reproduction technology (ART) is less complicated, since many natural boundaries are circumvented, con-

trolled, and optimised. Furthermore, the pregnancy rates with ART is higher, and therefore require lower numbers of patients to confirm a treatment effect.

In this thesis, ART is not classified as rational andrological treatment, but rather as palliative treatment of male factor infertility, since the symptom of poor sperm quality is treated, and not the underlying cause. Nevertheless, ART is effective in inducing pregnancy. A delivery rate per cycle of 15-17% has been reported for intracytoplasmic sperm injection (ICSI) [15], and the cumulative delivery rate after several cycles is about 50% [16]. The relatively high ongoing pregnancy rates with ART should not restrain us from careful investigation of the cause of poor semen quality. We have shown that in a proportion of men a treatable condition is found. These treatments may have a lower pregnancy rate per cycle, but the beneficial treatment effect is not confined to, for example, the three IVF cycles remunerated by most health insurance companies in the Netherlands. Some underlying causes require treatment, regardless of the infertility treatment, and should not be missed (e.g., testicular tumours and hypogonadism in 1/196 and 1/33 men, respectively, in our clinic). Also for ART, patient characteristics may be useful to predict successful treatment.

Understanding of male infertility is of critical importance in the application of ICSI, since it may have a genetic transmissible cause. In men with oligozoospermia or azoospermia a higher than normal frequency of 30% genetic disorders has been suggested [17]. Especially the genetic risk associated with ART requires careful examination of both the man and the woman, to detect hereditary diseases. ART bypasses the effective biological mechanisms of sperm selection [18,19]. Not only may gene defects involved in male infertility be transmitted, also gene defects underlying inheritable systemic diseases manifesting with infertility may be passed on to the offspring. Otherwise sterile patients with cystic fibrosis gene mutations leading to congenital absence of the vas deferens, Y chromosome microdeletions leading to spermatogenic impairment, and other karyotype abnormalities can reproduce through ICSI [20-24]. A higher incidence of chromosomal abnormalities has been detected among ICSI babies [25,26]. Apart from genetic risks, ART carries a higher risk of multiple pregnancy, reduced birth weight and associated risks. For example, an increased risk of hypospadias and cryptorchidism has been suggested in association with low birth weight and IVF [27,28].

The recognition of potential genetic risks has raised the interest in basic research on spermatogenesis and its genetic regulation. The number of identified genetic abnormalities associated with male infertility is rapidly increasing, which may shed more light on the complex genetic regulation of spermatogenesis. Studies on mice with gene mutations or gene knockouts and impaired fertility have identified many genes involved in spermatogenesis [29,30]. An overview of common genetic defects

associated with infertility in the human is given by several authors [9,31,32].

Genetic testing and counselling is important before ICSI is employed [17,33-35] and, where possible, a rational treatment improving spontaneous pregnancy rate should be used as first line approach. These treatments are generally cheaper, with a lasting effect, and may therefore be more cost-effective [36,37]. Classical andrological treatment may improve sperm quality and enable the choice of a less invasive ART in the further treatment of the couple.

Evaluation of diagnostic procedures

Two ancillary diagnostic modalities in the work-up of male infertility patients - serum inhibin B measurements and scrotal colour Doppler ultrasound (CDU) - were assessed in more detail.

Serum inhibin B measurements in infertile patients

The existence of inhibin, a testicular suppressor of FSH secretion, was first postulated in 1932 [38]. Later investigations suggested the Sertoli cell as the primary origin of inhibin in the male [39,40]. These observations led to the theory that (1) inhibin is the primary feedback signal for pituitary FSH secretion, and (2) inhibin is a marker of Sertoli cell function and spermatogenesis. These hypotheses were, however, not consistently confirmed with the inhibin radioimmunoassay (RIA) [41,42]. Since specific ELISAs for bioactive inhibins recently became available [43], the inhibin concept is being reinvestigated.

We found a strong negative correlation between serum FSH and inhibin B levels, confirming the negative feedback effect of inhibin B on FSH secretion (Chapter 4). This is a consistent finding in the recent literature [44-46]. We confirmed the postulated capacity of inhibin B to express the quality of spermatogenesis. We demonstrated a strong positive association between the serum inhibin B level and the spermatogenic status of the seminiferous tubules, assessed in testicular biopsies. Earlier studies on inhibin subunit mRNA expression in the germinal epithelium already suggested that inhibin production is stimulated by late phases in spermatogenesis in the rat [47-49]. Furthermore, we observed that serum inhibin B was correlated with total testicular volume, sperm concentration and total sperm count, consistent with recent publications [50-52]. The correlation of these indicators of spermatogenesis with inhibin B was stronger than with FSH. We have shown that inhibin B measurements may help in differentiating between obstructive and secretory azoospermia, and concluded that inhibin B is the best of the known endocrine markers of spermatogenesis.

After publication of Chapter 4, reports by others have supported our findings, and provided additional data confirming the value of inhibin B as marker of spermatoge-

nesis. Inhibin B seems to be secreted not directly in response to pituitary FSH, but only when late phases of spermatogenesis are present. Recently, a study suggested that inhibin B was produced by Sertoli cells in association with germ cells from the pachytene spermatocyte to early spermatid phase [53]. Another study revealed a positive correlation between serum inhibin B levels and intratesticular spermatid numbers [54].

The close correlation between effective spermatogenesis and serum inhibin B was illustrated by the parallel gradual decrease of sperm count and serum inhibin B levels during treatment with testosterone-enanthate, whereas serum FSH and LH levels had already collapsed much earlier [45]. Similarly, after discontinuing exogenous testosterone administration, FSH and LH levels were already restored to normal when inhibin B levels and sperm concentration were still recuperating, at equal pace.

Inhibin B levels may be useful as marker of prepubertal seminiferous tubule function [55]. Before puberty, inhibin B can be produced by Sertoli cells in the absence of germinal cells [53,56], and the serum inhibin B level may reflect the Sertoli cell number. In prepubertal rats, Sertoli cell multiplication and increasing inhibin B levels coincide. Experimental reduction and increase of Sertoli cell numbers corresponded with lower and higher serum inhibin B levels, respectively [57]. Decreased serum inhibin levels were detected in young cryptorchid boys with the less specific RIA for inhibin [58]. A different clinical application of inhibin B estimation in men would involve its use as a serum marker for Sertoli cell tumours, which are quite rare [59-61].

Serum inhibin B may prove an important outcome measure in studies on male fertility, and may provide important prognostic information. Semen analysis is subject to a large intraindividual variability [62], has many biases and confounders [63,64], and semen samples may be difficult to obtain. Serum inhibin B measurement seems more practical for population studies investigating the quality of spermatogenesis than semen analysis or the invasive testicular biopsy procedure. Inhibin B may prove to be a more sensitive marker for spermatogenesis than semen analysis.

In cases with azoospermia, sperm used for the ICSI procedure may be retrieved from a testicular biopsy (TESE: testicular sperm extraction) [65]. Serum FSH level and testicular volume are inaccurate predictors of successful TESE. The visualisation of spermatids in a diagnostic testicular biopsy is the best predictor of successful therapeutic TESE [66,67]. Since the serum inhibin B level appears to be correlated with the presence of spermatids in the seminiferous epithelium, it may provide a complementary, less invasive predictor of successful testicular sperm extraction. Recently, it was shown that serum inhibin B in combination with serum FSH measurement gave the best prediction of the presence of elongated spermatids in testis histology. However, the prediction of successful retrieval of sperm from the biopsies by TESE was not very accurate [68].

Scrotal colour Doppler ultrasonography

In Chapter 5 the value of routine scrotal CDU in male infertility patients has been described. Reports on scrotal CDU procedures in 1,372 infertile men were reviewed. The high incidence of 38% scrotal abnormalities, of which 67% were not palpable, underlines its diagnostic value. According to the WHO diagnostic classification [69], 48% (659/1,372) of the patients had idiopathic oligo-, astheno-, terato- or azoospermia. In 29% of these patients (191/659), a scrotal abnormality was detected with additional CDU. Based on the finding of testicular tumour and varicocele as possible determinants for abnormal semen parameters, the percentage of idiopathic semen abnormalities was reduced from 48 to 38%. In the WHO manual, varicocele and testicular tumours are described as two underlying causes of infertility [69]. Less is known on the clinical significance of testicular cysts, epididymal cysts and testicular microlithiasis. It would be worthwhile to study a control population of fertile men, for comparison of the prevalence of scrotal abnormalities, and to define reference values of normality (e.g., for testicular volume, testicular heterogeneity, epididymis size, and venous diameter and reflux in the pampiniform plexus).

The most striking finding in our study group was the presence of a testicular tumour in seven patients (circa 1/200); of seven tumours, only one was expected on the basis of physical examination. The rate of testicular tumours in our infertile population is higher than that reported for the general population. The reported increase in the incidence of testicular tumours in the general population is associated with an increase in other disorders of the reproductive tract, such as infertility, incidence of cryptorchidism and hypospadias, and decreased testicular volume [70-73]. We conclude that routine ultrasound screening of the scrotal organs in infertile males, a high-risk group for testicular tumours, may be warranted. The cost-effectiveness of screening for scrotal pathology (e.g., varicocele and testicular tumours) should be studied.

The most frequent finding was varicocele in 30% of the cases, of which only 40% was also palpable. The low sensitivity of palpation (40%) is in accordance with other reports [74], which emphasises that many varicoceles in the infertile population are undetected. The accuracy of palpation is disputed since a low inter-observer agreement and low specificity have been reported. Two experienced urologists agreed on the criteria for varicocele palpation and examined 134 men. They independently reported 19 and 32% clinical varicoceles, respectively, in the same men [75]. A low specificity of 33-76% has been reported [76-80]. Only Comhaire *et al.* reported a specificity of 95% [81], which is comparable to the specificity of 91% that we also report. The low accuracy of palpation makes it a poor screening method for varicocele, and grading into subclinical and clinical varicocele on the basis of clinical examination

appears inappropriate. The positive predictive value of 66% in our study illustrates that 34% of positive palpations are not even subclinical. CDU is a more accurate and objective screening method, and allows grading on the basis of venous diameter and reflux characteristics.

There is no gold standard to study the accuracy of CDU. Venography has gained acceptance as standard method to register retrograde flow in the spermatic venous system. Nevertheless, different procedures and criteria have been described for venography, which may also give false diagnoses [82]. Direct comparisons of CDU with venography reported a correspondence rate of 63 up to 98% [77,78,83,84]. These diverging results may be explained by a lack of standardisation of procedures and criteria for venography and CDU. We reviewed studies using venography and (Doppler) ultrasound for varicocele diagnosis, and observed notable differences in materials and methods [77,78,83-102]. Different procedures (supine, standing and sitting postures, employment of Valsalva manoeuvre), different criteria (for direction, reversal, and duration of flow, and for the diameter of veins in the pampiniform plexus), and dissimilar equipment (e.g., different frequency reach of echotransducers) were used. Using strict criteria, a sensitivity of 97% with a 94% specificity was reported for CDU compared with venography [77]. In this light, standardisation of the scrotal CDU procedure and accompanying criteria is important. A manner to evaluate diagnostic methods and to define criteria in the absence of a gold standard, is basing the criteria on the effect of varicocele repair on improvement of sperm quality, scrotal temperature [103], or serum inhibin B level. For example, Jarow *et al.* defined as cut-off level a venous diameter of greater than 3 mm, which predicted a positive outcome in terms of semen improvement [88].

Prospectively, scrotal CDU should include measurement of testicular volume, since this gives a more accurate estimation of testicular volume compared with clinical measurements [104-106].

Evaluation of varicocele treatment

We found increased sperm counts and motility after ligation of the varicoceles diagnosed with scrotal CDU, whether palpable or not (Chapter 6). There was no significant spontaneous change in sperm concentration or motility in the period prior to ligation. Varicocele ligation was less effective in men with small testicles or a history of cryptorchidism. In 44% of men with less than 1 million sperm per ejaculate presurgery, improvement to above this threshold for ICSI was achieved. Varicocele ligation may therefore allow a less invasive ART in the further treatment of the couple. Others have also reported semen improvement after ligation of varicoceles diagnosed with scrotal ultrasound or Doppler examination [88,94,107]. A smaller effect of

varicocele treatment in men with a history of cryptorchidism or small testicles has also been reported in terms of a smaller probability of conception compared with other subgroups [108].

Improvement of semen parameters is a consistent finding in most studies on treatment of both clinical and subclinical varicocele (as reviewed by Schlesinger *et al.* [36] and Marsman *et al.* [109]). This finding was confirmed by randomised controlled trials [110-113]. Nevertheless, the results of the randomised trials are contradictory and inconclusive with respect to the effect on spontaneous pregnancy rates. Unfortunately, data on pregnancy and confounding female factors were incomplete for our study population.

The differences in diagnostic procedures and criteria may, in part, explain the varying results of varicocele treatment between studies. Especially the low specificity of palpation may result in a high rate of false-positive clinical varicoceles, for which no treatment effect may be expected. This may also explain some of the variance between centres in the reported prevalence of clinical varicocele. In large series of subfertile men (>1,000 men), prevalences of 12 to 39% have been reported [114-118]. Because the result of palpation was not confirmed with a reference method, the true prevalence of clinical varicocele in these series is unknown.

Apart from differences in diagnostic procedures (summarised above in the section on Scrotal colour Doppler ultrasonography), the selection of patients, duration of follow-up, treatment modality, and investigation of the female partner may also differ. Several studies present exclusion criteria based on semen parameters, serum FSH level, age of the female partner, testicular volume, and difference between affected and contralateral testicular volume. Before continuing research on varicocele, we should, ideally, aim at a consensus on the definition of varicocele, and standardisation of diagnostic procedures.

Value of a standardised approach for clinical research and patient care

A thorough assessment of treatments and diagnostic techniques is one of the means to improve andrological care. Assessment of patient care requires the collection of reliable, complete and structured data. Whereas the prospective randomised controlled trial is often the optimal design for evaluation of treatments, the necessary resources are limited. For such studies, protocols and case record forms are used to promote complete and reliable data. Since the use of computerised structured data collection is becoming more widespread, the potential of data collected routinely, outside the framework of a specific trial, is increasing.

At the Andrology outpatient clinic, electronic forms for structured data entry have supported complete and concise collection of data. Clinical data were entered direct-

ly into the information systems by physicians during patient encounters, which prevents the errors which may occur when written data are entered off-line. The restructuring of initial data sources by the ORCA model has resulted in structured data. Although routine patient care may not primarily be seen as a potential source for research, routine data collection may result in data repositories with great value, for versatile purposes. Chapters 3, 5 and 6 demonstrated the value of these data for retrospective analysis.

Also prospective research is much facilitated by a structured and standardised data collection, as was shown by the evaluation of the significance of serum inhibin B determination in infertile men (Chapter 4). The value of this hormone as a marker for spermatogenesis could be studied easily, since all data for the research question were already gathered routinely. The only extra effort was the request of the inhibin B assay by the physician, and adding the codes for the new inhibin B assay to our information system.

A well-designed data set and collection of data in a structured CPR may decrease the gap between retrospective and prospective research, since retrospective research does not necessarily require laborious review of paper medical records. Furthermore, prospective trials may be easier to organise, since data are already collected in a structured manner. Evidently, the absence or lack of a proper control group remains a major drawback in retrospective studies. The electronic collection of patient data required a definition of the data set, investments in time to develop and get acquainted with the system, and disciplined use of structured data entry. The current results are a reward for these initial investments.

Although every standard has its imperfections, the strength of using a standard is comparability of the diagnoses, and the procedures leading to these diagnoses. The diagnostic classification is not exhaustive, but a more detailed diagnosis, for instance on the basis of advanced sperm function testing, can be described as a more specific subgroup of a standard WHO diagnosis.

Clinical studies in infertility medicine may require large numbers of patients that only can be recruited in multicentre studies within a reasonable time span. Conscientious use of a common data set would facilitate pooling of data and multicentre research.

Conclusions

The consistent use of a standard protocol for the examination of infertile men has resulted in a uniform patient approach. Collection of clinical data in a structured fashion led to a complete set of data on a large andrological population. The ORCA model proved suitable for semantic integration of separate existing databases. The transition to the ORCA structure solved ambiguity, redundancy and inconsistency in the original data.

The remodelled data were available and valuable for multiple purposes. General exploration of the data provided insight in the andrological population, and feedback on patient care. Three important questions were answered: (1) what is the prevalence of abnormalities and diagnoses, (2) for which proportion of patients is an andrological treatment available, and (3) what is the value of diagnostic procedures and treatments. Structured data collection provides an infrastructure for retrospective and prospective research.

Serum inhibin B estimation is the best of the known endocrine markers of spermatogenesis, and may have a role in the future andrological diagnostic and prognostic work-up.

Colour Doppler ultrasonography is the method of choice for varicocele screening. Varicoceles detected in this manner were treated effectively in terms of improved sperm count and sperm motility. A surplus value of scrotal ultrasound is the accurate estimation of testicular volume, and detection of other existing pathology, especially testicular tumours.

A standardised examination and reporting of male infertility is important (1) for accurate diagnoses, (2) for optimal treatment, (3) to identify subgroups with a rationale for treatment, and (4) for assessment of diagnostic procedures and therapy.

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Summary

It is estimated that about 15% of couples experience infertility during their reproductive life, while attempting to conceive. In about half of these couples, a male factor is responsible for the infertility, whether or not combined with a female factor. The medical field of andrology, dealing with male reproductive health and dysfunction, is relatively young. The cause of male infertility is frequently unknown and many diagnostic procedures and treatment modalities have not been evaluated in a thorough manner. Andrology should be further developed by increased understanding of infertility, assessment of existing diagnostics and therapies, and by development of new diagnostic and treatments methods. Such research requires complete, valid, unambiguous and structured data. Medical information systems can support structured collection and scientific analysis of patient data. In our Andrology Outpatient Clinic (University Hospital Dijkzigt-Rotterdam), patient data have been collected in a structured manner for more than 10 years, which implies a wealth of data for clinical research.

The aims of the current thesis were (1) to study the value of standardised examination and structured reporting of male infertility patients, and (2) to evaluate diagnostic procedures and treatment methods in andrology. The first chapter introduces the background and motivation for the present study, the aims and objectives, and our approach in addressing these aims.

We commenced with the development of a research information system for andrological data (described in Chapter 2). In the past decade, data were collected in separate electronic databases in different systems. The content of the data sources was analysed. Several barriers for research with the data were exposed. Some medical items were ambiguous, the existence of invalid data was revealed, and several items were redundant. We have categorised the problems with existing data, and described solutions that may be generally applicable for these problems. The Open Record for CAre (ORCA) is a computer-based patient record, which promotes accurate and structured data collection. For our andrology clinic, the data were validated and integrated, and restructured according to the ORCA model. All existing data could be transferred to the ORCA model and a 1:1 relationship between medical meaning and database representation was attained. The resulting research information system was used to further address our objectives, as described in the subsequent chapters.

In Chapter 3, a general overview of the andrological population is presented. The prevalence of WHO diagnoses for male infertility is given and treatment options were inventoried. A cause for infertility was identified in 52% of the study population on the basis of the WHO diagnostic guidelines alone. This percentage was increased to 66% by employing advanced diagnostic methods. The evaluation of additional diagnostic methods was addressed separately in the following chapters. The exploration

of diagnoses, diagnostic procedures and treatment options illustrates the value of a structured, standardised assessment of male infertility.

Chapter 4 describes a study on the value of the serum inhibin B level as a marker of spermatogenesis. Recently, specific assays for bioactive serum inhibins were introduced. With these assays, we confirmed the suggested role of inhibin B as primary testicular feedback signal for pituitary FSH (follicle-stimulating hormone) secretion, and as sensitive marker for spermatogenesis. We demonstrated a strong correlation between the serum inhibin B level and the status of the spermatogenetic epithelium in testicular biopsies. Discrimination of competent and incompetent spermatogenesis was better with inhibin B than with serum FSH. We conclude that the serum inhibin B level is the best of the known endocrine markers of spermatogenesis.

The diagnostic significance of colour Doppler ultrasonography examination of the scrotal contents is described in Chapter 5. In 38% of 1,372 infertile men, scrotal abnormalities associated with infertility were detected. Only 33% of the abnormalities detected during ultrasonography were uncovered by clinical palpation. In this study population, the percentage of men with idiopathic infertility was reduced from 48 to 38% on the basis of testicular tumours and varicocele detected with Doppler ultrasonography. The most striking finding was a testicular tumour in 7 of the 1,372 men (i.e., approximately 1/200); only one of these tumours was palpable. Infertile men have been suggested to be at risk for testicular tumours, and routine scrotal colour Doppler ultrasonography appears amenable in this group of men. The most frequent finding was varicocele, in circa 30% of the cases. The accuracy of varicocele palpation is questioned, since 34% of palpable varicoceles were false positive. We state that varicocele palpation is not an appropriate screening tool for varicocele. Scrotal colour Doppler ultrasonography is more objective and accurate for detection of varicocele. Moreover, it is useful for detection of other scrotal pathology (in particular testicular tumours) and accurate estimation of testicular volume, which gives an impression of the quality of spermatogenesis.

Surgical treatment of varicoceles detected with colour Doppler ultrasonography resulted in an increased number of spermatozoa in the ejaculate, and enhanced sperm motility (Chapter 6). The improvement in sperm quality was irrespective of the outcome of varicocele palpation. The beneficial effect on sperm quality was smaller in men with severely hypoplastic testicles or a history of testicular maldescent.

Finally, Chapter 7 presents an overall discussion of the results described in this thesis, and the conclusions.

Samenvatting

Naar schatting ondervindt 15% van de paren met kinderwens een tijdelijk of blijvend vruchtbaarheidsprobleem. In ongeveer de helft van deze paren is een oorzaak voor het uitblijven van zwangerschap te vinden bij de man, al dan niet gecombineerd met een oorzaak bij de vrouw. Andrologie is het medische vakgebied dat zich bezighoudt met de voortplantingsfuncties en -diskfuncties van de man. De oorzaak van gestoorde mannelijke vruchtbaarheid is in de meeste gevallen onbekend, en diagnostische en therapeutische methoden zijn veelal niet grondig geëvalueerd. De andrologie dient verder te worden ontwikkeld door onderzoek naar de fysiologie en pathologie van mannelijke fertiliteit, evaluatie van bestaande diagnostische en therapeutische methoden, en de ontwikkeling van nieuwe diagnostiek en behandelingen. Hiervoor zijn complete, accurate, ondubbelzinnige en gestructureerde gegevens vereist. Medische informatiesystemen kunnen gestructureerde dataverzameling en wetenschappelijke analyse van patiëntengegevens ondersteunen. Op de polikliniek Andrologie van het Academisch Ziekenhuis Dijkzigt-Rotterdam worden patiëntengegevens al meer dan 10 jaar routinematig gestructureerd vastgelegd. Deze gegevens kunnen zeer waardevol blijken voor klinisch-wetenschappelijk onderzoek.

Het doel van het onderzoek beschreven in dit proefschrift was (1) de waarde te bestuderen van een gestandaardiseerde inspectie en gestructureerde verslaglegging bij onvruchtbare mannen, en (2) evaluatie van diagnostiek en behandeling in de andrologie. Hoofdstuk 1 geeft een inleiding van de achtergronden en motieven van dit onderzoek, de doelstellingen en een uiteenzetting van onze aanpak.

Het onderzoek werd begonnen met de ontwikkeling van een research informatiesysteem voor andrologische medische gegevens (beschreven in Hoofdstuk 2). In het afgelopen decennium werd andrologische data verzameld in afzonderlijke databestanden. De inhoud van deze databases is geïnventariseerd, waarbij bleek dat er verschillende belemmeringen waren voor wetenschappelijk onderzoek met deze data. Sommige gegevens waren dubbelzinnig of incorrect. Deze problemen zijn gecategoriseerd en algemeen toepasbare oplossingen voor deze problemen zijn beschreven. ORCA (Open Record for CAre) is een elektronisch medisch dossier, ontwikkeld door het Instituut Medische Informatica (Erasmus Universiteit Rotterdam), dat een eenduidige, accurate en gestructureerde dataverzameling bevordert, waarmee bovengenoemde problemen kunnen worden voorkomen. ORCA bleek geschikt voor het integreren van bestaande andrologische patiëntgegevens. De overgang naar ORCA vereiste validatie, explicatie en herstructurering van de gegevens. Alle bestande data kon in ORCA worden overgenomen, waarbij een één-op-één relatie tussen medische betekenis en database representatie werd verkregen. Het research informatie systeem werd gebruikt voor de beantwoording van de overige vraagstellingen.

In Hoofdstuk 3 wordt een algemeen overzicht van de andrologische populatie gepresenteerd. De frequentieverdeling van diagnoses bij mannelijke infertiliteit en de behandelingsmogelijkheden zijn geïnventariseerd. Een oorzaak voor de infertiliteit werd bij 52% van de onderzochte mannen geïdentificeerd op basis van WHO (Wereld Gezondheidsorganisatie) richtlijnen. Dit percentage werd verbeterd tot 66% met aanvullende diagnostische methoden. De evaluatie van 2 van deze additionele diagnostische methoden en een andrologische behandeling is in meer detail beschreven in de aansluitende hoofstukken. De evaluatie van diagnoses, diagnostiek en behandeling illustreert de waarde van gestandaardiseerde en gestructureerde beoordeling van onvruchtbare mannen.

In Hoofdstuk 4 wordt de waarde beschreven van het bepalen van de concentratie van het hormoon inhibine B in serum, voor kwalificatie van de spermatogenese (het proces van zaadcel productie). Recent is een gevoelige methode ontwikkeld voor de bepaling van bioactief inhibine B. Met deze methode hebben we de hypothese bevestigd dat inhibine B de testiculaire terugkoppeling op FSH (follikel-stimulerend hormoon) secretie door de hypofyse verzorgd. Tevens bleek het serum inhibine B een gevoelige maat voor de kwaliteit van de spermatogenese te zijn. Wij hebben een sterke correlatie tussen serum inhibine B niveaus en de status van het spermatogenetisch epitheel in testisbiopten aangetoond. Het onderscheiden van competente en gestoorde spermatogenese was nauwkeuriger met inhibine B dan op basis van het serum FSH. Wij concluderen dat het serum inhibine B de meest geschikte endocriene indicator van de spermatogenese is.

De diagnostische waarde van kleuren Doppler echografie van de scrotale organen wordt beschreven in Hoofdstuk 5. Bij 38% van de 1372 onderzochte mannen werd een afwijking gevonden die met infertiliteit is geassocieerd. Slechts 33% van deze afwijkingen werd bij lichamelijk onderzoek gedetecteerd. Het percentage mannen met subnormaal zaad zonder aantoonbare oorzaak werd gereduceerd van 48% naar 38%, op basis van een testis tumor of een varicocèle die door echografie werd bemerkt. De meest opvallende bevinding bij echografie was een testistumor bij 7 van de 1372 infertiele mannen (circa 1/200). Slechts 1 van deze testistumoren was waargenomen bij lichamelijk onderzoek. Een verminderde vruchtbaarheid is mogelijk geassocieerd met een verhoogde kans op testis tumoren. Routinematige kleuren Doppler echografie van de scrotuminhoud bij infertiele mannen lijkt daarom zinvol. De meest voorkomende afwijking, bij circa 30% van de mannen, was een varicocèle (spatader in de balzak). De nauwkeurigheid van varicocèle palpatie is twijfelachtig, aangezien 34% van de voelbare varicocèles fout-positief bleek. Wij stellen dat palpatie geen geschikte methode voor varicocèle screening is. Kleuren Doppler echografie van de balzak is objectiever en nauwkeuriger bij varicocèle detectie. Bovendien kun-

nen hiermee andere scrotale afwijkingen worden opgemerkt (in het bijzonder testis tumoren) en kan het testis volume nauwkeurig worden bepaald, wat een impressie geeft van de kwaliteit van de spermatogenese.

Chirurgische behandeling van varicocèles gedetecteerd met kleuren Doppler echografie resulteerde in een toegenomen aantal spermatozoa in het ejaculaat, en een betere progressieve beweeglijkheid van spermatozoa (Hoofdstuk 6). De verbetering in zaadkwaliteit was onafhankelijk van het al dan niet voelbaar zijn van de varicocèle. Het positieve effect op spermakwaliteit was kleiner bij mannen met kleine testikels en bij mannen met een voorgeschiedenis van gestoorde testikel indaling.

Tenslotte bevat Hoofdstuk 7 een algemene discussie van de in dit proefschrift beschreven bevindingen, en de hieruit volgende conclusies.

Andrology Record

name patient: _____ date of birth: ___-___-19__ patient Id: _____

name partner: _____ date of birth: ___-___-19__ patient Id: _____

History taking and previous history

Date of history taking	___-___-____	
Referrer	☐ GP ☐ urology ☐ second opinion	☐ gynaecology ☐ internal medicine
Current relationship since:	___-___-____	
Infertile since:	___-___-____	
Infertility current relationship if secondary: -number of children -time to previous pregnancy	☐ primary ___ children ___ months	☐ secondary
Male infertility (incl. former relationships) if secondary: -number of children -time to previous pregnancy	☐ primary ___ children ___ months	☐ secondary
Female infertility (incl. former relationships) if secondary: -number of children -time to previous pregnancy	☐ primary ___ children ___ months	☐ secondary
Previous infertility investigations / treatment [1]	☐ no	☐ yes, i.e. _____
Contraception method	☐ condom ☐ OAC ☐ spermicide ☐ other, i.e.: _____	☐ IUD ☐ temperature curve ☐ vasectomy
Systemic disease	☐ no ☐ COPD ☐ neurologic disease ☐ other, i.e.: _____	☐ diabetes ☐ tuberculosis
History of medical treatment	☐ no	☐ yes
High fever in the past 6 months	☐ no	☐ yes
Surgery	☐ no ☐ hydrocelectomy ☐ prostatectomy ☐ urethral strictures ☐ varicocele ligation ☐ other, i.e.: _____	☐ inguinal hernia ☐ hypospadias ☐ vasectomy ☐ vaso-vasostomy ☐ bladder neck operation

[1] In the case of abnormal findings, details can be given in the textbox at the bottom of the page (description; treated when, how and where; recovery, etc.)

Remarks on history taking and previous history:

History taking and previous history continued

Urogenital infections (orchitis: see testis pathology)	☐ no ☐ pyelonephritis ☐ epididymitis ☐ other, i.e.: _____	☐ cystitis ☐ prostatitis
Sexually transmitted disease	☐ no ☐ syphilis ☐ other, i.e.: _____	☐ chlamydia ☐ gonorrhoea
Puberty development	☐ normal	☐ abnormal

		Left	Right
Epididymitis	☐ no	☐	☐
Testicular pathology			
torsio testis	☐ no	☐	☐
trauma	☐ no	☐	☐
pain	☐ no	☐	☐
mumps orchitis	☐ no	☐	☐
orchitis without mumps	☐ no	☐	☐
other	i.e.: _____		
Testicular maldescent	☐ no	☐	☐

Coitus frequency	___ times per	☐ week ☐ month
Timing of coitus around ovulation	☐ no	☐ yes
Erections	☐ normal	☐ abnormal
Ejaculations	☐ normal	☐ abnormal

Environmental factors

Profession	_____	
Environmental factor	☐ no ☐ heat ☐ other, i.e.: _____	☐ toxic substance
Smoking	☐ no ☐ yes, i.e. ___ cigarettes per ☐ other, i.e. __ _____ per	☐ day ☐ week ☐ _____ since: _____
Alcohol consumption	☐ no ☐ yes, i.e. ___ units per week	
Drug abuse	☐ no	☐ yes

Remarks on history taking and previous history:

Physical examination

Date of physical examination ___-___-____

General

Height _____ cm

Weight _____ kg

Blood pressure (mm Hg) _____/_____ systolic / diastolic
method:
☐ RR sitting ☐ RR standing
☐ RR supine
☐ other, i.e. _____

General physical examination ☐ normal ☐ abnormal

Signs of virilisation ☐ normal ☐ hypogonadism

Gynaecomastia ☐ no
☐ yes, Tanner stage ____

Urogenital tract

Penis ☐ normal ☐ scars
☐ plaques ☐ hypospadias
☐ ulcers ☐ other: _____

		Left	Right
Testis consistency	☐ both normal		
	non-palpable	☐	☐
	soft	☐	☐
	hard	☐	☐
	other: _____		
Testis location	☐ both normal		
	high scrotal	☐	☐
	retractile	☐	☐
	inguinal	☐	☐
	ectopic	☐	☐
	other: _____		
Testis volume		___ ml	___ ml
Epididymi	☐ both normal		
	thickened	☐	☐
	tender	☐	☐
	cyst(s)	☐	☐
	non-palpable	☐	☐
	other: _____		
Vas deferens	☐ both normal		
	thickened	☐	☐
	non-palpable	☐	☐
	other: _____		

Remarks physical examination:

Physical examination continued

		Left	Right
Varicocele	☐ none		
	0: subclinical	☐	☐
	I: palpable with Valsalva	☐	☐
	II: palpable	☐	☐
	III: visible	☐	☐
	other: _____		
Scrotal swelling	☐ none		
	inguinal hernia	☐	☐
	hydrocele ☐	☐	
	tender	☐	☐
	other: _____		
Inguinal examination	☐ normal		
	inguinal hernia	☐	☐
	lymphadenopathy	☐	☐
	surgical scars	☐	☐
	inguinal scars	☐	☐
	other: _____		
Rectal examination - prostate	☐ normal	☐ soft swelling	
	☐ hard swelling	☐ tender	
	other: _____		
Rectal examination – seminal vesicles	☐ normal (non-palpable)		
	☐ palpable		
Scrotal thermography	☐ normal	☐ abnormal	

Remarks physical examination:

Laboratory

Serum

	Unit (mM, U/l etc.)	1 dd / /	2 dd / /	3 dd / /	4 dd / /	5 dd / /
FSH						
LH						
testosterone						
SHBG						
inhibin B						
prolactin						
17β-oestradiol						

Genetic screening

Karyotype	☐ normal	☐ abnormal
CFTR gene mutation	☐ no	☐ yes
Y-chromosome microdeletion	☐ no	☐ yes

Other / details

Semen analysis

Date analysis	___ - ___ - ___	___ - ___ - ___	___ - ___ - ___	___ - ___ - ___
Technician				
Duration of abstinence (days)				
Time of production hh:mm	:	:	:	:
Time of analysis hh:mm	:	:	:	:
Volume (ml)				
Appearance of semen	☐ normal ☐ abnormal	☐ normal ☐ abnormal	☐ normal ☐ abnormal	☐ normal ☐ abnormal
Liquefaction	☐ normal ☐ abnormal	☐ normal ☐ abnormal	☐ normal ☐ abnormal	☐ normal ☐ abnormal
Consistency (viscosity)	☐ normal ☐ increased	☐ normal ☐ increased	☐ normal ☐ increased	☐ normal ☐ increased
Addition of chymotrypsin	☐ no ☐ yes	☐ no ☐ yes	☐ no ☐ yes	☐ no ☐ yes
pH				
Round cells (10^6/ml)				
Leucocytes (10^6/ml)				
Concentration (x 10^6/ml)				
Motility (microscopy) at 37°C (%)				
a: rapid progressive motility				
b: slow or sluggish progressive motility				
a+b: progressive				
c: non-progressive				
d: immotile				
Vitality (% alive)				
Morphology:				
Number of cells assessed				
Normal morphology				
Head abnormalities				
Neck / midpiece abnormalities				
Tail abnormalities				
Cytoplasmic droplets				
Percentage normal				
TZI index				
MAR IgG (% binding)				
MAR IgA (% binding)				
Agglutination	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
Other / remarks				

Scrotal colour Doppler ultrasonography

Date investigation: ___ - ___ - ___

Name radiologist _____

Venous diameter _____ mm

Increase in diameter with Valsalva / standing ☐ no ☐ yes ☐ standing ☐ Valsalva

Venous reflux in the pampiniform plexus
☐ no
☐ < 2 seconds with Valsalva
☐ > 2 seconds with Valsalva
☐ during normal respiration

Varicocele ☐ no ☐ yes

Testis volume Left: ___ ml Right: ___ ml

Testis
- homogeneity ☐ normal ☐ abnormal
- cyst(s) ☐ no ☐ yes
- processes ☐ no ☐ yes

Epididymi
- size ☐ normal ☐ abnormal
- cyst(s) ☐ no ☐ yes

Hydrocele ☐ no ☐ yes

Other / remarks

Venography

Date investigation: ___ - ___ - ___

Name radiologist _____

Conclusion ☐ no varicocele ☐ varicocele

Other / remarks

Testis biopsy score

Date biopsy ___ - ___ - ___

Name radiologist _____

Biopsy score _____

Other / remarks

Diagnosis

Semen classification

- ☐ normal
- ☐ oligozoospermia
- ☐ teratozoospermia
- ☐ asthenozoospermia
- ☐ antibody coated sperm
- ☐ agglutination
- ☐ leucospermia
- ☐ abnormal plasma
- ☐ azoospermia
- ☐ aspermia
- ☐ necrospermia
- ☐ globozoospermia
- ☐ other, _____

Clinical diagnosis

- ☐ sexual and/or ejaculary dysfunction
- ☐ immunological cause
- ☐ no demonstrable cause
- ☐ isolated seminal plasma abnormalities
- ☐ iatrogenic cause
- ☐ systemic cause
- ☐ congenital abnormalities
- ☐ acquired testicular abnormalities
- ☐ varicocele
- ☐ male accessory gland infection
- ☐ endocrine cause
- ☐ idiopathic oligozoospermia
- ☐ idiopathic asthenozoospermia
- ☐ idiopathic teratozoospermia
- ☐ idiopathic azoospermia
- ☐ obstructive azoospermia

Other / remarks

Therapy
Medication

_____
_____
_____

Varicocele treatment
-date ___ - ___ - ___
-method ☐ Palomo ☐ embolisation
other, i.e. _____

Assisted reproduction ☐ IUI ☐ IVF
☐ AID ☐ ICSI

Other / remarks

Pregnancy
Date of finding ___ - ___ - ___

Method of detection ☐ HCG test ☐ ultrasound
☐ other, i.e.: _____

Last menstruation date ___ - ___ - ___

Fertilisation ☐ spontaneous ☐ after therapy _____

Other / remarks

Dankwoord

Alhoewel er maar één naam op dit boekje staat, zijn uiteraard ook anderen belangrijk geweest bij de totstandkoming ervan, niet alleen direct voor het onderzoek, maar ook daarbuiten; promotor, co-promotoren en andere begeleiders, co-auteurs, de leescommissie, collega's, stagiaires, familie, vrienden, paranimfen, Cecile. Als ik hier namen zou gaan noemen, zou ik vast mensen vergeten, daarom:

Bedankt allemaal,

Frank

Curriculum Vitae

Frank Pierik werd geboren op 4 april 1970 te Rheden. In 1988 behaalde hij het VWO diploma aan het Thomas à Kempis College te Arnhem. Vervolgens begon hij aan de studie Biomedische Gezondheidswetenschappen aan de Katholieke Universiteit Nijmegen. Tijdens deze studie werden stages volbracht bij de vakgroep Toxicologie van de Katholieke Universiteit Nijmegen (onder leiding van Dr. P.J. Sessink en Dr. R.P. Bos), de afdeling Pathologie van het Rijksinstituut voor Volksgezondheid en Milieu te Bilthoven (onder leiding van Dr. W. Goettsch en Dr. J. Garssen), en het Virology Laboratory, Medical School, University of Edinburgh (onder leiding van Dr. M. Norval). In maart 1994 studeerde hij af als Gezondheidswetenschapper in de afstudeerrichting Toxicologie. Vanaf februari 1994 tot februari 1998 was hij aangesteld als AIO bij de Polikliniek/Laboratorium Andrologie (Dr R.F.A. Weber), Academisch Ziekenhuis Dijkzigt-Rotterdam en de instituten Medische Informatica (Prof Dr Ir J.H. van Bemmel) en Endocrinologie & Voortplanting (Prof Dr J.A. Grootegoed) van de Faculteit der Geneeskunde en Gezondheidswetenschappen, Erasmus Universiteit Rotterdam. In deze periode werd het onderzoek verricht dat in dit proefschrift is beschreven. Op dit moment is Frank Pierik werkzaam als postdoc op een samenwerkingsproject van de Polikliniek/Laboratorium Andrologie en het instituut Maatschappelijke Gezondheidszorg (Prof Dr P.J. van der Maas) van de Faculteit der Geneeskunde en Gezondheidswetenschappen, Erasmus Universiteit Rotterdam. In dit onderzoek wordt de relatie bestudeert tussen blootstelling aan omgevingsfactoren, met name de zogenaamde 'endocrine disrupters', en gestoorde testis indaling en hypospadie bij pasgeborenen jongetjes en de zaadkwaliteit bij volwassen mannen.

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