European Society of Human Reproduction and Embryology

COURSE 1

Paramedical Course

19 June 2005
Copenhagen / Denmark

Inhoud

Course 1 - Paramedical course

Program

Course Co-ordinator: E. Corrigan (UK) and H. Joris (B)

Morning sessions: "Working together"

09.00 - 09.10: Introduction – *L. Corrigan (UK)*

09.10 - 09.30: The role of the nurse in Denmark – *I.Rose (DK)*

09.30 - 10.00: Intra-uterine insemination – *A. Geril (B)*

10.00 - 10.30: Embryology – *A. Veiga (E)*

10.30 - 11.00: Coffee break

11.00 - 11.30: Ultrasound guided embryo transfer – *V. Robinson (UK)*

11.30 - 12.00: Q & A Discussion panel

12.00 - 13.00: Lunch break

Afternoon Hands-on Session: "Understanding techniques"

Participants will be subdivided into 4 different groups (max. 10 to 12 participants) who will rotate from one group session to another.

Group 1: Ultrasound scanning (1 hour) – *M. Hammar (S) & J. Hinks (UK)*

Group 2: Sperm preparation (1 hour) – *A. Van de Velde (B) and D. Molero (E)*

Group 3: Video presentations and discussions

– Embryology (30 min.) - *P. Wilson (UK)*
– Embryo transfer (30 min.) - *H. Birch (UK)*

Group 4: Data processing made simple (1 hour) – *W. Meul (B) & H. Van Ranst (B)*

Role of the IVF-nurse in Denmark

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Learning objectives

- To get information about how fertility treatment is organised in Denmark
- To gain knowledge about which tasks IVF-nurses in Denmark have
- To gain knowledge about IVF-nurses' expectations to development of their job

Introduction

The health care sector is very differentially organised in different countries. In Denmark we have a large public health care sector where most of the expenses are paid by the taxes and a smaller, private sector where the patients pay themselves for the treatment. The general practitioner is the gatekeeper and decides which patients need to be referred to specialists. Most of the health care staff is trained in the biomedical aspects of diseases and health (e.g., medical doctors, nurses, physiotherapists and laboratory staff). Only a few psychologists are working in the somatic secondary health system (hospitals, specialized clinics).

Fertility treatment

Among Western European countries, Denmark has the largest proportion of use of assisted reproduction treatment (ART) compared to the population (Nyboe Andersen et al, 2004). Fertility treatment is organised in 9 public and 11 private fertility clinics. These clinics perform the most advanced ART as IVF, ICSI, FER, oocyte donation (OD) as well as inseminations. Further, gynaecological clinics and wards (satellite clinics) perform inseminations and hormone induction. In 2000 16 Danish clinics reported 5278 IVF cycles, 3004 ICSI cycles, 1242 FER and 158 OD (Nyboe Andersen et al, 2004). Results from the Danish Fertility Society, 2004, show 11518 advanced treatments, 65.9% made in public clinics, and 34.1% in private.

Staff and organisation

The fertility treatment is separated from the traditional pregnancy and childbirth system.
The staffs at the fertility clinics consist of medical doctors, IVF-nurses, biologists, laboratory technicians, secretaries, hospital porters and cleaners. A few of the clinics have staff further educated as sex therapists, and a some have the possibility of referring the most stressed fertility patients to a psychologist. There are no mental health workers in the Danish health care system.

The purpose of this lecture is to present results about Danish IVF-nurses' different tasks. The results are based on a questionnaire study among all IVF-nurses working at Danish fertility clinics.

Material and methods

In the autumn of 2004 nurses employed at fertility clinics in Denmark received an open-ended questionnaire, where we asked the participants in groups to describe in their own words their tasks and how they expected the development of their job tasks in the future. In total, 23 fertility and gynaecological clinics (some with interest in IVF) received this open-ended questionnaire and nurses from 8 clinics responded. Some of the clinics have no nurses employed.

Based on these responses we developed a structured questionnaire measuring in detail the IVF - nurses' different medical, technical, informational, patient-centred support and care, research and development, and administrative tasks; assessment of time spent on the different tasks; assessment of whether there was time enough to take care of the patients and socio-demographic data. Further, an open-ended question about their expectations of and wishes for how their tasks should develop in the future was added.

Study population

In total, 76 IVF-nurses personally received the structured questionnaire, and 70 (92.6%) responded. All respondents were women. The majority (86.6%) of the IVF-nurses were between 30 and 49 years old, and 59.7% were between 40 and 49 years old.

Most (92.6%) had as minimum10 years experience as a nurse. Only one nurse had experience of less than 5 years. About 75% have worked in the fertility area less than 10 years. In private clinics only nurses with more than 10 years experience were employed. Furthermore, nurses in private clinics had significantly more years of experience as IVF-nurses. In total, 54 (79.4%) had their main job at a public fertility clinic and the remaining was primarily employed at a private clinic.

Results

All fertility clinics (both public and private) were represented in the statement.

Clinical tasks

The clinical tasks were organized in 5 subgroups: direct patient contact, invasive procedures, ultrasound scans, diagnostic procedures, and other clinical procedures.
The IVF-nurses estimated that they spend between 25 and 74% of their working time on clinical tasks.

Direct patient contact

Everybody participated in the direct patient contact with preparation for examination and treatment, observation during the treatment, and follow up after treatment. Thus, the nurse was the most present professional person for the patients during the treatment.

Invasive procedures (oocyte pick up, transfer, TESA, cyst puncture, insemination)
Nurses assisted in oocyte pick up, transfer, TESA, and cyst puncture but none of them performed these tasks independently. In total, 21.6% of the participants performed inseminations independently, whereas 63.1% assisted at this.

Ultrasound scans

Between 36 and 46 (54.5%-68.7%) of the nurses assisted at different types of scans during treatment, whereas 14 to 20 (20.9%-30.3%) never participated in scans. There were significant coherence between performing scans independently and performing insemination independently.

Diagnosing procedures (GU, HSU, Hysteroscopy)

The majority of the nurses had an assisting role at the examinations carried out in the clinic in connection with evaluation and diagnose.

Other clinical procedures

Pain management, application of venflon, blood sampling and acupuncture were examples of tasks that the nurses to a high degree handled by themselves.
All nurses working at clinics where oocyte pick up were performed (95.2%) took part in pain management, 65.1% independently. In total, 45 (65.2%) applied venflon, and 1/3 of the nurses performed acupuncture.

Pedagogic tasks

The pedagogic tasks were organized in 3 subgroups: information, dialogues, and education/ communication.
The IVF-nurses estimated that they spend between 25 and 74% of their working time on pedagogic tasks.

Information

The nurse was involved in informing the patient about all aspects of the treatment in the clinic as well as in instructing about the part of the treatment the patient should carry out by herself.
More than 90% of the nurses had information tasks in connection with examinations. All participants informed the patient about the medicine, which was part of the treatment, and all instructed the patient in self-administration of medicine. In total, 63 (92.6%) informed about pregnancy test, 65 (95.6%) replied telephone enquiries, and 28 (42.4%) replied e-mails.

Dialogues

About half of the nurses participated in the preliminary dialogue about the treatment, whereas less participated in the final dialogue.

In total, 91.9% of the participants carried out dialogues about lifestyle, and 45 (68.2%) had dialogues about alternative treatment.

Education/communication

More than 90% of the nurses were involved in composing written information material for the patients.

A part of the nurses had educational tasks that were not related to the patients. In total, 45 (67.2%) taught students from different health educations. The majority of these had their main job in a

public clinic, and there was thus a highly significant coherence between the type of clinic and education of students ($p<0.001$).

Psychosocial tasks

All the nurses thought they had an important role in patient-centred care during the different visits in the clinic, and most of them found that it was possible for them to fill in this role.

All participants estimated that it was an important part of the nurse's job to find the time to help the couples that had special need for psychological support.

In total 98.5% of the participants answered that it was important to have a dialogue with the patients about how they were influenced emotionally by the examination and treatment, and 88.2% thought it was important that the nurse advised about the handling of the emotional consequences of their childlessness and treatment.

Only a small group of the nurses found that advising about psychosocial problems was less important. More than 90% of those who found it important agreed that they had wholly or partly possibility of performing this task.

In total, 71.2% of the nurses found it important to talk with the couples about moral and ethical considerations connected with the treatment. Furthermore, 92.4% found it important to offer consultation personally or by telephone, and 93.4% of these wholly or partly agreed that they had this possibility.

Research and development

In total, 56 (83.6%) of the nurses were working in clinics with research activities, and 50 of these (89.3%) participated in research tasks. Not all nurses at a specific clinic participated equally in research activities. The research tasks were often delegated to one or more nurses (study nurse). There was a significant coherence between the type of clinic and the distribution of research tasks. All participants took part in the development of nursing.

Tasks related to the running of the clinic

To a varying degree all nurses participated in tasks determining the daily running of the clinic (purchase of articles, maintenance of medical instruments and the technical equipment, filling up utensils, cleaning-up etc.).

Administrative tasks

Work planning and organization were tasks that all nurses found to be included in their field of activity.

Expectations and wishes for the future

The nurses wanted to maintain their present tasks but would like more independent clinical tasks such as acupuncture, ultrasound scanning, and insemination. Approximately 1/3 of the participants considered tasks within health care to be a part of their future work area, e.g. dialogues about smoke stopping and weight control. Furthermore, they found great challenges within the psychosocial

area and would like more competence as regards communication, teaching, research and development.

Discussion

The purpose of the questionnaire was to illustrate the role of the IVF-nurse in Danish fertility clinics as widely as possible. The questions were designed to ask about all the tasks the nurses had mentioned in the preliminary investigation.

The high feedback rate expressed that nurses from both public and private clinics found it relevant to contribute to visualize the role of IVF-nurses in Denmark.

Most of them were employed in public clinics (although the number of clinics was almost equal), which could be connected with the fact that in general there were more nurses employed in public clinics than in private clinics.

An explanation of the fact that there were more IVF-experienced nurses employed in private clinics could be that the private clinics were dependent on the necessary competences being present. There was little room for training.

The questionnaire showed that the nurse, being the most present professional person, had a broad spectrum of tasks.

Technical, informational, and especially patient-centred care took up a lot of time. The majority of the nurses estimated that they spend between 25 and 74% of their working hours on clinical as well as pedagogic tasks. This could express that the pedagogic tasks rarely were detached from but rather integrated with the clinical tasks. Or it could be due to an overlap with the situations of a pedagogic character that at the same time acted as a psychosocial relief for the patients.

There were no big differences in the tasks the nurse performed whether she was employed in a public or a private clinic. All nurses had assisting tasks and independent tasks.

The invasive procedures were central in the course of the treatment at the fertility clinics, and on all Danish clinics it was the doctor's task to perform these. This illustrates the nurse's important role as the doctor's partner in these clinical procedures.

Very few nurses performed ultrasound scans independently. However, a group of nurses performed ultrasound scans of patients for insemination and of pregnant patients. This could be because the diagnostic or evaluating aspects of these scans were minor, as compared to scans of patients undergoing an IVF treatment. These nurses also performed the insemination themselves.

Many nurses mentioned insemination as a task that could be performed independently by nurses in the future, if they were trained properly. Cheung et al, 2003, have shown that as regards transfer no differences in pregnancy rate have been found to be dependent on which profession (trained doctors or nurses) performed the task. Barber et al, 1996 and 2000 claim that the success rate after transfer is connected with the nurses' qualifications. The most trained had the highest success rate.

All nurses performed several independent tasks, but directly asked about visions of the future the nurses called for even more independency. They found the work with the psychosocial part of childlessness especially interesting and important and would like to prioritize this part of the treatment higher.

As we in Denmark have no psychologists and mental health workers in the clinics, the IVF-nurses, being the most present professional person, found it natural to perform these tasks of patient-centred care. The nurses found their group competent to solve the tasks but expressed a wish of being able to seek advice from other professionally trained experts, e.g. psychologists.

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Intrauterine Inseminations by paramedicals

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Learning objectives

At the end of this presentation the participant will be able to

1 Understand the role of the paramedical in an insemination program in a multidisciplinary team setting.
2 Understand the different steps in the training of the paramedical

Lecture summary

Intra-uterine insemination (IUI) is one of the most frequent therapies used in the treatment of infertility. Because the insemination program is largely managed by the nursing staff (midwives and counsellors) it was only a small step for them to perform the technical act of the insemination. The management of the insemination program is in close collaboration with gynaecologists, psychologist, and laboratory technicians of the infertility centre.

“Because of their specific obstetric, nursing and psychological skills and as well as their knowledge and attitude with respect to reproductive events, midwives are a link in the fertility world”. This is laid down in the professional profile of Belgian midwives and protects them with respect to carrying out these specific tasks; more especially counselling and the technical performing of ultrasound follicular measurements and artificial insemination. The treating fertility gynaecologist handles diagnosis and determines treatment.

In case of immunological infertility, mild andrological, mild endometriosis and idiopathic infertility IUI with husband sperm can be performed. IUI with donor sperm is performed in lesbian couples, single women and azoospermic men. In our centre specific psychological counselling is obligatory for all requests with the use of donor sperm. During the entire treatment continuous patient support is available.

Description of tasks

After the gynaecologist has diagnosed the patient and stipulated the therapeutic measures, the patient is counselled by the midwife. During these counselling session and individual review of the treatment is given to the patient.
- Natural cycle
- Mild ovarian stimulation
- Monitoring by blood and ultrasound
- Insemination technique

- Possible side effects
- Actual chances of pregnancy
- Price of the treatment
- Signing of the medical legal documents

The ovulation is monitored by ultrasound and endocrine monitoring. These results are discussed on a daily basis for each patient during a multi-disciplinary team meeting. The instructions from the gynaecologist with respect to the further treatment are communicated to the patients by telephone.

The insemination technique

Transcervical intrauterine insemination is performed on an ambulatory basis. With the patient in the lithotomy position, the cervix is exposed with a bivalve speculum and cleansed with saline solution. A polyethylene catheter containing the washed and motile sperm fraction is atraumatically inserted into the uterine cavity. The semen is then instilled slowly, to avoid uterine spasm. The catheter is removed; the patient can leave and go home or resume work.

Outcome and follow-up

Fourteen days after the insemination a pregnancy test is performed. This can be done by detection of serum human chorionic gonadotropin (hCG); the results are communicated by telephone to the patient. If there is a pregnancy a first ultrasound is performed in the 7th week. The patient can start a new cycle if no pregnancy did occur.

The training of the midwives

In our centre the inseminations are performed by midwives since November 2001.

The first step in the training was a theoretical lesson by a gynaecologist. Then the midwives were trained in a practical session by the gynaecologist. The 10 first IUI had to be performed under supervision, and even now there is a continuous supervision by the gynaecologist.

A general rule is that after 6 cycles the patient needs a new consultation with her gynaecologist and that sperm results can only be discussed by the gynaecologist. There also is a double check of the sperm identity with the laboratory technicians and with the couple. In our centre we have an internal registration of the inseminations.

Conclusions

In the multidisciplinary team of gynaecologists, laboratory technicians and psychologist the midwives play an essential role in the insemination program. They are especially trained for this. We can also state that the results did not increase since November 2001, and that the patients acceptance is excellent. Most of them are satisfied with this changement in management of the IUI program.

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Embryology

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Learning objectives

- Different aspects to be considered when setting up an In Vitro Fertilization (IVF) laboratory in order to improve embryo quality and ART efficacy.
- Culture systems and methodology of gametes and embryo handling.
- Optimization of the results by choosing the best embryo for transfer.

Setting up an IVF laboratory

Different aspects have to be considered when setting up an IVF laboratory (1, 2, 3):

Localization; spatial and environmental requirements: The IVF laboratory has to be located in a secure low-traffic area, and to offer adequate space to ensure safe and comfortable working conditions. The access to the laboratory has to be limited to the people working there. It is recommended to distribute it in different working-areas, with a separate office space, and isolating the area for embryo handling from other laboratory activities (sperm processing, embryo freezing, frozen embryo storage, preimplantation genetic diagnosis (PGD), etc). The IVF laboratory has to be intercommunicated with oocyte retrieval and replacement rooms. Environmental factors such as air quality are very important. Air sampling and determination of volatile organic compounds (VOC) have to be determined inside and outside of the IVF laboratory area, and air filtration units must be installed when necessary. Periodical microbial sampling for aerobic bacteria and fungi should be also performed. Overpressure, controlled and consistent temperature and an adequate humidity level are required in the IVF laboratory.

Equipment: Specific clothing, including shoes, hats and masks, is necessary to enter the laboratory area. The laboratory equipment should be adequate for laboratory work and easy to clean and disinfect. The use of toxic cleaning materials is not permitted. Correct operation, maintenance and periodical calibration of instruments are required. Maintenance manuals of all equipment have to be available in the laboratory. Critical items of equipment, including incubators and frozen storage facilities, have to be available and provided with alarms. All IVF laboratories should have an automatic emergency generator backup in the event of power failure. The surfaces of microscopes and horizontal laminar flow cabinets have to be equipped with heating plates in order to maintain the temperature during oocyte and embryo manipulation. The number of incubators has to be determined according to the activity of the IVF program. Gas cylinders should be placed in a separate room with an automatic backup system. It is important to limit the incubator's openings. CO2 and temperature daily measurements are needed. It is essential to use embryo tested disposable materials (pipettes, dishes, tubes, etc).

Laboratory personnel: A laboratory supervisor/director, embryologists and auxiliary personnel are necessary. Sufficient technically qualified personnel for the size and volume of the program is required. Daily organization of the routine work in the laboratory is needed. Good communication between the clinical staff and the IVF laboratory personnel is essential to assure the appropriate techniques to be applied. The specialists in clinical embryology need to follow continuous education to be trained in new methodologies. Periodical audits are recommended to control if the work of embryologists is done in a standard and consistent way.

Culture systems

Physiological conditions for embryo development: Scientific background
The female reproductive tract has different nutritional environments and reflects the requirements of the early embryo (4).

Concentration of pyruvate, lactate, glucose, amino acids and oxygen, changes from the fimbrium to the uterus in a gradient manner. The pyruvate and lactate concentrations are at their highest point in the oviduct closest to the fimbrium, and decrease down the reproductive tract to reach its lowest level in the uterus. The opposite occurs with glucose: its concentration is low in the oviduct and high in the uterus. Different concentrations of oxygen and carbon dioxide are found in the oviduct compared to in the uterus, which reflects pH (5). Amino acids, that act as biosynthetic precursors, energy substrates, regulators of energy metabolism, antioxidants and chelators, as well as regulators of intracellular pH, are also present in high concentrations in the reproductive tract (8).
The embryo's requirements are also different depending on the developmental stage. The early pre-compacted embryo (before the 8-cell stage) has a low metabolic activity, and it generates energy from low levels of oxidation of pyruvate/lactate and amino acids. Cell proliferation and compaction are stimulated by the presence of non-essential amino acids. In contrast, the embryo post-compaction (8-cells and later stages) has a high metabolic activity, uses glucose as the preferred nutrient and requires both nonessential and essential amino acids for cell proliferation and differentiation as well as specific vitamins to maintain oxidation (6, 7, 8).

Culture conditions

Gametes and embryos need an environment as similar as in the female reproductive tract as possible to be able to develop optimally. In an optimal environment the embryos can minimize the amount of energy spent to maintain their internal environment.

Culture media systems try to reproduce the physiological conditions in the female reproductive tract.

The culture systems include different culture media formulated with the same core salt concentration to minimize osmotic or pH stress when moving from one medium to another. Osmolarity of all media has to be maintained between 285-290 mosm/Kg.

Different amino acids and different nutrients are added to compose the sequential media: non-essential amino acids and pyruvate and lactate for precompactation embryo culture media, essential and non-essential amino acids and glucose for post-compactation embryo culture media (6, 7, 8).
Media used to manipulate gametes and embryos under air conditions have to be buffered (Hepes, Mops) to avoid important pH variation. The intracellular pH of human embryos is around 7.2 and it seems that external pH does not control intracellular pH. Embryos have the capacity to regulate intracellular pH in a culture environment of pH 7.2 to 7.4. When embryos are cultured in a pH too

different from intracellular pH the rate of development is decreased (9, 10). The presence of a protein source (albumin) is also important to the embryo. Albumin works as pH buffer, colloid osmotic regulator and as carrier of growth factors, and also plays a role as scavenger, surfactant and nutrient. All media have to be supplemented with around 5% of human serum albumin (HSA).
Human oocytes and embryos are very sensitive to temperature variations. Culture temperature should be the physiological temperature: 37ºC. All media have to be equilibrated in the incubator overnight (at least 4 hours and no more than 18) before use.

Culture under oil is highly recommended to avoid evaporation and reduce CO2 diffusion, acting as physical barrier.

Despite good quality embryo culture systems are now commercially available, media can be homemade been as well. Ultrapure water is required and further mouse embryo tests are needed for quality control before use.

Methodology for gametes and embryo handling

Washing and disinfection of hands before handling any product is essential, and wearing non-toxic gloves when handling any biological fluid as blood, follicular fluid and semen, is recommended.
Identification and labelling of all biological samples, tubes and culture dishes is needed. It is important to introduce security measures, as a second viewer (controler), to minimize de risk of fatal errors when samples, tubes and dishes are labelled or when gametes and embryos are manipulated.

No mouth pipetting is allowed for gamete or embryo handling. Different sizes of pipettes have to be available for the different procedures: oocyte localization, oocyte denudation, oocyte and embryo moving, blastocyst manipulation, etc.

Manipulation time must be minimized during oocyte retrieval, oocyte denudation prior to intracytoplasmic sperm injection (ICSI) or at fertilization checking after conventional insemination, and at further embryo observations. Micromanipulation time during ICSI must be controlled and limited, thus no more than 6 oocytes should be injected per ICSI dish. ICSI methodology and tool optimization are needed for reach adequate results.

Microscopic examination must be performed at high magnification (x400) to enable detailed observation of pronuclear structures by fertilization checking on day 1. Reobservation should be done when it is needed. Isolated culture of zygotes with different patterns is recommended for later identification of the embryos with the highest implantation potential.

Detailed observation of the embryos must be performed also at high magnification at the inverted microscope. Quality of the embryos is scored following morphological criteria: number and symmetry of blastomeres, % and localization of cytoplasmic fragmentation and multinucleation. Cytoplasmic appearance and zona pellucida (ZP) alterations have to be recorded as well (Figure 1). When embryo freezing is required, adequate freezing protocols have to been applied for different embryo stages. Only good quality embryos, showing a correct cleavage rate and low fragmentation rate, or good quality blastocyst must be cryopreserved to achieve high embryo survival and implantation rates after thawing.

The details of all methodologies being in use have to be described and gathered in a manual of Standard Operation Procedures (SOP) that should be reviewed and revised annually. This manual has to be immediately available for the IVF laboratory personnel when it is needed.

Choosing the best embryo for replacement

Embryo replacement can be performed on day 2, 3 or 5-6 of embryo development. Careful embryo evaluation and selection must be done on an individual basis. Multiple pregnancy score, taking in account embryo quality and the age of patient, is required for avoiding multiple pregnancies. Routine discipline of controlled identification must be followed to avoid any possibility of mistake. Embryos are replaced in a small volume of replacement medium.

Oocyte evaluation

In the morphologic evaluation of the oocyte, cytoplasmic defects such as granularity and vacuolisation have been described. The morphology of the polar body has also been related to oocyte quality (11).

Zygote evaluation

Different classifications of zygotes depending on pronuclear evaluation have been described and related to embryo development and to chromosomal abnormalities. Pronuclear patterns that give rise to good quality embryos with high implantation potential have been identified (12).

Embryo evaluation

Many different scores have been described to quantify embryo quality and every laboratory classifies embryos according to the one that correlates the best with implantation rate.

Besides the morphological features of the embryo described previously, other aspects have been incorporated to select the best embryo for transfer. The early cleavage rate (at 25-29 hours after insemination or ICSI) correlates with higher rates of blastocyst development and pregnancy (13), even though an accelerated cleavage may be associated with embryo mosaicism. Multinucleation in one or more blastomeres can be observed on day 2 or 3. Such embryos have a decreased implantation potential and multinucleation is correlated with chromosomal abnormalities.

Blastocyst evaluation

Culture to the blastocyst stage has been used as a method to select the most viable embryo. Only good quality embryos on day 3 become blastocysts (14, 15). A good blastocyst has a well-defined and compact inner cell mass and a well-structured trophectoderm, and when expanded has between 150-200 cells. Embryos that reach blastocyst stage at day 5 have better prognosis than those at day 6. Few day 7 blastocyst can implant.

According to all this features, the best embryo for transfer has to be selected when it comes from a normal oocyte that showed a favourable pronuclear pattern, has 2 cells at the end of day 1, 4 to 6 cells at day 2, and 7-9 cells or shows signs of compactation on day 3, and that is able to achieve the blastocyst stage at day 5 of culture.

The collaboration between the clinical staff, the embryologists and the paramedical personnel in an IVF program is essential to obtain satisfactory results with the treatment of infertile couples. Every single step of the procedure has to be optimized in order to increase the pregnancy rate in a population that is becoming harder to treat successfully.

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Ultrasound guided embryo transfer

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(no text received)

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Learning Objectives

The aim of this course is to help participants to:
- Understand how a semen analysis is performed
- Know how the different parameters can be determined

Summary

Semen analysis aims to determine a number of parameters necessary for the physician to obtain objective information regarding the quality of a semen sample. Semen analysis is usually performed manually. Automated semen analysers have been introduced in the market more than a decade ago. However, not all tests can be performed with these instruments. The basic semen parameters to determine are: volume, concentration, motility and morphology. With these parameters, it is possible to have an idea about the fertilizing capacity of a semen sample. Additionally, other tests can be performed to obtain a complete picture on the quality of a semen sample. An important part of a semen analysis if the procedure to select sperm cells with the highest fertilizing capacity from the semen. This selection procedure can be performed in different ways.

It is known that semen quality can vary. Therefore, in order for the physician to get a complete picture of the quality of a semen sample, repeated analysis is strongly advised. The information from semen analysis and sperm selection procedure allows the treating doctor to determine the role of the semen sample and to discuss possible treatments with the couple seeking treatment.

The aim of this text is to summarize a number of tests that are routinely performed and how a selection procedure can be performed.

Introduction

Standardization of semen analysis begins with the method of collection of the ejaculate and its delivery to the laboratory. The World Health Organisation (WHO) recommends that at least two, and preferably three, semen samples be obtained over a period of at least a month to assess a man's

semen quality reliably. Factors influencing specimen collection include abstinence, specimen collection, split ejaculates and retrograde ejaculation.

Abstinence is a major source of variation in semen characteristics that must be controlled in order to determine true biological variation. Normally, a 3-day period of abstinence is recommended.

Specimen collection must be controlled carefully. Patients must be provided with clear, comprehensive instructions stating what should and should not be done.

1. Produce the specimen by masturbation directly into a sterile plastic jar provided by the laboratory. Jars whose lids have waxed cardboard, plastic or rubber liners must not be used.
2. Do not use contraceptive condoms, as the lubricant is spermicidal. Withdrawal (coitus interruptus) is also unacceptable, as the first, sperm-rich, part of the ejaculate is often lost. Men objecting to masturbation on religious or moral grounds may use silastic condoms such as the Seminal Collection Device (HDC Corporation, Mountain View, CA, USA).
3. Only remove the lid of the jar at the last moment before ejaculation, to minimize microbiological contamination, and replace it immediately after completing the collection.
4. Write your full name and a second identifier on the specimen jar, along with the date and time of collection.
5. Specimens not produced adjacent to the laboratory must be received as quickly as possible, certainly within an hour of collection, having been kept within the range of "room" to body temperature (25-37 ºC).

Split ejaculates

There is little need or justification for split ejaculates when collecting for assisted conception treatments. However, for diagnostic purposes, ejaculates may be collected in two (sometimes more) fractions so that the first fraction, which contains the majority of the spermatozoa suspended in primarily prostatic fluid.

Retrograde ejaculation

Occurs when spermatozoa are not ejaculated in the ante grade direction along the urethra but in the retrograde direction into the bladder. Recovery of the spermatozoa either by catheterization and washing of the bladder or by urination immediately after ejaculation. Alkalinization of the urine is commonly achieved by oral intake of sodium bicarbonate.

Semen analysis

The guidelines of the WHO should be considered as minimum standards for all semen analyses. Normal values are listed in figure 1.

Firgure 1

Nomenclature – semen variables	
Volume	≥ 2 mL semen
Aspermia	No ejaculate
Azoospermia	No spermatozoa in the ejaculate
pH	≥ 7.2
Sperm concentration	≥ $20x10^6$/ml (Normozoospermia)
Oligozoospermia	≤ 20 $x10^6$/ml
Severe Oligozoospermia	≤ 10 $x10^6$/ml
Total nº of spermatozoa	≥ $40x10^6$ ejaculated
Sperm motility	≥ 50% a+b or ≥ 25% a
• Asthenozoospermia	Motility below normal range
• Necrozoospermia	Only dead spermatozoa in the ejaculate
Sperm morphology	≥ 15% normal spermatozoa (WHO)
Teratozoospermia	< 15% normal forms
Sperm vitality	≥ 75% of alive
Leukocytes	< $1x10^6$/ml
Immunobead test	< 50% active
MAR tests	< 50% active with latex

Macroscopic examination

Macroscopic evaluation of a semen sample includes assessment of: liquefaction, appearance, volume, viscosity and pH.

Liquefaction: Liquefaction of a normal semen sample occurs within 1 h at ambient temperature.
Volume: Volume can be measured using a graduated pipette.

Viscosity: Viscosity is rated subjectively by observing the length of the tread when semen drops from a pipette by gravity.

pH: can be measured by spreading a drop onto a pH paper.

Microscopic examination

Sperm concentration

This term should be used instead of 'sperm density' to avoid confusion with the specific gravity of spermatozoa. Although sperm concentration has a positive association with the likelihood of achieving a pregnancy there are no absolute limits to distinguish fertile from infertile except for azoospermia.

Concentration can be determined by counting a number of fields in a haemocytometer or a counting chamber specifically developed for semen samples. By using a formula including the number of sperm cells and the number of squares counted, the result will express a certain number of spermatozoa per ml. For a correct assessment of concentration, minimal 200 cells should be counted if possible.

Sperm motility

The motility of ejaculated spermatozoa is an extremely important functional characteristic. Spermatozoa show different types of motility listed herafter:

1. Class a: rapid progressive motility (≥25 _m/s progression);
2. Class b: slow progressive motility (5-25 _m/s progression);
3. Class c: non-progressive motility (flagellar activity, but < 5 _m/s "space gain");
4. Class d: immotile (no flagellar activity).

A correct evaluation of motility characteristics may be influenced by the viscosity of the semen. Motility can be determined by using the same type of counting chamber as for the determination of concentration or by placing a drop of semen on a microscopic slide.

Sperm vitality

Vital staining differentiates between immotile and dead spermatozoa, with the percentage of vital cells usually slightly exceeding that of motile cells. Sperm vitality assessment is useful in samples where the motility is extremely low, allowing necrozoospermia to be distinguished from total sperm immotility (e.g. in Kartagener's syndrome).

Sperm morphology

The morphology of spermatozoa is extremely important in the complete evaluation of a semen sample but again there is no clear boundary between fertility and infertility. Normal sperm morphology is significantly related to in vitro tests of sperm function and in vivo conception and also fertilization in vivo.

The WHO 1992 classification for human sperm morphology, in which all borderline forms are considered abnormal, recognizes the following description of a normal human spermatozoon and four categories of defects. (Fig. 2)

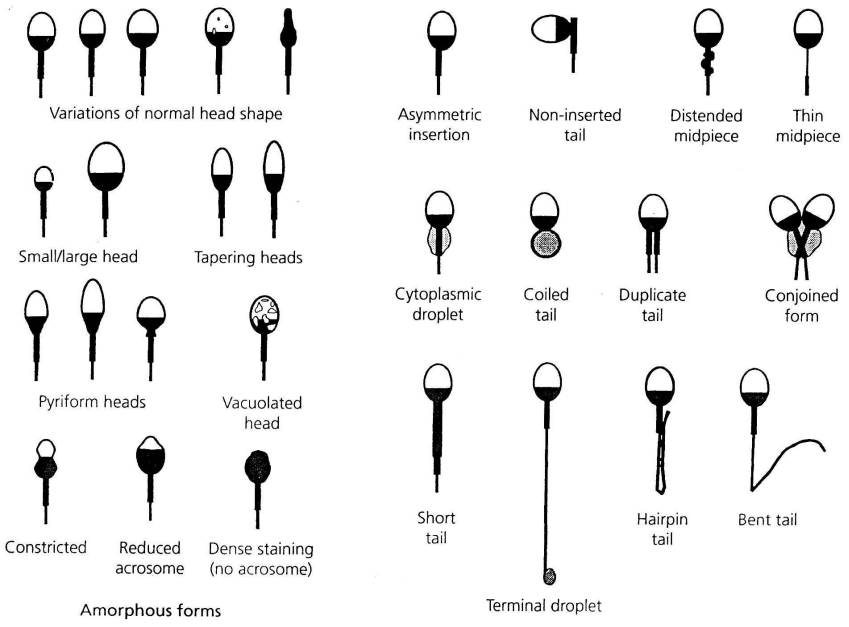

Figure 2: Drawings of normal and abnormal forms of human spermatozoa

Normal form: A normal, mature human spermatozoon has an oval head of regular outline (3-5 _m long, 2-3 _m wide) with clearly defined pale anterior (acrosomal) and darker posterior regions in stained preparations.

Head shape and size defects These include large, small, tapering, pyriform, amorphous, vacuolated (occupying > 20% of the head area), reduced acrosome (<40% of the head area), absent acrosome, double head, or any combination of these.

Necks and midpiece defects These include absent tail ("free head"), non-inserted or bent tail (tail forms an angle of about 90º to the long axis of the head), distended or irregular midpiece, abnormally thin midpiece, or any combination of these.

Tail defects These include short, multiple, hairpin, broken (angulation of ≥ 90º), irregular width, terminal droplet, coiled, or any combination of these.

Immature forms These are defined by the presence of cytoplasmic droplets of more than one-third the area of a normal sperm head. Cytoplasmic droplets may also be seen at other locations along the tail, or even at the tip of the tail.
Correct assessment of morphology is done after staining of fixed cells by using a larger magnification. Different staining procedures are available. Presence of seminal fluid can negatively influence the quality of the preparation after staining.

Antisperm antibodies

The spermatozoon evokes an immune response when exposed to the systemic immune defense system. Therefore, trauma, e.g. vasectomy, in the male genital apparatus or inflammatory reactions in the male or female genital tracts can evoke the production of sperm-directed antibodies. Depending on the nature and the location of the sperm antigen and on the concentration of antibodies, different effects can be seen:

1. Agglutination. The effect can be seen as agglutinates of moving sperm in the semen sample. (Fig. 3).
2. Cytotoxic effect. With serum (i.e. with active complement) sperm will be killed.
3. Other effects as hampering passage through cervical mucus, and zona binding and passage

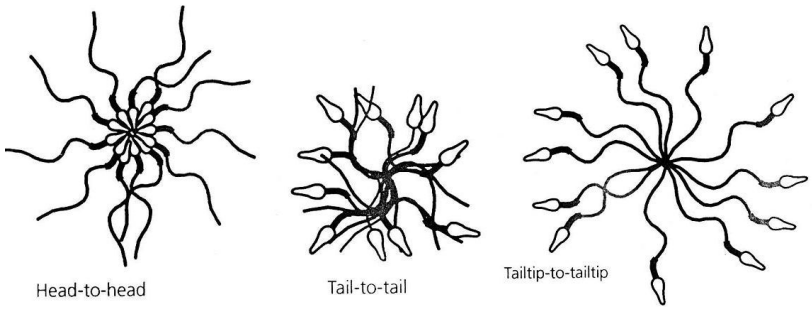

Figure 3: Agglutination patterns of human spermatozoa.

SpermMAR tests

This test uses latex particles coated with human Ig. Normally when sperm are mixed with these latex particles nothing happens. However, in the presence of anti-human Ig, there are two possibilities. If the sperm do not have antibodies on their surface they will be seen swimming without attached particles, whereas the particles (which do have antibodies on their surface) will be clumped together due to the antiserum. In contrast, if the sperm have antibodies on their surface, the anti-human Ig will bind together the Ig localized on the particles and the sperm. Motile sperm, swimming with attached particles, will then be seen.

Immunobead test

Antibodies bound to the human sperm surface can be visualized by other antibodies which have been raised against human IgG, IgA or IgM immunoglobulin molecules. Immunobeads are plastic particles with attached anti-human Ig antibodies. Thus, anti-IgG, -IgA or -IgM immunobeads detect sperm with anti-sperm antibodies of the IgG, IgA and IgM isotypes respectively.

Sperm preparation methods

For clinical procedures such as IUI or IVF, as well as for laboratory tests of sperm fertilizing ability, spermatozoa must be separated from the seminal plasma after ejaculation.

Many techniques for selection of spermatozoa have been described. The most commonly used procedures are the “swim-up” and “density gradient centrifugation”. These procedures are explained in detail.

Wash & Swim-up procedure

This method selects the spermatozoa based on motility, ideally selecting only vital spermatozoa. Swim-up from semen is the original sperm preparation method for selecting a motile subpopulation intended to replicate the migration of spermatozoa into cervical mucus in vivo. Aliquots of semen are taken as soon as the sample is liquefied and placed in round-bottomed tubes underneath a layer of medium. (Fig. 4).

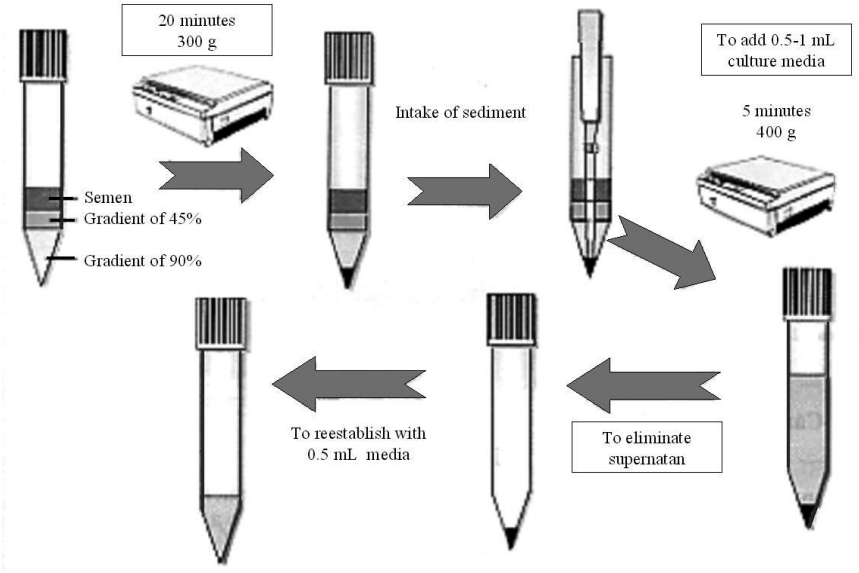

Figure 4: Wash & Swim-up technique

Density Gradient

Density gradient methods separate spermatozoa based upon their specific gravity or density (Fig. 5). Under centrifugal force the cells reach their equilibrium position on the density gradient (isopycnic point). Usually, discontinuous rather than continuous gradients with 2- or 3 layers are used. Besides density of the layers, centrifugal force and time of centrifugation influence the number of sperm cells that can be recovered from a given semen sample.

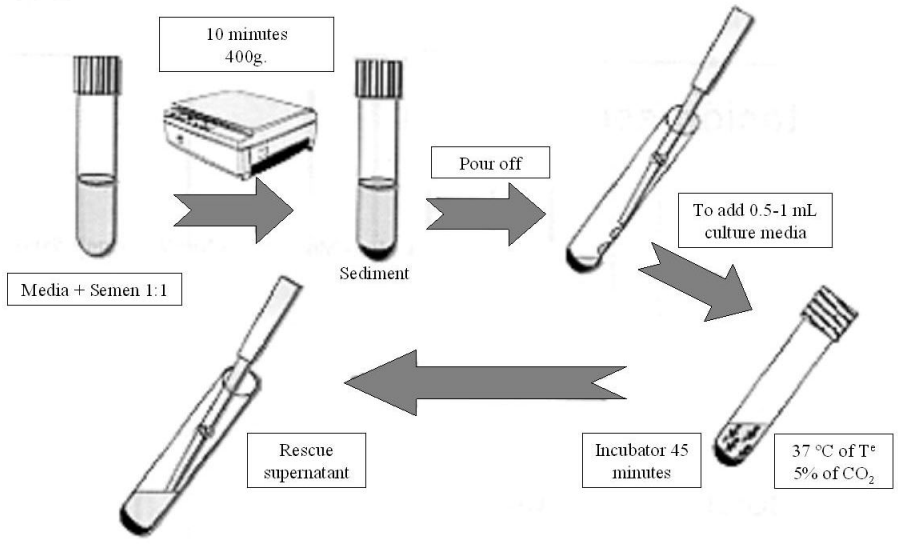

Figure 5: Density gradients

A centrifugation time of 20 minutes is commonly used. In case of a 2-layer gradient, the following combinations of gradients are often used: 95-45 % or 80-40 %.

References

1. WHO (1987), (1992), (1999) Laboratory Manual for the Examination of Human Semen. Cambridge University Press, Cambridge, U.K.
2. Kvist, U; and Björndahl, L. (2002). ESHRE Monograph: Manual on Basic Semen Analysis. Oxford University Press. 1-24.
3. Mortimer, D; and Mortimer, S.T. (1999). Laboratory investigation of the infertile male. The Bourn Hall Guide to Clinical and Laboratory Practice. Ed. P.R. Brinsden. Partenon Publishing. 53-81.
4. Gil-Salom, M; Romero, J; Mínguez, Y; Molero MªD; Remohí, J; Pellicer A. (1998). Testicular Sperm Extraction and Intracytoplasmic Sperm Injection: a Chance of Fertility in Nonobstructive Azoospermia. The Journal of Urology. Vol. 160. 2063-2067.
5. De Sousa, R; Molero, Mª-D; Remohi J. (1999). Inseminação Artificial com Sêmen Homólogo. Cap. 10. En: Manual Prático de Reprodução Humana. Eds. Rodriguez, L; Bonilla, F; Simón, C; Remohí, J. Revinter. 75-87
6. Sellés, E; y Cols. (2005). Capacitación Espermática. Cap. 22. En: Manual Practico de Esterilidad y Reproducción Humana. Eds. Remohí, J; Cobo, A; Romero, JL; Pellicer, A; Simón, C. 2ª Ed. McGraw-Hill·Interamericana. 283-287.

Dataprocessing made simple

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1. About the Author

When I started working at the fertility centre in 1985, the only computer there was an Apple IIe I designed several databases with FileMaker Pro, but we also had databases running in Dbase III and FoxPro. You can understand what trouble we had if we wanted to get the right combined data out of these various databases.

We now have a database in FileMaker that covers the whole range of our activities, from the intake of the patient, triggering of OPU, evaluation of the embryos up to the follow up of the children. I'm now rewriting everything for FileMaker 7 and adding some more requested features.

2. Why FileMaker

We looked at several database systems before we selected FileMaker. Of course I had already some experience with FileMaker, but other collegues of the centre also designed their own databases using Access, FoxPro and DbaseIII. So why to choose FileMaker over these other systems? Because of the easy way that FileMaker gives you access to their building tools and because you don't have to learn a programming language. FileMaker pro have its own scripting tools, but in the end it is just "point and click". Of course you have to invest time to learn to design a database, however this becomes a second nature in no time. And even when you find this hard, you can get pretty far without ever making one single script.

For those who want some more information, I can tell you that it is a relational database with interoperability for exchanging data via open standards (SQL, ODBC, JDBC, XML). So you can connect it to a large database system in your hospital without problem (the only problem is that you must get the approval of your IT department J). It is a cross-platform system which can be run on Citrix systems.

3. How to develop in FileMaker

This is not different than in other systems. You have to be prepared: What is my goal? What needs to be registered for what purpose? Who may register what and do I have to deploy my solution over the company network. What about security?

The problem

Let's say that your boss wants to do a study. He asks you to collect the data and later he want to publish his findings. He wants to do a study comparing agonist and antagonists. In the antagonist arm he wants to compare starting day 6 of stimulation versus starting day 1 of stimulation with the antagonists. He wants 20 patients in each arm, which makes 60 patients in total.

How to tackle the problem?

1. The needed fields

- Name
- First Name
- Patients File number (the patient unique registration number)
- Type of patient (agonist, antagonist D1, antagonist D6)
- Date start treatment (LMP or day start suppression)
- Date start stimulation
- Date start antagonist (not needed for agonist)
- Total dose hMG in units
- Length stimulation in days
- Date of ovum pickup
- Number of COC
- Number transferred
- Number frozen
- Outcome

Of course you can gather of lot more data but for this example it is enough. We will also make a database with one file. We will not tackle the relational capabilities. This would get us to far for an introduction. Maybe next year?

2. Type of fields

You have several types of fields at your disposal.

Field type	Data type	Information
Text	Can hold 2GB of information	Sorting is alphabetical
Number	Stores up to 800 digits	Sorts as numbers
Date	Gregorian calendar 1/1/0001 to 31/12/4000	Use 4-digit years as good practice!
Time	Hours, minutes and seconds	Sorting by 24 hour clock
Timestamp	Combines date and time	Example: 28/02/2005 2:00 PM
Container	Holds all binary information Image, sound , movie, pdf file,...	No sorting of course
Calculation	Stores the result of a formula based on other fields in your system	End result can be text, number, date, time or container
Summary	Returns information on the current found set of records	Examples are average, maximum, ...

The result for our fields is:

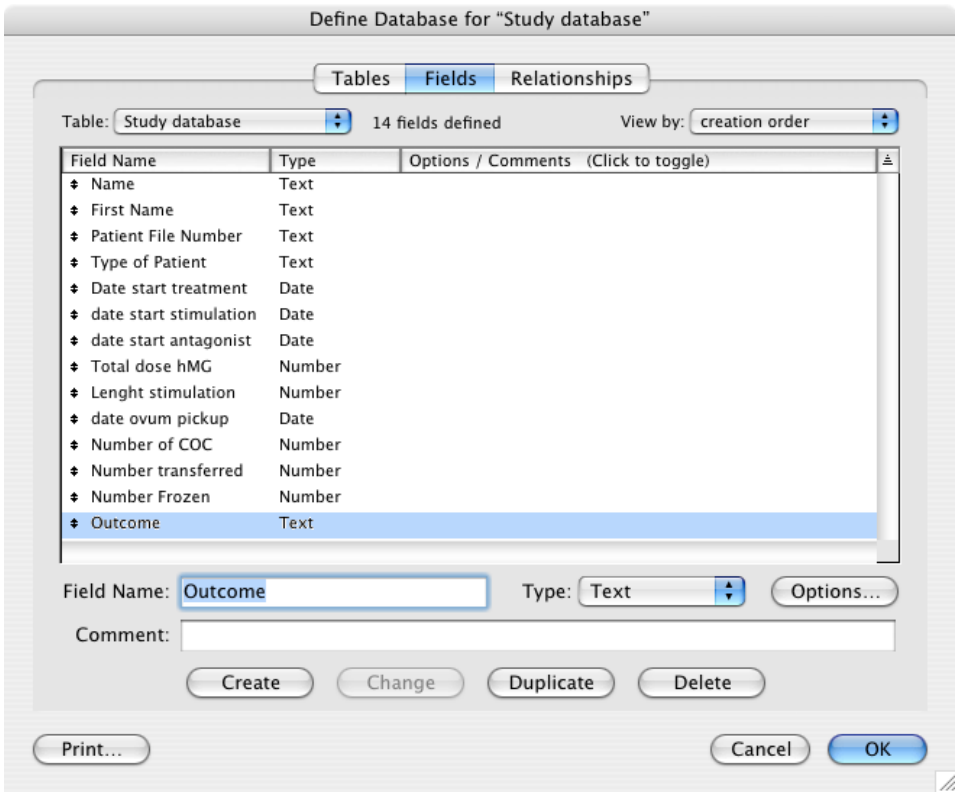
- Name: text field
- First Name: Text field
- Patients File number: can be text or a number field depending on the system of the hospital
- Type of patient: text field
- Date start treatment: date field
- Date start stimulation: date field
- Date start antagonist: date field
- Total dose hMG: number field
- Length stimulation in days: number field
- Date ovum pickup: date field
- Number of COC: number field
- Number transferred: number field
- Number frozen: number field
- Outcome: text field

3. Defining your database

Launch FileMaker Pro 7 by double clicking the FileMaker Pro icon on the desktop.

FileMaker will display a dialog. You can begin working with one of the templates or create a new empty file. Choose “Create a new empty file”. Click “OK”. A new dialog appears. Give the database a name and choose a location to save your database.
Name it “Study database.fp7” and save it to the desktop.

A define field dialog lets you enter the fields we need for the database. Enter the field one by one in the dialog; choose for each field the type of field and click create. When every field is entered it should look something like the dialog below.



When you make a mistake in a field, enter the new name and then click “change”.

You will see that there is a “options” button. This is field-dependant. It will change with every field type. Here you can make the database system enter default values, make a list of values to use with the field (example: the “Type of Patient” could hold a list that lets the user choose between “Agonist – Antagonist D1 – Antagonist D6”). By introducing such a list you limit what the user can enter and the consistency of your data will be better. You can also limit the input on a number field. If you don’t want to enter more than 3 in the field “Numbered transferred”, you can do this with in the options for that field.

When you are done click “OK”. If for some reason you need to make corrections in the define database dialog, you can enter it again by choosing: File->define->database

You should now be in “Browse” mode. This means you are ready to enter data in your database. Now try to enter some data into the database.

4. Some basic functions

- To create a new record: Records->New Record
- To find records: view->find mode
- To delete a record: Records->delete record
- To sort: Records->Sort Records…
- To change the layout: View->layout mode
- To have an excel-like view: View->Table mode

5. Homework

Now that you have created a database, you are going to change the layout so that you can make your database more attractive. Try to match the layout to the one on the next page. We will go into detail during the workshop in Copenhagen.

Go in to Layout mode and use the tools from the toolbar. Make the buttons  You don’t have to make scripts.

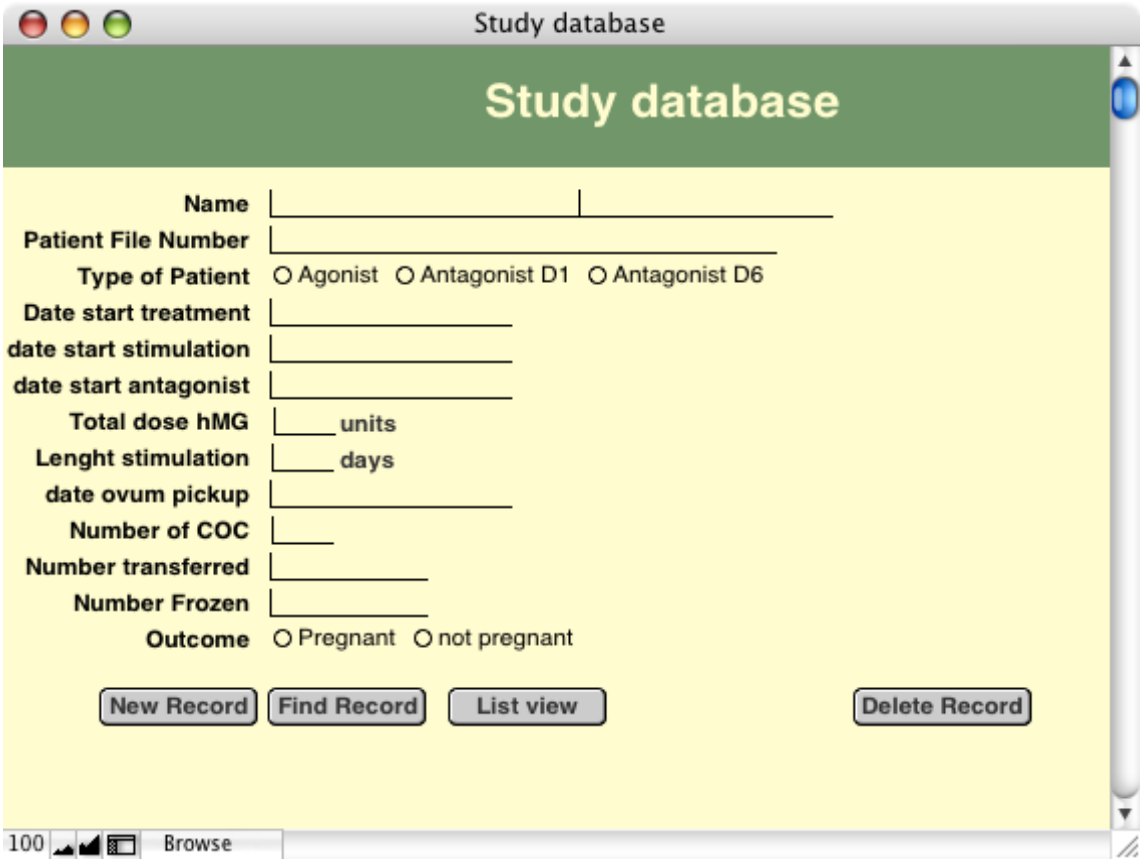
References

If you want to know more about FileMaker:

- Using FileMaker Pro 7: the only FileMaker 7 book you need. Steve Lane, Bob Bowers, Scott Love, Chris Moyer. Que 2005

- Website of John Mark Osborne: free tips and resources, http://www.databasepros.com/

- FileMaker Advisor: A great magazine about FileMaker http://filemakeradvisor.com/



Happy FileMaking!