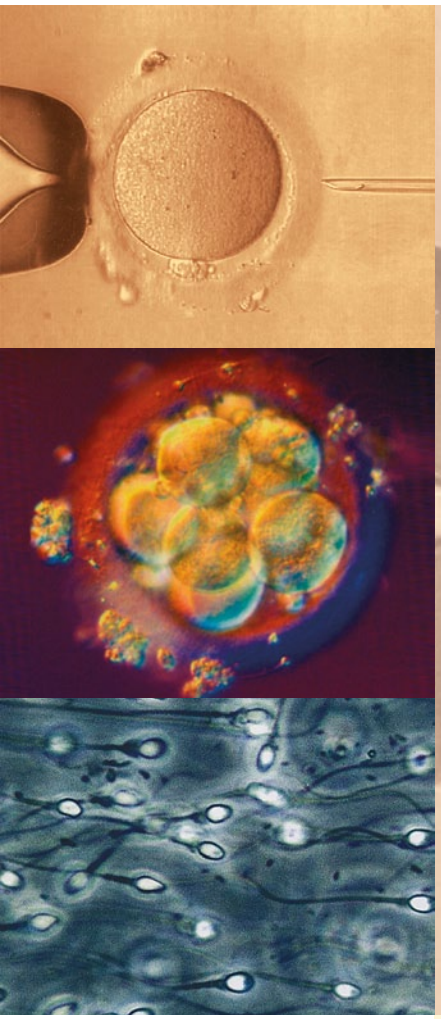
Journal für

Reproduktionsmedizin und Endokrinologie

– Journal of Reproductive Medicine and Endocrinology –

Andrologie • Embryologie & Biologie • Endokrinologie • Ethik & Recht • Genetik
Gynäkologie • Kontrazeption • Psychosomatik • Reproduktionsmedizin • Urologie



Carl Djerassi and the World Health Organisation Special Programme of Research in Human Reproduction

Benagiano G, Merialdi M

J. Reproduktionsmed. Endokrinol 2011; 8 (Sonderheft 1), 10-13

Offizielles Organ: AGRBM, BRZ, DVR, DGA, DGGEF, DGRM, DIR, EFA, OEGRM, SRBM/DGE

Indexed in EMBASE/Excerpta Medica/Scopus

Krause & Pachernegg GmbH, Verlag für Medizin und Wirtschaft, A-3003 Gablitz

Journal für

Pneumologie

e-Abo

Datenschutz:

Ihre Daten unterliegen dem Datenschutzgesetz und werden nicht an Dritte weitergegeben. Die Daten werden vom Verlag ausschließlich für den Versand der PDF-Files des Journals für Pneumologie und eventueller weiterer Informationen das Journal betreffend genutzt.

Lieferung:

Die Lieferung umfasst die jeweils aktuelle Ausgabe des Journals für Pneumologie. Sie werden per E-Mail informiert, durch Klick auf den gesendeten Link erhalten Sie die komplette Ausgabe als PDF (Umfang ca. 5–10 MB). Außerhalb dieses Angebots ist keine Lieferung möglich.

Abbestellen:

Das Gratis-Online-Abonnement kann jederzeit per Mausklick wieder abbestellt werden. In jeder Benachrichtigung finden Sie die Information, wie das Abo abbestellt werden kann.

Das e-Journal Journal für Pneumologie

- ✔ steht als PDF-Datei (ca. 5–10 MB) stets internetunabhängig zur Verfügung
- ✔ kann bei geringem Platzaufwand gespeichert werden
- ✔ ist jederzeit abrufbar
- ✔ bietet einen direkten, ortsunabhängigen Zugriff
- ✔ ist funktionsfähig auf Tablets, iPads und den meisten marktüblichen e-Book-Readern
- ✔ ist leicht im Volltext durchsuchbar
- ✔ umfasst neben Texten und Bildern auch eingebettete Videosequenzen.

www.kup.at/pneumologie

Carl Djerassi and the Word Health Organisation Special Programme of Research in Human Reproduction

G. Benagiano, M. Merialdi

Many hormonal contraceptives appropriate for use in Developing countries are not of interest to major Western, international pharmaceutical companies. For this reason, in the mid-seventies the World Health Organisation, in response to great demand for improved long-acting injectable hormonal contraceptives, particularly in Africa and Asia, sponsored a chemical synthesis programme of long-acting progestational and androgenic steroids with Carl Djerassi as one of its main leaders.

Almost 300 esters of norethisterone, levonorgestrel and testosterone were prepared by this programme in university-based research laboratories situated mainly in developing countries and then screened by the US National Institutes of Health in animal models.

This paper describes the overall strategy of this research and development activity and the philosophy that served as an engine for a unique non-profit collaborative work.

Among progestins three compounds, levonorgestrel-butanoate, cyclopropylcarboxylate and cyclobutyl-carboxylate, proved to be particularly long-acting when administered as microcrystalline suspensions, although – for financial reasons – only one, levonorgestrel-butanoate is being further developed.
J Reproduktionsmed Endokrinol 2011; 8 (Special Issue 1): 10–3.

Key words: WHO, Carl Djerassi, human reproduction

Introduction

Among Carl Djerassi's many contributions to science, probably the least known is the work he carried out with the oldest and best known research programme ever sponsored by the World Health Organisation (WHO): "The Special Programme of Research, Development and Research Training in Human Reproduction", commonly known as HRP.

As recounted by Kessler [1], WHO's involvement in the field known today as "Reproductive Health", dates back sixty years when, in 1951, the Government of India requested assistance from the Organisation's Regional Bureau for South-East Asia to conduct pilot studies on the Rhythm Method of family planning in urban and rural areas [2]. This was not an easy decision, since at the time the very idea of family planning (let alone that of birth control) was rejected by many of its Member States, with arguments such as that "family planning will result in ageing of the population and cause a decrease in productivity" [3]. For this reason, it took a decade before the topic of family planning would be brought into WHO's agenda through an extensive review of research needs. Named *Biology of Reproduction*, the document reporting this review unfortunately remained unpublished, although it set the tone for all later developments and was instrumental for the appointment, in 1963, of the first staff member charged exclusively with research in reproduction. Finally, in December 1965 a separate Human Reproduction Unit was created within the Division of Family Health. Five years later, WHO convened a meeting on the "Promotion of research in reproductive biomedicine including fertility control". This meeting identified a number of obstacles to the development of new fertility-regulating methods; notable among them an insufficient knowledge of reproductive processes; difficulties in interfering with physiological reproductive functions without causing adverse effects; the scarcity of scientists working in the area (with almost none in developing countries); lack of interest by the pharmaceutical industry with an ensuing paucity of funds. The most important decision reached during the meeting was to conduct a feasibility project to create an "Expanded Programme of Research, Development and Research Training in Human Reproduction" [1]. When, in 1971, this expanded programme was formally established, one of its objectives was the creation of Task Forces for mission-oriented research aimed at creating new methods for the control of human fertility. One of these Task Forces, created in 1973, dealt with long-acting agents for the regulation of human fertility [4].

The Task Force on Long-Acting Agents for the Regulation of Human Fertility

When the new Task Force initiated its activities, the group of scientists involved in its work quickly realised that only two long-acting formulations were available for national and international programmes: the microcrystalline suspension of 17α-acetoxy-6α-methyl-pregn-4-ene-3,20-dione, known with the acronym DMPA, or depot-medroxy-progesterone acetate (administered at a dose of 150 mg), and an oily solution of 19-norpregn-4-en-20-yn-3-one,17α-[(1-oxoheptyl)oxy], known with the acronym NET-EN or norethisterone enanthate (administered at a dose of 200 mg). To properly evaluate these two long-acting agents (commercially known as Depo Provera and Noristerat), the Task Force initiated a series of multicentre, multinational, comparative trials. These studies proved that the duration of action of DMPA is 3 months, or even longer, whereas that of NET-EN is 2 months [5–8]. At the same time, it became clear that both agents had shortcomings, especially in terms of bleeding patterns. In

Received and accepted: February 24, 2011
Correspondence: Professor Giuseppe Benagiano, Department of Reproductive Health and Research, World Health Organisation, Geneva, Switzerland; e-mail: Giuseppe.Benagiano@uniroma1.it

addition, DMPA came under strong criticism by consumer groups that considered its use dangerous for the health of women. Although these objections were carefully analysed and disproven [9], a number of Governments and Family planning programme administrators felt that there was a need for additional formulations having better characteristics and a duration of action of up to 6 months. As already mentioned, there was a complete lack of interest by the pharmaceutical industry in this area of research and this reality made it both desirable and necessary to find a new avenue for the development of such substances.

Therefore, the Task Force decided to initiate a programme for the chemical synthesis and screening of a large number of steroid derivatives, with the aim of developing several long-acting formulations (monthly, three-monthly, and six-monthly) for use both in females and males. To organise the work, a preparatory expert meeting was organised in 1975 to look into the feasibility of creating a WHO-sponsored Chemical Synthesis Programme (CSP). It goes without saying that Carl Djerassi was among the scientists invited to this meeting and he quickly became the engine moving the entire synthetic work.

The WHO Chemical Synthesis Programme (CSP)

As it is easily understood, the development of new injectable contraceptive compounds requires a concerted effort to synthesise novel long-acting steroid derivatives, screen them and carry out animal biological evaluation to determine which compound would be suitable for testing in the human. This type of work had never been carried out outside the system created by pharmaceutical companies and it was not at all sure that it could be carried out by the newly proposed WHO programme. This question was posed during the already mentioned preparatory meeting held in 1975 and the answer given by participants was positive for two main reasons: first, it was felt that new compounds were needed and, second, participants were convinced that the synthetic work could be combined with an institution building exercise in developing countries. Indeed, this programme could conceivably serve as a model for other drug development efforts aimed at obtaining compounds outside the traditional pharmaceutical industry mechanism.

The preliminary work carried out by the Task Force-promoted CSP has been described in an article in *Science* by Crabbe, Diczfalusy and Djerassi [10] where it was pointed out that the development of drugs for tropical parasitic diseases such as leishmaniasis, schistosomiasis, and onchocerciasis might follow the same path.

Under the guidance of Carl Djerassi, a group of internationally recognised steroid chemists with past or current experience in the pharmaceutical industry attended a meeting held under WHO auspices at Stanford University. Besides Carl Djerassi, then professor at the Department of Chemistry, Stanford University, Stanford, CA, USA, the following attended: P. Crabbe (Department of Chemistry, University of Missouri, Columbia, MO, USA) who became the Coordinator of the CSP; S. Archer (School of Science, Rensselaer Polytechnic Institute, Troy, NY, USA); G. Benagiano (representing the WHO Secretariat in Geneva); E. Diczfalusy (Karolinska Institute, Stockholm, Sweden); J. Fried (Department of Chemistry, University of Chicago, Chicago, IL, USA); T. Higuchi (Department of Pharmaceutical Chemistry, Kansas University, Lawrence, KA, USA). This group compiled an initial list of approximately 150 hypothetical steroid derivatives that they believed could be synthesised. They also proposed 15 laboratories for participation in the CSP. After a site visit by WHO staff and consultants, laboratories in Australia, Brazil, Bulgaria, the (then) German Democratic Republic, Iran, Israel, Mexico, Nigeria, Poland, Singapore, Spain, and Sri Lanka were selected; each laboratory was supplied with the appropriate literature material and chemicals and funded with sums between US$ 10,000 and US$ 15,000. It was stipulated that patent rights would remain with WHO and a requirement was made that they were to provide a minimum of 5 g of the purified steroid derivatives assigned to them.

Design of the Programme

From the outset it was decided that, to expand the number of long-acting agents, rather than synthesise novel steroid moieties, the CSP should aim at designing new injectable derivatives of well-known steroids. Three target areas were selected [11]:

- 3–6 months progestin-only injections
- monthly oestrogen-progestin injections
- long-acting testosterone-progestin combinations.

Besides testosterone, 2 well-known progestin moieties known to be effective as contraceptives and to be safe in human use were selected: norethisterone and levonorgestrel.

It was also decided that to prolong the duration of action of these three steroids, the chemically active compound was to be transformed into a "prodrug" that would slowly release the active moiety. To this aim, the free 17-hydroxyl group was to be esterified by inserting an appropriate acid chain; then, after administration, enzymatic hydrolysis would convert the "prodrug" into an active contraceptive agent [10]. Obviously, the rate of hydrolysis would become the criterion to determine whether a compound would be suitable as a long-acting agent.

The coordinating group of scientists decided the nature of the new ester chains to be prepared on the basis of several criteria: first and foremost, a high lipoid solubility leading to accumulation in the body fat. The group was convinced that by a judicious choice of the ester chain, e.g. aliphatic, alicyclic or aromatic substituents, or some suitable combination, release characteristics of the desired type might be obtained. They were also convinced that formulation might exert an overriding influence on the duration of action of individual compounds.

A preliminary review of the literature furnished by Schering AG, Berlin (now part of Bayer AG) indicated that a variety of 17-steroid esters and ethers had already been reported, such as normal fatty acid ester chains from C_2 to C_{12}, laurate, adamantylcarboxylate, bis-succinate, cyclopentylpropanoate, cyclohexylcarboxylate, phenylpropanoate, p-alkoxyphenylpropanoate, polyphosphate, cyclopentyl ether, cyclooctyl ether, tetrahydropyranyl ether, etc. Therefore the synthetic work was directed only to esters not previously described.

The first parent progestin selected was norethisterone since there was considerable laboratory and clinical experience with it and it was no longer protected by patents. Levonorgestrel was subsequently included because of its high progestational potency, although it was later found that esterification was more difficult with this steroid.

As detailed by Crabbe, Diczfalusy and Djerassi [10], after completion of the synthetic work, the samples were submitted to quality control, purification whenever necessary and formulation. The steroid esters were then submitted for bioassay to the Contraceptive Development Branch of the Center for Population Research, US National Institute of Child Health and Human Development (NICHD), where the biological evaluation of all progestational compounds was carried out using the suppression of oestrus in female rats, using DMPA and NET-EN as standards for comparison. Tests for prolonged androgenic activity were carried out in castrated male rats, using as the measure of biological activity the weight increase of the ventral prostate. In this case testosterone enanthate was used as a standard. Finally, the most promising derivatives were tested in subhuman primates.

Synthesis of Long-Acting Derivatives

The 12 laboratories participating in the CSP produced 213 esters and some 10 ethers of norethisterone and levonorgestrel. With regard to the androgen derivatives, some 72 testosterone esters were prepared. Each centre was given a list of 16 to 20 ester structures, and was invited to submit a research proposal. As expected, each centre had its own specific problems, ranging from inexperienced personnel, to lack of sophisticated equipment and instruments, lengthy regulations imposed on import of chemical reagents and small equipment items and even frequent power failures. In this respect, the direct involvement of the WHO secretariat proved to be critical in solving these contentious issues by ordering directly the chemicals and shipping them through WHO international channels.

Thus, the organisation of such a network proved to be difficult and the coordinator of the CSP had to act as a 'general trouble shooter'; in the long run however, this was the only way to organise such a unique programme. Technically, the work was challenging and presented numerous difficulties since most of the acid chains encompassing over 100 different chemical structures, had not been described in the chemical literature. A particularly difficult task was that of achieving the required high purity (about 99%) [10].

Progress in the work was carefully monitored and *ad hoc* consultations took place in November 1977 and in January 1979 in Geneva and in January 1980 in Jena, then in the German Democratic Republic.

The synthetic and screening work, as well as some aspects of the quality control, formulation, studies of the rate of enzymatic hydrolysis and the biological evaluation in animals of the progestin derivatives, has been fully reported in an *ad hoc* issue of the international journal *Steroids* [12]. Compounds were tested either as oily solutions or aqueous microcrystalline suspensions. In general, levonorgestrel esters were usually longer acting than the norethisterone esters and aqueous suspensions were better than oily solutions, although the duration of action was highly dependent on the crystal size of these aqueous suspensions.

From all data collected it was concluded that the biological activity of the levonorgestrel esters increases from formate to butanoate and then decreases slowly when the chain becomes longer. In the cyclic esters the activity increases up to the cyclobutyl ring and then decreases with larger rings. In addition, the introduction of substituents on the cyclopropyl and cyclobutyl rings leads to less active compounds [11].

Interestingly, overall there was a lack of correlation between structure or in vitro hydrolysis rates and biological activity; this seems to indicate that the duration of action results from the chemical nature of the side chain, the formulation (e.g. particle size, vehicle), as well as the intrinsic properties of the steroid nucleus.

Further Work With Selected Progestin Esters

Several compounds proved to be longer acting than DMPA and NET-EN, three being of particular interest: levonorgestrel butanoate, cyclopropyl-carboxylate and cyclobutyl-carboxylate. Their duration of action in the rat oestrus suppression test, when given at a dose of 16 mg in aqueous microcrystalline suspension, was longer than 91 days; in addition, one fourth of the dose of these three esters gave a similar duration of activity as DMPA.

Pharmacokinetics data in the rhesus monkey of both the levonorgestrel cyclobutyl-carboxylate and butanoate have shown that after intramuscular injection of 20 mg of either compound, measurable levels of levonorgestrel (1–2 ng/ml) are found in the circulation more than 100 days after injection, although the cyclopropyl-carboxylate gave rise to a far shorter period in which measurable levels of levonorgestrel could be observed [13].

For financial reasons, in the nineties, the WHO programme decided to proceed with only one of these compounds – levonorgestrel butanoate (LNG-B): this ester can provide contraceptive protection at a dose of 5–10 mg every three months and would therefore impose a lower body burden than DMPA, possibly resulting in less ovarian suppression, less amenorrhoea and a more rapid return of fertility. Because it is easy to synthesise, production costs are anticipated to be low, and a favourable public sector price can be negotiated. During 1996, based on the results of pharmacokinetic/pharmacodynamic studies, a dose of 12.5 mg of a micronized formulation of LNG-B was tested in comparison with 200 mg of NET-EN, each given in a two-monthly regimen. Both drugs were found to be equally effective. Both induced steroid-related side-effects, but at one year the average weight gain of users was greater with NET-EN and the vaginal bleeding patterns observed with LNG-B tended to be less disturbed than with NET-EN. Subsequent studies in cynomolgus monkeys found that with larger particles, peak blood levels are reduced, the duration of action is increased and the overall steroid dosage required for three months of contraceptive coverage is reduced. An optimal particle size was determined and a manufacturing process was established. Pharmacokinetic/pharmacodynamic studies in women suggested that the final dosage required for

three months of contraceptive coverage will be 10 mg or less. Based on these results, an Investigational New Drug Application was filed with the United States Food and Drug Administration (USFDA) for this compound [13]. In parallel with these studies, the programme has also investigated its potential in combination with an androgen ester (testosterone buciclate), as a method for male contraception. Progress however came to a halt in the year 2000 because of difficulties in obtaining supplies for clinical trials. Commercial companies involved in this work insisted that WHO provide indemnification against any and all third party claims, a responsibility the Organisation could not assume [14]. Fortunately, in 2003, representatives of WHO, NICHD, the Contraceptive Research and Development Programme known with the acronym CONRAD, the Concept Foundation, a non profit entity dedicated to fertility regulation and representatives from academia and industry unanimously agreed to resume activities with LNG-B. In 2004, a confidentiality agreement was signed between WHO, NICHD and CONRAD for a collaborative effort to further develop this compound and during 2006, extensive reevaluation of the physical and chemical properties has been performed [15]. At present CONRAD is working with NICHD in the final stages of manufacturing a clinical batch with a new formulation for a clinical study to be started in the spring of 2011 by NICHD

Lessons to be Learned

In the foreword to the issue of *Steroids* reporting the results of CSP, the then Editor Albert Segaloff pointed out that the complexity of the world in which we live makes it exceedingly difficult to introduce new chemical entities as drugs. He went on acknowledging that *"WHO has put together a truly excellent international effort to project, synthesise a large series of esters of these two progestational agents and then test them for purity and their duration of action in animals"*. Segaloff concluded: *"It is my belief that this has been an outstanding effort in cooperation and gives me great pleasure to present in this issue of Steroids a complete summary of their effort. It is to be hoped that these newly developed long-acting esters will prove to be equally effective in their clinical trials!"* [12].

For sure the WHO-sponsored CSP has become an example of how a multinational cooperative non-profit project in drug chemical synthesis can be established outside traditional pharmaceutical channels. It is a model of particular relevance to the developing world, especially in areas where, for whatever reason, the pharmaceutical industry shows no interest, such is the case of tropical diseases and pesticides designed specifically for tropical pests.

Obviously, not everything was rosy: the CSP has taken longer than it would have if compounds were to be synthesised in the steroid laboratories of a large pharmaceutical firms; also the subsequent processing was slower and had to be stopped for years for lack of financial resources. At the same time, in terms of direct funding from the WHO, the NIH and, more recently, CONRAD, the programme has proved to be much cheaper.

In conclusion, to use the words of Carl Djerassi and the two other Coordinators of the CSP: "The WHO program illustrates how a multinational cooperative project in drug chemical synthesis can be established outside the traditional pharmaceutical channels – a model that is of particular relevance to lesser developed nations".

Conflict of Interest

There is no conflict of interest regarding this paper.

References:

1. Kessler A. Establishment and early development of the programme. In: Khanna J, Van Look PFA, Griffin D (eds). Reproductive Health: A key to a brighter future. World Health Organisation, Geneva, 2000; 43–59.

2. World Health Organisation. Final report on pilot studies on family planning. Vol. I and II. WHO Regional Office for South-East Asia, New Delhi, 1954.

3. World Heath Organisation. Work of the 5th World Health Assembly: Population issues. WHO Chron 1952; 6: 183–4.

4. Benagiano G. Twenty five years of changing perceptions: from population policies to reproductive health; celebrating 25 years of HRP. In: Kovács L, Resch BA (eds). Research on Human Reproduction. Albert Szent-Györgyi Medical University Press, Szeged, Hungary, 1998; 36–46.

5. WHO Task Force on Long-Acting Systemic Agents for the Regulation of Fertility (Benagiano G, Diczfalusy E, Goldzieher JW, Gray R, Apelo R, Bergstein N, de Souza JC, Devi PK, Kesserü E, Koetsawang S, Ojo OA, Gupta AN, Pretnar A, Toppozada HK). Multinational comparative clinical evaluation of two long-acting injectable contraceptive steroids: norethisterone oenanthate and medroxy-progesterone acetate: 1. Use-effectiveness. Contraception 1977; 15: 513–33.

6. WHO Task Force on Long-Acting Systemic Agents for the Regulation of Fertility (Benagiano G, Diczfalusy E, Diethelm P, Goldzieher JW, Gray R, Apelo R, Bergstein N, de Souza JC, Devi PK, Kesseru E, Koetsawang S, Ojo OA, Gupta AN, Pretnar A, Toppozada HK). Multinational comparative clinical evolution of two long-acting injectable contraceptive steroids: norethisterone oenanthate and medroxyprogesterone acetate. 2. Bleeding patterns and side effects. Contraception 1978; 17: 395–406.

7. WHO Task Force on Long-Acting Systemic Agents for the Regulation of Fertility (Gray RH, Diethelm P, Benagiano G, Annus J, Toppozada HK, Koetsawang S, Aimakhu VE, Khan T, Pretnar A, Chatterjee TK, Molitor-Pfeffer MP, Apelo R, Lichtenberg R, Crosignani PG, De Souza JC, Gomez-Rogers C, Haspels AA) Multinational comparative clinical trial of long-acting injectable contraceptives: norethisterone enantathe given in two dosage regimens and depot-medroxyprogesterone acetate. A preliminary report. Contraception 1982; 25: 1–11.

8. WHO Task Force on Long-Acting Systemic Agents for the Regulation of Fertility (Toppozada HK, Koetsawang S, Aimakhu VE, Khan T, Pretnar-Darovec A, Chatterjee TK, Molitor-Pfeffer PM, Apelo R, Lichtenberg R, Crosignani PG, de Souza JC, Garcia Huidobro M, Haspels AA, Annus J, Benagiano G, Diethelm P, Gray RH). Multinational comparative clinical trial of long-acting injectable contraceptives: norethisterone enantathe given in two dosage regimens and depot-medroxyprogesterone acetate. Final report. Contraception 1983; 28: 1–20.

9. Benagiano G, Fraser I. The depo-provera debate: commentary on the article "Depo-Provera, a critical analysis". Contraception 1981; 24: 493–528.

10. Crabbe P, Diczfalusy E, Djerassi C. Injectable contraceptive synthesis: an example of international cooperation. Science 1980; 209: 992–4.

11. Crabbe P, Archer S, Benagiano G, Diczfalusy E, Djerassi C, Fried J, Higuchi T. Design of the WHO Chemical Synthesis Programme. Steroids 1983; 41: 243–54.

12. Steroids 1983; 41, entire March 1983 issue; 242–425.

13. Special Programme of Research, Development and Research Training in Human Reproduction. Annual Technical Report 1996. World Health Organisation, Geneva, 1997; 41–3.

14. Department of Reproductive Health and Research. Annual Technical Report 2000. World Health Organisation, Geneva, 2001; 103.

15. Department of Reproductive Health and Research. Biennial Technical Report 2005–2006. World Health Organisation, Geneva, 2007; 32–3.

Mitteilungen aus der Redaktion

Besuchen Sie unsere Rubrik

☑ Medizintechnik-Produkte

Neues CRT-D Implantat
Intica 7 HF-T QP von Biotronik

Artis pheno
Siemens Healthcare Diagnostics GmbH

Philips Azurion:
Innovative Bildgebungslösung

Aspirator 3
Labotect GmbH

InControl 1050
Labotect GmbH

e-Journal-Abo

Beziehen Sie die elektronischen Ausgaben dieser Zeitschrift hier.

Die Lieferung umfasst 4–5 Ausgaben pro Jahr zzgl. allfälliger Sonderhefte.

Unsere e-Journale stehen als PDF-Datei zur Verfügung und sind auf den meisten der marktüblichen e-Book-Readern, Tablets sowie auf iPad funktionsfähig.

☑ Bestellung e-Journal-Abo