Voor mijn ouders

CHAPTER 1

Introduction

Since the discovery and marketing of the first combined oral contraceptive (COC) in early 1960s, hormonal contraceptives have proven very useful in birth control as well as addressing a diversity of female health issues.

Although the first generation of COCs containing 50 μg or more ethinyl estradiol (EE) elicited an increased risk of cardiovascular events, contraceptive hormones were not abandoned. Their efficacy in birth control and convenience of use enabled more women to make career while raising a family. Moreover, COCs have been conveniently used worldwide for treating acne, cycle control and reducing discomfort and blood loss accompanying every menstrual bleeding. Hence, we may conclude that COCs have been affecting women's quality of life favourably for decades now.

This success story, however, does have a shady side. The history of serious cardiovascular side effects of oral contraceptives goes back just as long as that of its accomplishments and has motivated extensive basic, epidemiological and clinical research on this field. Great efforts have been made to clarify the pathophysiology of COC-induced cardiovascular disease, identify subgroups at risk, investigate the actual absolute risks involved and identify surrogate markers for cardiovascular disease which should facilitate getting a drug-specific risk estimate during the pre-marketing phases of drug development.

Based on the type of blood vessels affected, cardiovascular disease caused by COCs can be divided into two categories: venous thromboembolism (VTE) and arterial disease. VTE consists of deep venous thrombosis and pulmonary embolism and is rare in healthy young women. VTE risk is elevated in a number of physiological, pathological and iatrogenic conditions such as pregnancy, puerperium, COC use, obesity, smoking, surgery, immobilization, cancer and thrombophilic disorders.

VTE incidence among healthy women of 18-49 years old is about 1 in 10.000 per year, and in users of COC of the same age 2 to 3 in 10.000 per year [1]. Of the two components each COC contains, namely an oestrogen and a progestin, the former is held responsible for the COC-related increased risk of cardiovascular side effects [2-5]. The extent of the effects of oral oestrogen on this risk has been speculated to be greater due to the first-pass of this hormone through the liver as there is evidence that plasma concentration of many coagulation and inflammation proteins synthesized by the liver are affected more intensely by oral oestrogens employed as hormonal replacement therapy [6-8].

In 1970, Inman reported a dose-dependent relationship between the VTE incidence in COC users and oestrogen dose for the first-generation preparations [9]. Consequently, EE dose was adjusted in the next generation of COCs to 20 or 30 μg. Subsequently, the extent of enhancement in risk associated with these low-dose preparations was found to be associated with the type of progestin employed [10-13]. More androgenic progestins such as levonorgestrel would counteract the unfavourable effects of EE on hepatic protein synthesis to a larger extent than the less-androgenic progestins such as desogestrel [14;15]. One of the proteins subjected to this mechanism of alteration is sex-hormone binding globulin (SHBG) [16-19]. Its increment was shown to correlate with the VTE risk associated with different types of COC [20]. Thus, it is suggested that SHBG could serve as a surrogate marker for steroid-induced risk of VTE.

Arterial disease is a collective term used for manifestations of cardiovascular disease in the coronary and systemic arteries. It is caused by atherosclerosis, a chronic low-grade inflammatory process of the arterial wall leading to formation of atheromatous plaques which eventually may either result in (partial) occlusion of the affected artery (as in myocardial infarction) or get ruptured, disseminate, and embolise downstream arterial branches (as in ischemic stroke). Among risk factors for arterial disease in general population are smoking, obesity, hypertension, diabetes, and elevated ratio of plasma total cholesterol (TC) to high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides, C-reactive protein (CRP), D-dimer and fibrinogen [21-32].

Arterial disease in women of reproductive age and otherwise healthy is rare and generally reveals itself as arterial thromboembolic events such as myocardial infarction and stroke in previously asymptomatic individuals. According to data collected in large studies, the annual incidence of myocardial infarction and stroke in this group is 0.2 to 1 and 1.1 in 10.000 women, respectively [33-36]. Some of the studies conducted in this field have found evidence of COC use being associated with a mild to moderate increased risk of arterial disease [21;36-38] while others found no additional risk unless there were cardiovascular risk factors like hypertension and smoking present [39-42]. Additional studies analysed the risk separately for different types of COCs and found a greater risk involved with use of the second-generation versus the third-generation COCs and non-users [43-46], or no difference at all [2;40;42]. Those who believe in risk modification by either all COCs or a specific type, have proposed possible pathways such as synergistic effect with hypertension, deleterious alteration of lipid metabolism and triggering or aggravating a state of low-grade inflammation [29;30;47-54].

Similar to other metabolic effects COCs exert, alterations in lipid metabolism are subjected to the oestrogenic-androgenic balance of different preparations. For instance, EE elevates HDL and triglycerides and lowers LDL. However when it is combined with an androgenic progestin some of these effects are attenuated or even reversed [18;55].

In the beginning of this century, pharmaceutical companies set about exploring a relatively new path by developing non-oral combined hormonal contraceptives (CHCs). Although they were mainly developed to eliminate the risk of missing a pill or insufficient absorption of hormones due to gastrointestinal pathology or antibiotic intake, it was anticipated that avoiding first-pass of high hormone concentrations through the liver might attenuate the related adverse effects on hepatic protein synthesis [7] and consequently reduce the risk of cardiovascular disease involved with CHCs. Moreover, continuous administration might lower the exposure of the liver to oestrogens even further by limiting the required daily EE dose [56]. Previously, non-oral delivery of sex-hormones had widely been implemented in post-menopausal women for hormone replacement therapy (HRT) and studied extensively for their effects on coagulation and lipids. In 2000, Mattson and colleagues reported that compared to oral oestradiol (E_2), intranasal E_2 does not exert metabolic adverse effects on lipids and angiotensinogen, while it is equally effective in alleviating

postmenopausal symptoms [7] and Hall collected evidence of transvaginal delivery of E_2 not affecting markers of coagulation [57]. Transdermal route was also found to affect markers of coagulation and inflammation less unfavourably, than the oral route [58-63]. Furthermore, there was already some experience acquired with administration of contraceptive hormones using a vaginal ring in the 1990s. Compared to the oral route, transvaginal administration of contraceptive oestrogen and progestins was associated with a more favourable lipid profile [64].

Another matter relevant to this topic is the extended COC use. Users often choose such extended-regimen to eliminate their cyclic bleeding, alleviate cycle-related symptoms or increase contraceptive efficacy. However, little is known about the risk of cardiovascular disease of such regimen, compared to the traditional three-weeks on one-week off intake. Since reaching new steady state takes different amounts of time for each of the affected coagulation proteins, the coagulation system could be out of balance during the initial phase of pill intake as well as the pill-free week [65]. From this point of view, it would be advantageous not to subject the coagulation system to monthly ups and downs. Consequently, continuous pill intake might attenuate the COC-induced VTE risk. On the other hand, continuous exposure to hormonal contraceptives could result in accumulation of their effects on hepatic protein synthesis and other metabolic processes, and thereby impose a higher risk of cardiovascular adverse disease.

This thesis means to address the differential metabolic effects of different levels of androgenecity, and routes and regimens of delivery of contraceptive hormones. Chapter 2 demonstrates the effects of the vaginal route of delivery of EE and Nestorone®, a non-androgenic progestin, on haemostasis variables and sex-hormone binding globulin (SHBG) in comparison to a COC containing EE and the androgenic levonorgestrel. In Chapter 3, we investigate the effects of the same treatments on lipids and angiotensinogen in an attempt to learn more about the impact of different delivery routes and androgenicity on the hepatic protein synthesis. Chapter 4 looks into the effects of the abovementioned preparations on measures of inflammation such as C-reactive protein and the possible explanations for these effects.

Subsequently, discriminant analysis (DA) is employed on the data collected in the first three chapters to identify variables with the strongest discriminative power of the treatments (Chapter 5). This study wis an open-minded attempt to learn more about the path through which CHCs affect the risk of cardiovascular disease in their users. It is based on the assumption that the variable or the combination of variables explaining the greatest part of the difference between an androgenic and a less-androgenic preparation might be the product(s) of the process that is also responsible for the difference in risk of cardiovascular disease that users of the mentioned CHCs are subjected to. This analysis leads to formulation a number of discriminating functions that subsequently are applied to a large set of data on seven different COCs with different degrees of androgenicity, for validation (Chapter 6). Considering the results of the DA on the data of the non-androgenic vaginal ring versus the androgenic COC could become compromised by effects exerted due to possible specific characteristics of Nestorone® beside its potential of modifying the oestrogenic effects in accordance with its androgenicity, we will also perform DA on the abovementioned dataset of the study on seven different COCs (Chapter 6).

In Chapter 7, a COC containing EE and levonorgestrel that has been developed for continuous use (Lybrel®) is compared for effects on haemostasis, SHBG, lipids, carbohydrate and bone metabolism to the cyclically used and in composition closely comparable COC (Loette®). We aim to find out whether the extended-regimen COC could create an unfavourable cardiovascular risk profile through accumulation of metabolic effects.

REFERENCES

1 Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ, III. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Intern Med 1998 March 23;158(6):585-93.

2 Jick H, Jick S, Myers MW, Vasilakis C. Risk of acute myocardial infarction and low-dose combined oral contraceptives. Lancet 1996 March 2;347(9001):627-8.

3 The Coronary Drug Project. Findings leading to discontinuation of the 2.5-mg day estrogen group. The coronary Drug Project Research Group. JAMA 1973 November 5;226(6):652-7.

4 Gerstman BB, Piper JM, Tomita DK, Ferguson WJ, Stadel BV, Lundin FE. Oral contraceptive estrogen dose and the risk of deep venous thromboembolic disease. Am J Epidemiol 1991 January;133(1):32-7.

5 van Kesteren PJ, Asscheman H, Megens JA, Gooren LJ. Mortality and morbidity in transsexual subjects treated with cross-sex hormones. Clin Endocrinol (Oxf) 1997 September;47(3):337-42.

6 Kiran H, Kiran G, Ekerbicer HC, Guven AM, Kilinc M. Effects of oestrogen replacement therapy on serum C-reactive protein levels in hysterectomised women. Aust N Z J Obstet Gynaecol 2004 April;44(2):131-4.

7 Mattsson LA, Christiansen C, Colau JC, Palacios S, Kenemans P, Bergeron C et al. Clinical equivalence of intranasal and oral 17beta-estradiol for postmenopausal symptoms. Am J Obstet Gynecol 2000 March;182(3):545-52.

8 Vongpatanasin W, Tuncel M, Wang Z, Arbique D, Mehrad B, Jialal I. Differential effects of oral versus transdermal estrogen replacement therapy on C-reactive protein in postmenopausal women. J Am Coll Cardiol 2003 April 16;41(8):1358-63.

9 Inman WH, Vessey MP, Westerholm B, Engelund A. Thromboembolic disease and the steroidal content of oral contraceptives. A report to the Committee on Safety of Drugs. Br Med J 1970 April 25;2(5703):203-9.

10 Effect of different progestagens in low oestrogen oral contraceptives on venous thromboembolic disease. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet 1995 December 16;346(8990):1582-8.

11 Spitzer WO, Lewis MA, Heinemann LA, Thorogood M, MacRae KD. Third generation oral contraceptives and risk of venous thromboembolic disorders: an international case-control study. Transnational Research Group on Oral Contraceptives and the Health of Young Women. BMJ 1996 January 13;312(7023):83-8.

12 Jick H, Kaye JA, Vasilakis-Scaramozza C, Jick SS. Risk of venous thromboembolism among users of third generation oral contraceptives compared with users of oral contraceptives with levonorgestrel before and after 1995: cohort and case-control analysis. BMJ 2000 November 11;321(7270):1190-5.

13 Kemmeren JM, Algra A, Grobbee DE. Third generation oral contraceptives and risk of venous thrombosis: meta-analysis. BMJ 2001 July 21;323(7305):131-4.

14 An open label, randomized study to evaluate the effects of seven monophasic oral contraceptive regimens on hemostatic variables. Outline of the protocol. Oral Contraceptive and Hemostasis Study Group. Contraception 1999 June;59(6):345-55.

15 Tans G, Curvers J, Middeldorp S, Thomassen MC, Meijers JC, Prins MH et al. A randomized cross-over study on the effects of levonorgestrel- and desogestrel-containing oral contraceptives on the anticoagulant pathways. Thromb Haemost 2000 July;84(1):15-21.

16 van der Vange, N., Blankenstein MA, Kloosterboer HJ, Haspels AA, Thijssen JH. Effects of seven low-dose combined oral contraceptives on sex hormone binding globulin, corticosteroid binding globulin, total and free testosterone. Contraception 1990 April;41(4):345-52.

17 Blode H, Wuttke W, Loock W, Roll G, Heithecker R. A 1-year pharmacokinetic investigation of a novel oral contraceptive containing drospirenone in healthy female volunteers. Eur J Contracept Reprod Health Care 2000 December;5(4):256-64.

18 Knopp RH, Broyles FE, Cheung M, Moore K, Marcovina S, Chandler WL. Comparison of the lipoprotein, carbohydrate, and hemostatic effects of phasic oral contraceptives containing desogestrel or levonorgestrel. Contraception 2001 January;63(1):1-11.

19 Rosen MP, Breitkopf DM, Nagamani M. A randomized controlled trial of second- versus third-generation oral contraceptives in the treatment of acne vulgaris. Am J Obstet Gynecol 2003 May;188(5):1158-60.

20 Odlind V, Milsom I, Persson I, Victor A. Can changes in sex hormone binding globulin predict the risk of venous thromboembolism with combined oral contraceptive pills? Acta Obstet Gynecol Scand 2002 June;81(6):482-90.

21 Croft P, Hannaford PC. Risk factors for acute myocardial infarction in women: evidence from the Royal College of General Practitioners' oral contraception study. BMJ 1989 January 21;298(6667):165-8.

22 Jonsdottir LS, Sigfusson N, Gudnason V, Sigvaldason H, Thorgeirsson G. Do lipids, blood pressure, diabetes, and smoking confer equal risk of myocardial infarction in women as in men? The Reykjavik Study. J Cardiovasc Risk 2002 April;9(2):67-76.

23 Folsom AR, Aleksic N, Catellier D, Juneja HS, Wu KK. C-reactive protein and incident coronary heart disease in the Atherosclerosis Risk In Communities (ARIC) study. Am Heart J 2002 August;144(2):233-8.

24 Koenig W, Sund M, Frohlich M, Lowel H, Hutchinson WL, Pepys MB. Refinement of the association of serum C-reactive protein concentration and coronary heart disease risk by correction for within-subject variation over time: the MONICA Augsburg studies, 1984 and 1987. Am J Epidemiol 2003 August 15;158(4):357-64.

25 Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. Am J Epidemiol 1996 September 15;144(6):537-47.

26 Lagrand WK, Visser CA, Hermens WT, Niessen HW, Verheugt FW, Wolbink GJ et al. C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? Circulation 1999 July 6;100(1):96-102.

27 Lowe GD, Yarnell JW, Sweetnam PM, Rumley A, Thomas HF, Elwood PC. Fibrin D-dimer, tissue plasminogen activator, plasminogen activator inhibitor, and the risk of major ischaemic heart disease in the Caerphilly Study. Thromb Haemost 1998 January;79(1):129-33.

28 Maresca G, Di Blasio A, Marchioli R, Di Minno G. Measuring plasma fibrinogen to predict stroke and myocardial infarction: an update. Arterioscler Thromb Vasc Biol 1999 June;19(6):1368-77.

29 Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 1998 August 25;98(8):731-3.

30 Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000 March 23;342(12):836-43.

31 Lowe GD, Yarnell JW, Rumley A, Bainton D, Sweetnam PM. C-reactive protein, fibrin D-dimer, and incident ischemic heart disease in the Speedwell study: are inflammation and fibrin turnover linked in pathogenesis? Arterioscler Thromb Vasc Biol 2001 April;21(4):603-10.

32 Marz W, Scharnagl H, Winkler K, Tiran A, Nauck M, Boehm BO et al. Low-density lipoprotein triglycerides associated with low-grade systemic inflammation, adhesion molecules, and angiographic coronary artery disease: the Ludwigshafen Risk and Cardiovascular Health study. Circulation 2004 November 9;110(19):3068-74.

33 Mann JI, Vessey MP, Thorogood M, Doll SR. Myocardial infarction in young women with special reference to oral contraceptive practice. Br Med J 1975 May 3;2(5965):241-5.

34 Petitti DB, Sidney S, Quesenberry CP, Jr., Bernstein A. Incidence of stroke and myocardial infarction in women of reproductive age. Stroke 1997 February;28(2):280-3.

35 Heinemann LA, Lewis MA, Spitzer WO, Thorogood M, Guggenmoos-Holzmann I, Bruppacher R. Thromboembolic stroke in young women. A European case-control study on oral contraceptives. Transnational Research Group on Oral Contraceptives and the Health of Young Women. Contraception 1998 January;57(1):29-37.

36 Mant D, Villard-Mackintosh L, Vessey MP, Yeates D. Myocardial infarction and angina pectoris in young women. J Epidemiol Community Health 1987 September;41(3):215-9.

37 Rosenberg L, Hennekens CH, Rosner B, Belanger C, Rothman KJ, Speizer FE. Oral contraceptive use in relation to nonfatal myocardial infarction. Am J Epidemiol 1980 January;111(1):59-66.

38 Ischaemic stroke and combined oral contraceptives: results of an international, multicentre, case-control study. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet 1996 August 24;348(9026):498-505.

39 Sidney S, Petitti DB, Quesenberry CP, Jr., Klatsky AL, Ziel HK, Wolf S. Myocardial infarction in users of low-dose oral contraceptives. Obstet Gynecol 1996 December;88(6):939-44.

40 Acute myocardial infarction and combined oral contraceptives: results of an international multicentre case-control study. WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. Lancet 1997 April 26;349(9060):1202-9.

41 Dunn N, Thorogood M, Faragher B, de Caestecker L, MacDonald TM, McCollum C et al. Oral contraceptives and myocardial infarction: results of the MICA case-control study. BMJ 1999 June 12;318(7198):1579-83.

42 Rosenberg L, Palmer JR, Rao RS, Shapiro S. Low-dose oral contraceptive use and the risk of myocardial infarction. Arch Intern Med 2001 April 23;161(8):1065-70.

43 Dunn NR, Arscott A, Thorogood M. The relationship between use of oral contraceptives and myocardial infarction in young women with fatal outcome, compared to those who survive: results from the MICA case-control study. Contraception 2001 February;63(2):65-9.

44 Lewis MA, Spitzer WO, Heinemann LA, MacRae KD, Bruppacher R. Lowered risk of dying of heart attack with third generation pill may offset risk of dying of thromboembolism. BMJ 1997 September 13;315(7109):679-80.

45 Tanis BC, van den Bosch MA, Kemmeren JM, Cats VM, Helmerhorst FM, Algra A et al. Oral contraceptives and the risk of myocardial infarction. N Engl J Med 2001 December 20;345(25):1787-93.

46 Spitzer WO, Faith JM, MacRae KD. Myocardial infarction and third generation oral contraceptives: aggregation of recent studies. Hum Reprod 2002 September;17(9):2307-14.

47 Krauss RM, Burkman RT, Jr. The metabolic impact of oral contraceptives. Am J Obstet Gynecol 1992 October;167(4 Pt 2):1177-84.

48 Godsland IF, Crook D, Simpson R, Proudler T, Felton C, Lees B et al. The effects of different formulations of oral contraceptive agents on lipid and carbohydrate metabolism. N Engl J Med 1990 November 15;323(20):1375-81.

49 Godsland IF, Crook D. Update on the metabolic effects of steroidal contraceptives and their relationship to cardiovascular disease risk. Am J Obstet Gynecol 1994 May;170(5 Pt 2):1528-36.

50 Norris LA, Bonnar J. The effect of oestrogen dose and progestogen type on haemostatic changes in women taking low dose oral contraceptives. Br J Obstet Gynaecol 1996 March;103(3):261-7.

51 Woodward M, Lowe GD, Rumley A, Tunstall-Pedoe H. Fibrinogen as a risk factor for coronary heart disease and mortality in middle-aged men and women. The Scottish Heart Health Study. Eur Heart J 1998 January;19(1):55-62.

52 Kluft C, Leuven JA, Helmerhorst FM, Krans HM. Pro-inflammatory effects of oestrogens during use of oral contraceptives and hormone replacement treatment. Vascul Pharmacol 2002 August;39(3):149-54.

53 Williams MJ, Williams SM, Milne BJ, Hancox RJ, Poulton R. Association between C-reactive protein, metabolic cardiovascular risk factors, obesity and oral contraceptive use in young adults. Int J Obes Relat Metab Disord 2004 August;28(8):998-1003.

54 Godsland IF, Crook D, Simpson R, Proudler T, Felton C, Lees B et al. The effects of different formulations of oral contraceptive agents on lipid and carbohydrate metabolism. N Engl J Med 1990 November 15;323(20):1375-81.

55 Kemmeren JM, Algra A, Grobbee DE. Effect of second and third generation oral contraceptives on lipid metabolism in the absence or presence of the factor V Leiden mutation. J Intern Med 2001 November;250(5):441-8.
56 Timmer CJ, Mulders TM. Pharmacokinetics of etonogestrel and ethinylestradiol released from a combined contraceptive vaginal ring. Clin Pharmacokinet 2000 September;39(3):233-42.
57 Hall G, Blomback M, Landgren BM, Bremme K. Effects of vaginally administered high estradiol doses on hormonal pharmacokinetics and hemostasis in postmenopausal women. Fertil Steril 2002 December;78(6):1172-7.
58 De Lignieres B, Basdevant A, Thomas G, Thalabard JC, Mercier-Bodard C, Conard J et al. Biological effects of estradiol-17 beta in postmenopausal women: oral versus percutaneous administration. J Clin Endocrinol Metab 1986 March;62(3):536-41.
59 Faguer dM, Conard J, Guyene TT, Sitt Y, Denys I, Arnoux-Rouveyre M et al. Comparative metabolic study of percutaneous versus oral micronized 17 beta-oestradiol in replacement therapy. Maturitas 1989 December;11(4):275-86.
60 Vehkavaara S, Silveira A, Hakala-Ala-Pietila T, Virkamaki A, Hovatta O, Hamsten A et al. Effects of oral and transdermal estrogen replacement therapy on markers of coagulation, fibrinolysis, inflammation and serum lipids and lipoproteins in postmenopausal women. Thromb Haemost 2001 April;85(4):619-25.
61 Post MS, van der Mooren MJ, Stehouwer CD, van Baal WM, Mijatovic V, Schalkwijk CG et al. Effects of transdermal and oral oestrogen replacement therapy on C-reactive protein levels in postmenopausal women: a randomised, placebo-controlled trial. Thromb Haemost 2002 October;88(4):605-10.
62 Post MS, van der Mooren MJ, van Baal WM, Blankenstein MA, Merkus HM, Kroeks MV et al. Effects of low-dose oral and transdermal estrogen replacement therapy on hemostatic factors in healthy postmenopausal women: a randomized placebo-controlled study. Am J Obstet Gynecol 2003 November;189(5):1221-7.
63 Lacut K, Oger E, Le Gal G, Blouch MT, Abgrall JF, Kerlan V et al. Differential effects of oral and transdermal postmenopausal estrogen replacement therapies on C-reactive protein. Thromb Haemost 2003 July;90(1):124-31.
64 Alvarez-Sanchez F, Brache V, Jackanicz T, Faundes A. Evaluation of four different contraceptive vaginal rings: steroid serum levels, luteal activity, bleeding control and lipid profiles. Contraception 1992 October;46(4):387-98.
65 Kluft C, Meijer P, Laguardia KD. Similarities in the rate of induction of changes in hemostasis variables by a transdermal contraceptive patch (ORTHO EVRA®) vs oral contraceptives in healthy female subjects. Fertil Steril 2006;86(3):s16.