

PROSTAGLANDINS, MENSTRUATION, AND MENSTRUAL DISORDERS

Pages with reference to book, From 120 To 126

Sheilth A. Saeed, Amin Suria (The Aga Khan University Medical College, Karachi.)

Abstract

Prostaglandins play an important role in the mechanism of human menstruation. Furthermore aberrant prostaglandin production may be an intimate component of the pathogenesis of menstrual disorders such as dysmenorrhoea, menorrhagia and perhaps the premenstrual syndrome. Inhibitors of prostaglandin biosynthesis available today have proven universally useful in the 'treatment of dysmenorrhoea and menorrhagia. The development of such drugs and endogenous inhibitors of prostaglandin synthase (EIPS) with greater specificity and also perhaps antagonists of the actions of specific prostaglandins is currently a high priority. It may be anticipated that the development of these newer ranges of natural and synthetic drugs will allow treatments of menstrual disorders with greater efficacy, and reduced sideeffects. (JPMA 36:120,1986).

PROSTAGLANDINS-AN INTRODUCTION

In 1930 two gynecologists, Kurzrok and Lieb¹ working in New York, examined the effects of human seminal fluid on strips of uterine muscle in organ baths. Uteri from fertile women with a history of sterility contracted. In addition, however, the same strip could relax in response to a sample from a different donor. In essence, these findings reflected what is a continuing trend in the prostaglandin (PG) literature for both interesting yet contradictory results. Shortly afterwards von Euler² proposed the name prostaglandin for one of the active principles in extracts from prostate glands. It was not until 1966, however, that the absolute chemical structure of prostaglandins was defined by Nugteren, van Dorp, Bergstrom, Hamberg and Samuelsson³. The initial steps in the elucidation of the fatty acid precursors for prostaglandin biosynthesis came in 1964 when the conversion of arachidonic acid (a C20 fatty acid precursor) into PGE₂ by homogenates of sheep vesicular gland was reported.^{4,5} The main sources of these precursors are glycerophospholipids from which the fatty acids are released primarily by the actions of phospholipases A₂ and

C. Thromboxane A₂ and prostacyclin are two newly discovered^{6,7} prostanoids (prostaglandinlike; usually referring to prostaglandins and thromboxanes) with potent, generally opposing cardiovascular actions. The widely used aspirin-like drugs (e.g. indomethacin, naproxen, mefenamic acid, ibuprofen) all act by of inhibiting prostaglandin synthesis⁸. In our laboratories, we have demonstrated that human plasma and serum contain endogenous inhibitors of prostaglandin synthase (EIPS) and arachidonate-induced platelet aggregation (EIPA)⁹.

Prostaglandins have many and varied properties which make them of interest; three in particular will be described. Firstly, they have potent cardiovascular actions with PGE₂ generally a vasodilator and PGF₂ a vasoconstrictor.¹⁰ Prostacyclin and thromboxane A₂ also have, respectively, potent vasodilator and vasoconstrictor properties. Secondly, PGE₂ may relax and PGF₂ contract smooth muscle although both contract uterine smooth muscle and hence their use for the induction of abortion^{11,12} and term labor^{13,14}. Prostacyclin is thought either to be without effect on uterine contractility or to be inhibitory of contractility whereas thromboxane A₂ may induce uterine contractions^{15,16} Thirdly, prostacyclin is a powerful inhibitor of platelet aggregation⁷ whilst thromboxane A₂ is potently proaggregatory⁶.

Prostaglandins and Mensfruation

Our understanding of the process of menstruation remains limited; much of what is known has been deduced from the classical studies of Markee. in the 1930's^{17,18} In these studies Markee transpiated pieces of endometrium to the anterior chamber of the eye of the rhesus monkey (Macca Mulatta) and observed rhythmic vascular changes within the explants throughout the menstrual cycle. Each period of menstrual bleeding was preceded by the following events; a period of endometrial regression resulting in further coiling of the endometrial spiral arterioles andeventually vascular stasis, followed by a period of marked vasoconstriction lasting from 4-24 hours preceding actual haemorrhage. Based on his observation, Markee suggested that a substance or substances produced by the regressing endo. metrium was responsible for this constriction of the spiral arterioles.

It is interesting that other investigators subsequently evaluated the nature of active substances in menstrual fluid, especially one known as menotoxin or menstrual toxin¹⁹. As little as 0.02 ml of menstrual discharge could consistently be lethal when administered to immature male rats. The greatest concentration of menstrual toxin was in washed endometrial debris, the equivalent of only 0.005 ml being lethal. The nature of the toxin has still not been clearly established.

In 1957 Pickles²⁰, reported the presence of a smooth muscle stimulant in human menstrual fluid. The major components of the stimulant were identified subsequently as PGE2 and PGF2a^{21,22} Concentrations of PGE2a and PGF2a in endometrium taken at different stages of the menstrual cycle have been determined by several methods (Table I).

TABLE I
Some Studies of the Prostaglandin content of Human Endometrium during the Menstrual Cycle.

| Authors | Method of measurement | No. of subjects studied | PGE ₂ | Luteal Vs. Proliferative | |
|---------------------------|---------------------------------------|-------------------------|------------------|--------------------------|---------------------------------------|
| | | | | PGF _{2a} | PGF _{2a} PGE ₂ |
| Pickles et al (23) | Bioassay | 17 | ↑ | ↑ | ↑ |
| Downie et al (24) | Bioassay | 44 | → | ↑ | ↑ |
| Singh et al (25) | Transmission Densitometry | 30 | → | ↑ | ↑ |
| Green and Hagenfeldt (26) | Gas-chromatography/ mass spectrometry | 5 | N'M' | ↑ | - |
| Evitt et al (27) | Radioimmunoassay | 47 | → | ↑ | ↑ |
| Willman et al (28) | Radioimmunoassay | 62 | ↑ | ↑ | → |
| Maathuis and kelly (29) | Gas-chromatography/ mass spectrometry | 45 | → | ↑ | ↑ |

N.M. = Not measured

In parenthesis, number of reference

Greater amounts of prostaglandins are found in endometrium taken during the luteal phase of the cycle

when compared with amounts in endometrium from the proliferative phase of the cycle. A significant shift in the PGF to PGE ratio, favouring PGF, has often been reported. Endometria obtained during menstruation have been found to contain even greater amounts of prostaglandins as reported by Downie et al (1974)²⁴ These findings are consistent with the enhanced local vasoconstrictive activity thought to precede menstruation. The changes in endometrial prostaglandin content with the phase of the menstrual cycle are not due to alterations in prostaglandin catabolism since the activity of NAD⁺-dependent 15-hydroxy-prostaglandin dehydrogenase in endometrium is increased during the luteal phase of the cycle³⁰.

Analysis of measurements of prostaglandin concentrations in peripheral plasma during the menstrual cycle have generally not revealed any significant trends during the cycle^{31,33} although in one study³⁴, a pre-ovulatory and a premenstrual increase in circulating levels of 13,14-dihydro-15-keto-PGF₂α (PGFM) (metabolite of prostaglandin-F) was reported. Administration of prostaglandins to nonpregnant women will induce uterine contractions³⁵ this effect occurs in early pregnancy and is the basis for the use of prostaglandins for menstrual regulation or post-conceptual therapy^{36,37} Interestingly although administration of PGF₂α is inhibitory of uterine cervical contractility^{38,39} The latter observations taken in conjunction with the recent observation of striking amounts of prostaglandins in cervical mucus⁴⁰ may be suggestive of a role for prostaglandins in sperm migration. Presently there is no firm evidence that naturally occurring prostaglandins (or analogues thereof) are luteolytic when administered to women.

Prostaglandins and Dysmenorrhoea

Derived from the Greek, meaning “difficult monthly flow” the word dysmenorrhoea has come to mean painful menstruation associated with lower abdominal pain. Many women who suffer from this disorder also suffer from a complex of symptoms that may include headache, weakness, depression, dizziness, paresthesias, nausea, vomiting and abdominal bloating. The symptoms usually appear just before, or at the onset of menstrual flow and persist at their worst during the first day or two of menstruation. In some women, lower abdominal pain can begin several days before menstruation starts and can continue throughout the period of menstruation.

Dysmenorrhoea is classified as primary if it occurs in the absence of pelvic disease. Patients complaining of primary dysmenorrhoea are usually young, nulliparous and have had symptoms since shortly after menarche. Dysmenorrhoea is classified as secondary if it is related to some form of pelvic pathology such as endometriosis, fibroids and pelvic inflammatory disease or to the presence of an intrauterine contraceptive device. Further classification of dysmenorrhoea into spasmoidic (usually primary) or congestive (often secondary) may not be useful, particularly since the elements of congestive dysmenorrhoea e.g. weight gain, abdominal bloating and breast tenderness, are consistent with the premenstrual syndrome or premenstrual tension. Moreover the actual period of menstruation is usually painfree in congestive dysmenorrhoea and hence is difficult to associate with painful menstruation.

Although accurate assessments of the incidence of dysmenorrhoea are not available, several recent studies from the USA, Great Britain and Sweden have provided useful information and calculations⁴¹⁻⁴⁵ Approximately 50-60% of women of reproductive age experience painful periods. As many as 30% of such women suffer severe pain that can be incapacitating⁴³⁻⁴⁴

Endometria from dysmenorrhoeic women contain greater amounts of prostaglandins than endometria from women without dysmenorrhoea^{45,46,47} Moreover concentrations PGFM in peripheral plasma taken during menstruation are significantly higher in women with dysmenorrhoea than in eumenorrhoeic women⁴⁷ It has been suggested that the increased production of prostaglandins within the uterus could lead to greater uterine activity (and pain) and that sufficient prostaglandins may be secreted into the systemic circulation to cause some of the other symptoms of dysmenorrhoea⁴². An alternative

explanation for the wide-spread symptoms of dysmenorrhoea could be the generation of a noxious substance that reaches the systemic circulation during the period of menstruation. Such a factor has recently been described⁴⁸. Treatment of dysmenorrhoea with non-steroidal anti-inflammatory drugs (prostaglandin synthase inhibitors, PGSI) does indeed reduce parameters of uterine pressure and work^{49,50}. The first reported trial of a PGSI for the treatment of primary dysmenorrhoea was by Schwartz and colleagues⁵¹. Flufenamic acid (125 mg tid or qid) afforded symptomatic relief in all 16 patients in the study during 31 treatment cycles. Since this publication the results of many more controlled, trials of different PGSI for the treatment of dysmenorrhoea have been published⁵². It should be noted that fenamates could hold an advantage over other PGSI for the treatment of dysmenorrhoea since they may antagonize the actions of prostaglandins, in addition to being inhibitory of prostaglandin biosynthesis⁵³. Overall, approximately 70-80% of women complaining of primary dysmenorrhoea obtain significant relief by ingesting various PGSI (e.g. Table-II).

TABLE II
Comparison of efficacy of Flufenamic Acid to
Indomethacin in the Treatment of Dysmenorrhoea.

| | Flufenamic acid | Indome- thacin |
|--|--------------------|-------------------|
| Significant pain relief | 82% | 75% |
| Significant reduction in vomiting | 66% | 53% |
| Significant reduction in Diarrhoea | 52% | 44% |
| Side Effects attributable to drug (Ref. 52) | 9% | 18% |

A significant advantage of this form of treatment is that drug administration can be limited to a few days per month and by restricting use to the period of menstruation fears of effects on an unknown pregnancy can be alleviated.

Endogenous Inhibitor of Prostaglandin Synthase in Dysmenorrhea We have recently demonstrated the presence of endogenous inhibitors of prostaglandin synthase (EIPS) and arachidonate-induced plate aggregation (EIPA) in human blood plasma and serum⁹.

EIPS activity as measured by the effect of plasma on the net conversion of arachidonic acid to prostanoids has been determined in plasma of women with dysmenorrhea⁵⁴.

TABLE III
Eips activity of Human Plasma (0.5% v/v) against the
synthesis of PGE₂ by BSV Microsome Prostaglandin
synthase.

| Day(s) of cycle | With Dysmenorrhea (n = 7) | Without Dysmenorrhea (n = 7) |
|-----------------|-----------------------------------|--------------------------------------|
| 1 | 50.5 (± 2.2) | 48.4 (± 3.1) |
| 10-13 | 55.1 (± 4.4) | 52.3 (± 2.1) |

The results given in Table III show no significant differences in EIPS activity of plasma obtained from women who experience dysmenorrhea when compared to those who did not experience it.

Values are the mean (± S.E.) decrease in PGE₂ production, expressed as a percent of controls. BSV; Bull Seminal Vesicle (ReP 54).

It is possible, however, that because of the potency of EIPS activity in human plasma, the methodology used in the previous study⁵⁴ was not sufficiently sensitive to detect other plasma factors in dysmenorrhea which may modulate normal plasma EIPS activity. Alternatively, autocooids, such as vasoactive amines, kinins and opioids, may act in concert with prostaglandins to cause the symptoms that are associated with dysmenorrhea.

Prostaglandins and Menorrhagia

Iron deficiency anaemia may be indicative of heavy blood loss at menstruation and is more common in women whose loss is more than 80 ml per menses⁵⁵. Thus the most widely used definition of menorrhagia is that of a menstrual blood loss of 80 ml or greater per menses. A woman's menstrual blood loss usually varies by 20-40% between menses although greater variations may occur in women with menorrhagia⁵⁶. There is a much larger variation in menstrual blood loss between individuals. Intriguingly, it has been found that there is a significant correlation between menstrual blood loss in monozygotic twins which cannot be demonstrated for dizygotic twins⁵⁶.

It is unfortunate that often the main criteria for diagnosing menorrhagia is the patient's statement that she has heavy periods. It has been shown that women may not make reliable judgements of the volume of their menstrual blood loss. In one study 40% of women with menstrual blood loss exceeding 80 ml considered their periods to only moderately heavy or scanty whilst 14% of women with a measured loss of less than 20ml judged their periods to be heavy⁵⁵. In a recent study⁵⁷ it was shown that there was no significant relationship between menstrual blood loss and the patients' subjective assessment of that loss, the duration of menstruation, uterine weight or endometrial surface area. It has been demonstrated that women with menorrhagia have periods of menstruation that are no longer than women without menorrhagia⁵⁸.

Excessive bleeding from the uterine cavity may be preceded by ovulatory or anovulatory menstrual cycles. In the majority of women whose menstrual loss has been assessed objectively, no significant

differences from normal can be shown in the pattern of pituitary or ovarian hormone secretion⁵⁹. In a recent study⁶⁰, the endogenous concentrations of prostaglandins F₂ a (PGF₂ a) and E (PGE) were measured during the luteal phase of the menstrual cycle in the endometrium from 14 women with unexplained menorrhagia measured menstrual blood loss in excess of (50ml) and 15 women with normal menses (blood loss 50 ml or less). Although there was no significant differences in the PGF₂ a/PGE ratio between the two groups, this ratio was significantly lower in the endometrium from eight of the women whose blood loss exceeded 90ml ($p < 0.05$). There was a significant inverse correlation between the PGF₂ a ratio and blood loss ($r = 0.36$, $p < 0.025$). The synthetic capacity of the endometrium was assessed by incubation of the tissue with C₄ arachidonic acid. Endometria from nine women with unexplained menorrhagia synthesized more PGE₂ than PGF₂ a, whereas the converse was true with 11 control endometria. Consequently the PGF₂ a/PGE₂ ratio was significantly reduced in the former group.

These results are suggestive that excessive blood loss may be associated with a shift in the endometrial conversion of prostaglandin endoperoxide from PGF₂ to PGE₂.

The same group of investigators⁶¹ subsequently have found that endometria from women with excessive menstrual blood loss are more effective than endometria from women with normal menstrual blood loss at enhancing the production of 6-keto-prostaglandin F₁ a (the degradation product of prostacyclin) by a control preparation of myometrium. These workers suggested that an enhanced ability of the uterus to generate prostacyclin, a potent vasodilator and inhibitor of platelet aggregation, could influence the degree and duration of menstrual bleeding.

In 1976, Anderson and colleagues⁶² published the results of a trial of PGSI (mefenamic acid and flufenamic acid) for the treatment of menorrhagia due to dysfunctional uterine bleeding. In every patient mean blood loss was reduced during treatment; the pre-treatment value was (mean * SEM) 119 ± 10 ml (18 cycles in six patients) and the treatment value was 60 ± 7 ml (17 cycles in six patients). The presence of an intrauterine contraceptive device is associated with increased menstrual blood loss and there is evidence that the PGE content of endometrium is also increased under these conditions⁶³. Mefenamic acid has been used successfully to reduce menstrual blood-loss in women using intrauterine contraceptive devices⁶⁴. This treatment however does not alter total duration of bleeding or non-collectable spotting. Expressed as a percentage of the mean pretreatment volume, the reduction in loss was not significantly different for those women who were light losers (less than 80 ml) compared with heavy losers (80 ml or more), but it was greater in volume terms among the latter. The successful use of PGSI in the treatment of menorrhagia lends credence to the view that the mechanism of this disorder involves altered prostaglandin production.

REFERENCES

1. Kurzrok, R. and Lieb, C.C. Biochemical studies of human semen. II. The action of semen on the human uterus. *Proc. Soc. Exp. Biol. Med.*, 1930; 28: 268.
2. Von Euler, U.S. Uber die spezifische blutdrucksenkende Substanz des menschlichen prostata and samenblasenskreter. *Kiln. Wochenschr.*, 1935; 14: 1182.
3. Nugteren, D.H., Van Dorp D.A., Bergstrom, S., Hamberg, M. and Vonkeman, H. The biosynthesis of prostaglandins. *Biochim. Biophys. Acta*, 1984;90: 204.
4. Van Dorp, D.A., Beerthuis, R.K., Nugteren, D.H. and Vonkeman, H. The biosynthesis of prostaglandins. *Biochim. Biophys. Acta*, 1984; 90, 204-207.
5. Bergstrom, S., Danielsson, H. and Samuelsson B. The enzymatic formation of prostaglandin E₂ from arachidonic acid prostaglandins and related factors 32. *Biochim. Biophys. Acta*, 1964; 90: 207.
6. Haxnberg, M., Svensson, J. and Samuelsson, B. Thromboxanes; a new group of biologically active

- compounds derived from prostaglandin endoperoxides. Proc. Natl. Acad. Sci. USA., 1975; 72: 2994.
7. Moncada, S., Gryglewski, R., Bunting, S. and Vane, J.R. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature, 1976; 263: 663.
 8. Vane, J.R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature (New Biol.), 1971;231: 232.
 9. Saeed, S.A. and Suria, A. Endogenous inhibitor(s) of platelet aggregation. JPMA., 1985; 34:41.
 10. Gryglewski, R.J. Prostaglandins and vascular wall, in prostaglandins and thromboxanes. Edited by Berti, F. New York, Plenum Press, 1977, 265- 273.
 11. Karim, S.M. and Filshie, G.M. Therapeutic abortion using prostaglandin F_{2a} Lancet, 1970 ; I: 157.p.
 12. Roth-Brandel, U., Bygdeman, M., Wiqvist, N. and Bergstrom, S. Prostaglandins for induction of therapeutic abortion. Lancet, 1970; 1: 190.
 13. Karim, S.M., Trussell, R.R., Patel, R.C. and Hfflier, K. Response of pregnant human uterus to prostaglandin F_{2a} - induction of labour. Br. Med. J., 1968;4:621.
 14. Embrey, M.P. PGE compounds for induction of labour and abortion. Ann. N.Y. Acad. Sci., 1971; 180:51 8.
 15. Wilhelmsson, L, Wikland, M. and Wiqvist, N. PGH₂ TXA₂ and PGI₂ have potent and differentiated actions on human uterine contractility. Prostaglandins, 1981;21: 277.
 16. Makarainen, L. and Ylikorkala, O. Lack of effect of circulating prostacyclin on contractility of the non-pregnant human uterus. Br. J. Obstet. Gynecol., 1982;89: 402.
 17. Markee, J.E. Menstruation in intra-ocular endometrial transplants in the Rhesus monkey. Contr. EmbryoL, 1940; 177, 233-308.
 18. Markee, J.E. The relation of blood flow to endometrial growth and the inception of menstruation, in menstruation and its disorders. Edited by Engle, E.T. Springfield, Thomas, 1950.
 19. Smith, O.W. Menstrual toxin. Am. J. Obstet. Gynecol, 1947;54: 201.
 20. Pickles, V.R. A plain-muscle stimulant in the menstruum. Nature (Lond.), 1957; 180: 1198.
 21. Pickles, V.R. and Hall, W.J. Some physiological properties of the menstrual stimulant' substances A₁ and A_{2j} Reprod. Fertil., 1963; 6~ 315.
 22. Eglington, G, Raphael, R.A., Smith, G.N., Hall, W.J. and Pickles, V.R. Isolation and identification of two smooth muscle stimulants from menstrual fluid. Nature, (Lond.), 1963;200: 993.
 23. Pickles, V.R., Hall, W.J., Best, F.A. and Smith, G.N. Prostaglandins in endometrium and menstrual fluid from normal and dysmenorrhic subjects. J. Obstet. Gynaecol. Br. Commonw., 1965; 72: 185.
 24. Downie, J., Poyser, N.L. and Wunderlich, M. Levels of prostaglandins in human endometrium during the normal menstrual cycle. J. Physiol., 1974;236: 465.
 25. Singh, E.J., Baccarini, I.M. and Zuspan, F.P. Levels of prostaglandins F₂₈ and E₂ in human endometrium during the menstrual cycle. Am. J. Obstet. Gynecol, 1975;121: 1003.
 26. Green, K. and Hagenfeldt, K. Prostaglandins in the human endometrium; gas chromatographic mass spectrometric quantitation before and after IUD insertion. Am. J. Obstet. Gynecol, 1975; 122: 611.
 27. Levitt, M.N., Tobon, H. and Josimovich, J. B. Prostaglandin content of human endometrium. Fertil. Steril., 1975;26 : 296.
 28. Willman, E.A., Collins, W.P. and Clayton, S.G. Studies in the involvement of prostaglandins in uterine symptomatology and pathology. Br. J. Obstet. Gynaecol, 1976;83: 337.
 29. Maathuis, J.B. and Kelly, R.W. Concentrations of prostaglandins F_{2a} and E₂ in endometrium throughout the human menstrual cycle, after the administration of clomiphene or an oestrogen progestogen pill and in early pregnancy. J. Endocrinol., 1978; 77: 361.
 30. Casey, M.L., Hemseil, D.L., MacDonald, P.C. and Johnston, J.M. NAD⁺-dependent 15-hydroxy-prostaglandin dehydrogenase activity in human endometrium. Prostaglandins, 1980; 19: 115.
 31. Kindahl, H., Granstrom, E., Edqvist, L.E. and Eneroth, P. Prostaglandin levels in peripheral plasma

- during the reproductive cycle, in advances in prostaglandin and thromboxane research. Edited by Sainuelsson, B. and Paoletti, R. New York, Raven Press, 1976; V.2, p.667.
32. Ghodgaonkar, R.B., Dubin, N.H., Blake, D.A. and King, T.M. 13, 14-Dihydro-15-keto-prostaglandin F_{2a} concentrations in human plasma and amniotic fluid. *Am. J. Obstet. Gynecol.*, 1979; 134: 265.
 33. Schlegel, W., Urdinola, J. and Schneider, H.P. Radioimmunoassay for 13, 14-dihydro-15-keto-prostaglandin F_{2a} and its application in normo- and anovulatory women. *Acta Endocrinol.*, 1982; 100: 98.
 34. Koullapis, E.N. and Collins, W.P. The concentration of 13, 14-dihydro-15-oxo-prostaglandin F_{1α} in peripheral venous plasma throughout the normal ovarian and menstrual cycle. *Acta Endocrinol.*, 1980;93: 123.
 35. Roth-Brandel, U., Bygdeman, M. and Wijkvist, N. Effect of intravenous administration of prostaglandin E₁ and F_{2a} on the contractility of the non-pregnant uterus in vivo. *Acta Obstet. Gynecol Scand.*, 1970;49(Suppl.5) :19.
 36. Csapo, A.I. The prospects of PG's in postconceptional therapy. *Prostaglandins*, 1973; 3: 245.
 37. Mackenzie, I.Z., Davies, A.J., Embrey, M.P. and Guillebaud, J. Very early abortion by prostaglandins. *Lancet*, 1979; 1, 1223-1 226.
 38. Coutinho, E.M. and Darze, E. Spontaneous contractility and the response of the human uterine cervix to prostaglandins F_{2a} and E₂ during the menstrual cycle. *Am. J. Obstet. Gynecol.*, 1976; 126: 224.
 39. Bryman, I., Lindblom, B. and Norstrom A. Extreme sensitivity of cervical musculature to prostaglandin E₂ in early pregnancy. *Lancet*, 1982; 2: 1471.
 40. Charbonnel, B., Kremer, M., Gerozissis, K. and Dray, F. Human cervical mucus contains large amounts of prostaglandins. *Fertil. Steril.*, 1982;38: 109.
 41. Ylikorkala, O. and Dawood, M.Y. New concepts in dysmenorrhea. *Am. J. Obstet. Gynecol.*, 1978; 130:833.
 42. Anderson, A.B.M. The role of prostaglandin synthetase inhibitors in gynaecology. *Practitioner*, 1981;225: 1460.
 43. Klein, J.R. and Litt, I.F. Epidemiology of adolescent dysmenorrhea. *Pediatrics*, 1981; 68: 661.
 44. Svanberg, L. and Ulmsten, U. The incidence of primary dysmenorrhea in teenagers. *Arch. Gynecol.*, 1981;230: 173.
 45. Andersch, B. and Milsom, I. An epidemiologic study of young women with dysmenorrhea. *Am. J. Obstet. Gynecol.*, 1982; 144: 655.
 46. Lundstrom, V. and Green, K. Endogenous levels of prostaglandin F_{2a} and its main metabolites in plasma and endometrium of normal and dysmenorrhic women. *Am. J. Obstet. Gynecol.*, 1978; 130: 640.
 47. Halbert, D.R., Demers, L.M., Fontana, J. and Jones, D.E.D. Prostaglandin levels in endometrial jet wash specimens in patients with dysmenorrhea before and after indomethacin therapy. *Prostaglandins*, 1975;10: 1047.
 48. Irwin, J., Morse, E. and Riddick, D. Dysmenorrhea, induced by autologous transfusion. *Obstet. Gynecol.*, 1981;58: 286.
 49. Lundstrom, V., Green, K. and Wijkvist, N. Prostaglandins, indomethacin and dysmenorrhea. *Prostaglandins*, 1976;11: 893.
 50. Smith, R.P. and Powell, J.R. Intrauterine pressure changes during dysmenorrhea therapy. *Am. J. Obstet. Gynecol.*, 1982; 143: 286.
 51. Schwartz, A., Zor, U., Lindner, H. and Naor, S. Primary dysmenorrhea; alleviation by an inhibitor of prostaglandin synthesis and action. *Obstet. Gynecol.*, 1974;44: 709.
 52. Elder, M.G. The treatment of primary dysmenorrhoea with Prostaglandin synthetase inhibitors. *Res. Clin. Forums*, 1979; 1: 55.

53. Bennett, A. and Sanger, G.J. Prostanoids and their relationships to the non-pregnant uterus. *Res. Chin. Forums*, 1979;!: 2!.
54. Strickland, D.M., Cox, K., Saeed, S.A. and Mitchell, M.D. Prostaglandin synthase inhibitory activity in plasma of women with dysmenorrhea. *J. Reprod. Med.*, 1984; 29: 331.
55. Hallberg, L., Hogdahl, A., Nilsson, L. and Rybo, G. Menstrual blood loss a population study. Variation at different ages and attempts to define normality. *Acta Obstet Gynecol. Scand.*, 1966; 45: 320.
56. Rybo, G. Variations of menstrual blood loss. *Res. Chin. Forums*, 1982;4: 81.
57. Chimbira, T.H., Anderson, A.B.M. and Turnbull, A.C. Relation between measured menstrual blood loss and patient's subjective assessment of loss, duration of bleeding, number of sanitary towels used, uterine weight and endometrial surface area. *Br. J. Obstet. Gynaecol.*, 1980;87: 603.
58. Haynes, P.J., Hodgson, H., Anderson, A.B.M. and Turnbull, A.C. Measurement of menstrual blood loss in patients complaining of menorrhagia. *Br. J. Obstet. Gynaecol.*, 1977;84: 763.
59. Haynes, P.J., Anderson, A.B.M. and Turnbull, A.C. Patterns of menstrual blood loss in menorrhagia. *Res. Chin. Forums*, 1979;!: 73.
60. Smith, S.K., Abel, M.H., Kelly, R.W. and Baird, T. Prostaglandin synthesis in the endometrium of women with ovular dysfunctional uterine bleeding. *Br. J. Obstet. Gynaecol.*, 1981 ; 88: 434.
61. Smith, S.K., Kelly, R.W., Abel, M.H. and Baird, T. A role for prostacyclin (PG 12) in excessive menstrual bleeding. *Lancet*, 198!;!: 522.
62. Anderson, A.B., Haynes, P.J., Guillebaud, J. and Turnbull, A.C. Reduction of menstrual blood-loss by prostaglandin-synthetase inhibitors. *Lancet*, 1976;!: 774.
63. Hillier, K. and Kasonde, J.M. Prostaglandin E and F concentrations inhuman endometrium after insertion of intrauterine contraceptive device. *Lancet*, 1976;!: 15.
64. Guillebaud, J., Anderson, A.B.M. and Turnbull, A.C. Reduction by mefenamic acid of increased menstrual blood loss associated with intrauterine contraception. *Br. J. Obstet. Gynaecol.*, 1978; 85: 53.