References

Adams, N.R. 1995. Organizational and activation effects of phytoestrogens on the reproductive tract of the ewe. Proc Soc Exp Biol Med 208: 87-91.

Adlercreutz, H., Yamada, T., Wahala, K., and Watanabe, S. 1999. Maternal and neonatal phytoestrogens in Japanese women during birth. Am J Obstet Gynecol 180: 737-743.

Anderson, J.J.B., Ambrose, W.W., and Garner, S.C.1998. Biphasic effects of genistein on bone tissue in the ovariectomized, lactating rat model. Proc Soc Exp Biol Med 217: 345-350.

Anuntalabhochai, S., and Jesrichai, S. 1986. Effects of high dosage of a local Thai plant, white gwow (*Pueraria mirifica*), on courtnix quails: II. Changes in calcium, total protein and cholesterol concentrations in blood serum. J Sci Fac CMU 13: 29-37 (in Thai).

Arjmandi, B.H., et al. 1998. Bone-sparing effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. Am J Clin Nutr 68: 1364s-1368s.

Baird, D.D., et al. 1995. Dietary intervention study to assess estrogenicity of dietary soy among postmenopausal women. J Clin Endocrinol Metab 80: 1685-1690.

Barnes, S., Coward, L., Kirk, M., and Sfakianos, J. 1998. HPLC-mass spectrometry analysis of isoflavones. Proc Soc Exp Biol Med 217: 254-262.

Bennet, A., Lacaze, J.C., Caron, P., Berrada, R., Barbe, P., and Louvet, J.P. 1991. Correlations between mean LH levels and LH pulse characteristics: differences between normal and anovulatory women. Clin Endocrinol (Oxf) 35: 431-437.

Bennetts, H.W., Underwood, E.J., and Shier, F.L. 1946. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Aust Vet J 22: 2-12.

Benson, G.K., Cowie, A.T., and Hosking, Z.D. 1961. Mammogenic activity of miroestrol. J Endocrin 21: 401-409.

Berga, S.L., Mortola, J.F., Girton, L., Suhs, B., Laughlin, G., and Pham, P. 1989. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 68: 301-308.

Bernstein, L. et al. 1990. Serum hormone levels in pre-menopausal Chinese women in Shanghai and white women in Los Angeles: results from two breast cancer case-control studies. Cancer Causes Control 1: 51-58.

Blum, S.C., Heaton, S.N., Bowman, B.M., Hegsted, M., and Miller, S. 2003. Dietary soy protein maintains some indices of bone mineral density and bone formation in aged ovariectomized rats. J Nutr 133: 1244-1249.

Boker, L.K., Schouw,Y.T.V., De Kleijn, M.J.J.D., Jacques, P.F., Grobbee, D.E., and Peeters, P.H.M. 2002. Intake of dietary phytoestrogens by Dutch women. Nutr Epidemiol 132: 1319-1328.

Bounds, D.G., and Pope, G.S. 1960. Light-absorption and chemical properties of miroestrol, the oestrogenic substance of *Pueraria mirifica*. J Chem Soc : 3696-3705.

Boyar, R., Finkelstein, J.W., Roffwarg, H., Kapen, S., Weitzman, E.D., and Hellman, L. 1972. Synchronization of augmented luteinizing hormone secretion with sleep during puberty. N Engl J Med 287: 582-586.

Bray, M.J., et al. 2001. Short-term estradiol replacement in postmenopausal women

selectively mutes somatostatin's dose-dependent inhibition of fasting growth hormone secretion. J Clin Endocrinol Metab 86: 3143-3149.

Bulintarathikul, Y. 1978. Effects of *Pueraria mirifica* crude extract on the serum calcium and the tibial cartilagenous plate in the gonado-parathyroidectomized weanling rat. Master's Thesis, Department of Zoology, Graduate School, Chulalongkorn University.

Burton, J.L., and Wells, M. 2002. The effect of phytoestrogens on the female genital tract. J Clin Pathol 55: 401-407.

Busby, M.J., et al. 2002. Clinical characteristics and pharmacolinetics of purified soy isoflavones: single-dose administration to health men. Am J Clin Nutr 75: 126-136.

Cano, A., and Aliaga, R. 1995. Characteristics of urinary luteinizing hormone (LH) during the induction of LH surge of different magnitude in blood. Hum Reprod 10: 63 -67.

Cantero, A., Sancha, J.L., Flores, J.M., Rodriguez, A., and Gonzalez, T. 1996. Histopathological changes in the reproductive organs of manchego ewes grazing on lucerne. Zentralbl Vereinarmad [A] 43: 325-330.

Casanova, M., You, L., Gaido, K.W., Archibeque-Engle, S., Janszen, D.B., and Heck, H. 1999. Developmental effects of dietary phytoestrogens in spraque-dawley rats and interactions of genistein and daidzein with rat estrogen receptor α and β in vitro. Toxicological Science 51: 236-244.

Cassidy, A., Bingham, S., and Setchell, K.D.R. 1994. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr 60: 333-340.

Cassidy, A., Bingham, S., and Setchell, K.D.R. 1995. Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. Br J Nutr 74: 587-601.

Casson, P.R., Elikind-Hirsch, K.E., Buster, J.E., Hornsby, P.J., Carson, S.A., and Snabes, M.C. 1997. Effect of postmenopausal estrogen replacement on circulating androgens. Obstet Gynecol 90: 995-998.

Chansakaow, S., et al. 2000. Isoflavonids from *Pueraria mirifica* and their estrogenic activity. Planta Med 66: 572-575.

Chansakaow, S., Ishikawa, T., Seki, H., Sekine, K., Okada, M., and Chaichantipyuth, C. 2000. Identification of deoxymiroestrol as the actual rejuvenating principle of " Kwao Keur " *Pueraria mirifica*. The known miroestrol may be an artifact. J Nat Prod 63: 173-175.

Chongthammakun, S., and Terasawa, E. 1993. Negative feedback effects of estrogen on luteinizing hormone-releasing hormone release occur in pubertal, but not prepubertal, ovariectomized female rhesus monkeys. Endocrinology 132: 735-743.

Chuaychoo, A., Junyatum, U., Anuntalabhochai, S., and Smitasiri, Y. 1984. Toxic effects of white Gwow (*Pueraria mirifica*) in Japanese quails. J Sci Fac CMU 11: 46-55 (in Thai).

Colborn, T., Dumanaski, D., and Myers, J.P. 1996. Our stolen future. New York: Penquin Books.

Delmas, P.D., Stenner, D., Wahner, H.W., Mann, K.G., and Riggs, B.L. 1983. Increase in serum bone gamma-carboxyglutamic acid protein with aging in women. Implications for the mechanism of age-related bone loss. J Clin Invest 71: 1316-21.

Draper, C.R., Edel, M.J., Dick, I., Randall, A.G., Martin, G.B., and Prince, R. 1997. Phytoestrogens reduce bone loss and bone resorption in oophorectomized rats. J Nutr 127: 1795-1799.

Duncan, A.M., Berz, B.E., Xu, X., Nagel, T., Phipps, W., and Kurzer, M.S. 1999. Soy isoflavones exert modest hormonal effects in premenopausal women. J Clin Endocrinol Metab 84: 192-197.

Duncan, A.M., Underhilll, K.E.W., Xu, X., Lavalleur, J., Phipps, W.R., and Kurzer, M.S. 1999. Modest hormonal effects of soy isoflavones in postmenopausal women. J Clin Endocrinol Metab 84: 3479-3484.

Faber, K.A. and Hughes, J.R. 1991. The effect of neonatal exposure to diethylstilbestrol, genistein, and zearalenone on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. Biol Reprod 45: 649-653.

Franke, A.A., Custer, L.J., Cerna, C.M., and Narala, K. 1995. Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. Proc Soc Exp Biol Med 208: 18-26.

Fredricks, G.K., Kincaid, R.L., Bondioli, K.R., and Wright, R.W. 1981. Ovulation rates and embryo degeneracy in female mice fed the phytoestrogen, coumestrol. Proc Soc Exp Biol Med 167: 237-241.

Frohman, L.A. 1995. Endocrinology and metabolism. Third edition. New York: McGraw-Hill.

Gao, Y.H., and Yamaguchi, M. 1999. Anabolic effect of daidzein on cortical bone in tissue culture: comparison with genistein effect. Mol Cell Biochem. 194: 93-97.

Gao, Y.H., and Yamaguchi, M. 1999. Inhibitory effect of genistein on osteoclast-like

cell formation in mouse marrow cultures. Biochem Pharmacol 58: 767-772.

Gennari, C., Agunsdei, D., Civitelli, R., Nardi, P., and Montagnani, M. 1987. Effects of estrogens on vitamin D metabolism in postmenopausal osteoporotic women. P. Fioretti et al (eds.), Postmenopausal Hormonal Therapy: Benefits and Risks. pp. 243-253. New York: Raven Press.

Gianotti, J., et al. 2003. Suppression and recovery of LH secretion by a potent and selective GnRH-receptor antagonist peptide in healthy early follicular-phase women are mediated via selective control of LH secretory burst mass. Clinical Endocrinology 59: 526-532.

Gibori, G., Anrczak, E., and Rothchild, I. 1977. The role of estrogen in regulation of luteal progesterone secretion in the rat after day 12 of pregnancy. Endocrinology 100: 1483-1495.

Gill, S., Sharpless, J.L., Rado, K., and Hall, J. 2002. Evidence that GnRH decreased with gonadal steroid feedback but increase with age in postmenopausal women. J Clin Endocrinol Metab 87: 2290-2296.

Goldstein, D., et al. 1982. Correlation between estradiol and progesterone in cycles with luteal phase deficiency. Fert Steril 37: 348-354.

Granner, D.K. 1988. Hormones that regulate calcium metabolism. In R.K. Murry, D.K. Granner, P.A. Mayes, V.W. Rodwell (eds.), Harpers's Biochemistry, pp. 502-523. California: Appleton and Lange.

Gustafsson, J.A. 1999. Estrogen receptor β - new dimension in estrogen mechanism of action. J Endocrinol 163: 379-383.

Harrison, M.R., Phillippi, P.P., Swan, K.F., and Henson, M.C. 1999. Effect of genistein on steroid hormone production in the pregnant rhesus monkey. Proc

Soc Exp Biol Med 222: 78-86.

Head, K.A. 1997. Isoflavones and other soy constituents in human health and disease. Alt Med Rev 2: 433-450.

Hodgen, G.D., Wilks, J.W., Vaitukaitis, J.L., Chen, H-C., Papkoff, H., and Ross, G.T. 1976. Anew radioimmunoassay for follicle-stimulating hormone in macaques: ovulatory menstrual cycles. Endocrinology 99: 137-145.

Hopert, A.C., Beyer, A., Frank, K., Strunck, E., Wunsche, W., and Vollmer, G. 1998. Characterization of estrogenicity of phytoestrogens in an endometrial-derived experimental model. Environ Health Perspect 106: 581-586.

Horn-Ross, P.L., et al. 2000. Assessing phytoestrogens exposure in epidemiologic studies: development of a database (United States). Cancer Causes Control 11: 289-298.

Hotchkiss, J., Dierschke, D.J., Butler, W.R., Fritz, G.R., and Knobil, E. 1982. Relation between levels of circulating ovarian steroids and pituitary gonadotropin content during the menstrual cycle of the rhesus monkey. Biol Reprod 26: 241-248.

Hoyodom, M. 1971. Constituents of the tuberous roots of *Pueraria mirifica*. Master's thesis. Department of Chemistry, Graduate School, Chulalongkorn University (in Thai).

Hughes, C.L., Chakinala, M.M., Reece, S.G., Miller, R.N., Schomberg, D.W., and Basham, K.B. 1991. Acute and subacute effects of naturally occurring estrogens on luteinizing hormone secretion in the ovariectomized rat: part 2. Repro Toxicol 5: 133-137.

Ingham, J.L., Markham, K.R., Dziedzic, S.Z., and Pope, G.S. 1986. Puerarin 6-o-β-

apiofuranoside, A C-glycosylisoflavone o-glycoside from *Pueraria mirifica*. Phytoechemistry 25: 1772-1775.

Ingham, J.L., Tahara, S., and Dziedzic, S.Z. 1986. A chemical investigation of *Pueraria mirifica*. Z Naturforsch 41c: 403-408.

Ingham, J.L., Tahara, S., and Dziedzic, S.Z. 1988. Minor isoflavones from the roots of *Pueraria mirifica*. Z Naturforsch 44c: 724-726.

Ingham, J.L., Tahara, S., and Dziedzic, S.Z. 1989. Coumestans from the roots of *Pueraria mirifica*. Z Naturforsch 43c: 5-10.

Ishimi, Y., et al. 1999. Selective effects of genistein, a soybean isoflavones, on B-lymphopoiesis and bone loss caused by estrogen deficiency. J Endocrinol 140: 1893-1900.

Johnson, M.H., and Everitt, B.J. 1997. Essential Reproduction. 4th ed. London: Blackwell Science

Jones, H.E.H., and Pope, G.S. 1960. A study of the action of miroestrol and other oestrogens on the reproductive tract of the immature female mouse. J Endocrin 20: 229-235.

Karr, S.C., Lampe, J.W., Hutchins, A.M., and Slavin, J.L. 1997. Urinary isoflavonoid excretion is dose dependent at low to moderate levels of soy-protein consumption. Am J Clin Nutr 66: 46-51.

Kashemsanta, L., Suvatabandhu, K., Bartlett, S., and Pope, G.S. 1957.The oestrogenic substance (miroestrol) from the tuberous roots of *Pueraria mirifica*. Proceeding of the Ninth Pacific Congress Scientific Association 9: 37 (in Thai).

Kettle, L.M., et al. 1991. Follicular arrest during the midfollicular phase of the menstrual cycle: A gonadotropin-releasing hormone antogonist imposed

follicular-follicular transition. J Clin Endocrinol Metab 73: 644-649.

Khosla, S., Atkinson, E.J., Melton, L.J., and Riggs, B.L. 1997. Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: A population-based study. J Clin Endocrinol Metab 82: 1522-1527.

King, R.A. 1998. Daidzein conjugates are more bioavailable than genistein conjugates in rats. Am J Clin Nutr 68(suppl): 1496s-1499s.

King, R.A., and Bursill, D.B. 1998. Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. Am J Clin Nutr 67: 867-872.

Knight, D.C., and Eden, J.A. 1996. A review of the clinical effects of phytoestrogens. Obstet and Gynecol 87: 897-904.

Krajewski, S.J., Abel, T.W., Voytko, M.L., and Rance, N.E. 2003. Ovarian steroids differentially modulate the gene expression of gonadotropin-releasing hormone neuronal subtypes in the ovariectomized cynomolgus monkey. J Clin Endocrinol Metab 88, 665-662.

Kuiper, G.G.J.M, et al. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 138: 863-870.

Kurzer, M.S. 2000. Hormonal effects of soy isoflavones: studies in premenopausal and postmenopausal women. J Nutr 130: 660S-661S.

Lampe, J.W. 2003. Isoflavonid and lignan phytoestrogens as dietary biomarkers. J Nutr 133: 956s-964s.

Langkalichan, Y., and Smitasiri, Y. 1985. Effects of white gwow (*Pueraria mirifica*) on

reproduction in male albino rat. 11th Congress on Science and Technology of Thailand 11: 334.

Levy, J.R., Faber, K.A., Ayyash, L., and Hughes, C. 1995. The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. Proc Soc Exp Biol Med 208: 60-66.

Li, H., Chen, J., Overstreet, J.W., Nakajima, S., and Lasley, B.L. 2002. Urinary follicle-stimulating hormone peak as a biomarker for estimating the day of ovulation. Fertil Steril 77: 961-966.

Liggins, J., Grimwood, R., and Bingham, S.A. 2000. Extraction and quantification of lignan phytoestrogens in food and human samples. Analytical Biochemistry 287: 102-109.

Lindsay, R., Bush, L.T., Grady, D., Speroff, L., and Lobo, R.A. 1996. Estrogen replacement in menopause. J Clin Endocrinol Metab 81: 3829-3838.

Lu, L.J., and Anderson, K.E. 1998. Sex and long-term soy diet affected the metabolism and excretion of soy isoflavones in humans. Am J Clin Nutr 68: 1500s-1504s.

Lu, L.J., Anderson, K.E., Grady, J.J., Kohen, F., and Nagamani, M. 2000. Decrease ovarian hormones during a soya diet: implications for breast cancer prevention. Cancer Res 60: 4112-4121.

Lu, L.J.W., Anderson, K.E., Grady, J.J., and Nagamani, M. 1996. Effects of soya consumption for one month on steroid hormones in premenopausal women: implications for breast cancer risk reduction. Cancer Epidemiol Biomarkers Prev 5: 63-70.

MacLatchy, D.L., and Kraak, G.J.V.D. 1995. The phytoestrogen β-sitosterol alters the

reproductive endocrine status of goldfish. Toxicol Apppl. Pharmacol. 134: 305-312.

Mais, V., Kazer, R.R., Cetel, N.S., Rivier, J., Vale, W., and Yen, S.S. 1986. The dependency of folliculogenesis and corpus luteum function on pulsatile gonadotropin secretion in cycling women using a gonadotropin-releasing hormone antagonist as a probe. J Clin Endocrinol Metab 62: 1250-1255.

Makela, S., Poutanen, M., Lehtimaki, J., Kostian, M.L., Santti, R., and Vihko, R. 1995. Estrogen-specific 17 beta-hydroxysteroid oxidoreductase type 1 (E.C. 1.1.1.62) as a possible target for the action of phytoestrogens. Proc Soc Exp Biol Med 208: 51 - 59.

Malaivijinond, S., and Varavudhi, P. 1998. Evidence for morphine-induced galactorrhea in male cynomolgus monkeys. J Med Primatol 27: 1-9.

Malaivijitnond , S., et al. 2000. Changes of aged related factors in various age of cynomolgus monkeys and after treated with *Pueraria mirifica* phytoestrogens. COE International Symposium Development and Aging of Primates : 68.

Martin, P., Horwitz, K.B., Ryan, D.S., and McGuire, W.L. 1978. Phytoestrogens interaction with estrogen receptors in human breast cancer cells. Endocrinology 103: 1860-1867.

McGarvey, C., et al. 2001. Phytoestrogens and gonadotropin-releasing hormone pulse generator activity and pituitary luteinizing hormone release in the rat. Endocrinology 142: 1201-1208.

Mckane, W.R., Khosla, S., Risteli, J., Robins, S.P., Muhs, J.M., and Riggs, B.L. 1997. Role of estrogen deficiency in pathogenesis of secondary hyperparathyroidism and increased bone resorption in elderly women. Proc Assoc Am Physicians 109: 174-180.

McKenna, M.J., and Frame, R. 1987. Hormonal influences on osteoporosis. The American Journal of Medicine 82(suppl 1B): 61-66.

Medlock, K.L., Branham, W.S., and Sheehan, D.M. 1995. Effects of coumestrol and equol on the developing reproductive tract of the rat. Proc Soc Exp Biol Med 208: 67-71.

Mei, J., Yeung, S.S.C., and Kung, A.W.C. 2001. High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. J Clin Endocrinol Metab 86: 5217-5221.

Montgomery, G.W., Martin, G.B., Bars, J.L., and Pelletier, J. 1985. Gonadotrophin release in ovariectomized ewes fed different amounts of coumestrol. J Reprod Fertil 73: 457-463.

Muangdet, N., and Anuntalabhochai, S. 1985. Effects of low doses of white gwow (*Pueraria mirifica*) of female Japanese quails. J Sci Fac CMU 12: 29-41 (in Thai).

Muangman, V., and Cherdshewasart, W. 2001. Clinical Trial of the phytoestrogen-rich Herb, *Puerarira mirifica* as a crude drug in the treatment of symptoms in menopausal women. Siriraj Hosp Gaz 53: 300-309.

Murkies, A.L., Wilcox, G., and Davis, S.R. 1998. Phytoestrogens. J Clin Endocrinol Metab 83: 297-303

Nagata, C., Takatsuka, N., Inaba, S., Kawakami, N., and Shimizu, H. 1998. Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. J Natl Cancer Inst 90: 1830-1835.

Nakai, Y., Plant, T.M., Hess, D.L., Keogh, E.J., and Knobil, E. 1978. On the sites of the negative and positive feedback action of estradiol in the control of

gonadotropin secretion in the rhesus monkey. Endocrinology 102: 1008-1014.

Nicholls, J., Lasley, B.L., Nakajima, S.T., Setchell, K.D.R., and Schneeman, B.O. 2002. Effects of soy consumption on gonadotropin secretion and acute pituitary responses to gonadotropin-releasing hormone in women. J Nutr 132: 708-714.

O'Byrne, K.T., et al. 1993. Ovarian control of gonadotropin hormone-releasing hormone pulse generator activity in the rhesus monkey: duration of the associated hypothalamic signal. Neuroendocrinology 57: 588-592.

Onoe, Y., Miyaura, C., Ohta, H., Nozawa, S., and Suda, T. 1997. Expression of estrogen receptor β in rat bone. J Endocrinol 138: 4509-4512.

Overlie, I., Moen, M.H., Holte, A., and Finset, A. 2002. Androgens and estrogens in relation to hot flushes during the menopausal transition. Maturitas 41: 69-71.

Pacifici, R. 1988. Editorial: cytokines, estrogen and postmenopausal osteoporosis – the second decade. Endocrinology 139: 2659-2661.

Panriansaen, R. 2000. Characterization of *Pueraria mirifica* populations from various parts of Thailand. Master's Thesis, Chulalongkorn University.

Park, J.S., Goldsmith, L.T., and Weiss, G. 2002. Age-related changes in the regulation of luteinizing hormone secretion by estrogen in women. Exp Bio Med 227: 445-464.

Patisaul, H.B., Whitten, P.L., and Yong, L.J. 1999. Regulation of estrogen receptor beta mRNA in the brain: opposite effect of 17 β-estradiol and the phytoestrogen, coumestrol. Molecular Brain Research 67: 165-171.

Pau, K.Y., Gliessman, P.M., Hess, D.L., Ronnekleiv, O.K., Levine, J.E., and Spies, H.G. 1990. Acute administration of estrogen suppresses LH secretion without altering GnRH release in ovariectomized rhesus macaques. Brain Research

517: 229-235.

Persky, V.W., et al. 2002. Effect of soy protein on endogenous hormones in postmenopausal women. Am J Clin Nutr 75: 145-153.

Petrakis, N.L., et al. 1996. Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women. Cancer Epidemiol Biomarker Prev 10: 785-794.

Phipps, W.R., Martini, M., Lampe, J., Slavin, J.L., and Kurzer, M. 1993. Effect of flax seed ingestion on the menstrual cycle. J Clin Endocrinol Metab 77: 1215-1219.

Pinkerton, J.V., and Santen, R. 1999. Alternatives to the use of estrogen in postmenopausal women. Endocr Rev 20: 308-320.

Pisetpakasit, R. 1976. A Pharmaconostical study of *Pueraria mirifica*. Master's thesis, Department of Pharmacognosy. Graduate School. Chulalongkorn University (in Thai).

Pope, G.S., Grundy, H.M., Jones, H.E.H., and Tait, S.A.S. 1958. The estrogenic substance (miroestrol) from the tuberous roots of *Pueraria mirifica*. J Endocrin 17: 15-16.

Potter, S.M., Baum, J.A., Teng, H., Stillman, R.J., Shay, N.F., and Erdman, J.W. 1998. Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 68 (suppl): 1375s-1379s.

Register, B., Bethel, M.A., Thompson, N., Blohm, W.P., Ayyash, L., and Hughes, C. 1995. The effect of neonatal exposure to diethylstilbestrol, coumestrol and β-sitosterol on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat. Proc Soc Exp Biol Med 208: 72-77.

Rhoades, R., and Pflnzer, R. 1996. Human physiology. Third edition, Tokyo:

Saunders College Publishing.

Rhodes, L., et al. 1997. Effects of administration of testosterone, dihydrotestosterone, estrogen and fadrozole, an aromatase inhibitor, on sex colour in intact male rhesus macaques. J Reprod Fertil 111: 51-57.

Riggs, R.L., Khosla, S., and Melton, L.J. 2002. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23: 279-302.

Santell, R.C., Chang, Y.C., Nair, M.G., and Helferich, W.G. 1997. Dietary genistein exerts estrogenic effects upon the uterus, mammary gland, and the hypothlamic/pituitary axis in rats. J Nutr 127: 263-269.

Scheweiger, U., Laessle, R.G., Tuschl, R.J., Broocks, A., Krusche, T., and Pirke, K.M. 1989. Decreased follicular phase gonadotropin secretion is associated with impaired estradiol and progesterone secretion during the follicular and luteal phases in normally menstruation women. J Clin Endocrinol Metab 68: 888-892.

Schmidt, P.J., Murphy, J.H., Haq, N., Danaceau, M.A., and Clari, L.S. 2002. Basal plasma hormone levels in depressed perimenopausal women. Psychoneuroendocrinology. 27: 907-920.

Setchell, K.D.R. 1998. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. Am J Clin Nutr 68(suppl): 133s-1346s.

Setchell, K.D.R., et al. 2003. Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women. Am J Clin Nutr 77: 411-419.

Setchell, K.D.R., Lawson, A.W., Mitchell, F.L., Adlercreutz, H., Kirk, D.N., and Axelson, M. 1980. Lignans in man and in animal species. Nature 287: 740-742.

Sheehan, D.M., Medlock, K.L., and Burroughs, C.D. 1996. Permanent effects of neonatal coumestrol and equol treatment in rats. Second international Symposium on the Role of Soy in Preventing and Treating Chronic Diseases, Brussells, Belgium 2: 307.

Shimizu, H., Ross, R.K., Bernstein, L., Pike, M.C., and Henderson, B.E. 1990. Serum oestrogen levels in postmenopausal women: comparison of American whites and Japanese in Japan. Br J Cancer 62: 451-453.

Shughrue, P.J., Lane, M.V., Scrimo, P.J., and Merchenthaler, I. 1998. Comparative distribution of estrogen receptor-α (ER-α) and β (ER-β) mRNA in the rat pituitary, gonad, and receptor tract. Steroids 63: 498-504.

Shutt, D.A., and Cox, R.I. 1972. Steroid and phytoestrogen binding to sheep uterine receptors in vitro. J Endocrinol 52: 299-310.

Silverberg, S.J., and Bilezikian, J.P. 1994. Parathyroid function and responsiveness in osteoporosis. In J.P., Bilezikian, R., Marcus, and M.A., Levine (eds.), The Parathyroid Basic and Clinical Concepts, pp 805-812. New York: Raven Press.

Smitasiri, Y., and Sakdarat, S. 1995. Improvement and development of suitable form of *Pueraria mirifica* for birth control in pigeon. Available from: http://www.science. cmu.ac.th/ s.report/page14. Html (in Thai).

Smitasiri, Y., Junyatum, U., Songjitsawad, A., Sripromma, P., Trisrisilp, S., and Anuntalabhochai, S. 1986. Postcoital antifertility effects of *Pueraria mirifica* in rats. J Sci Fac CMU 13: 19-28 (in Thai).

Smitasiri, Y., Pangjit, S., and Anuntalabhochai, S. 1989. Inhibition of lactation in lactation rats with *Pueraria mirifica* compared with estrogen. J Sci Fac CMU 16: 7-11 (in Thai).

Smith, E.L., Hill R.T., Lehman, I.R., Lefkowitz, R.J., Handler, P., and White, A. 1983. Principles of biochemistry: mammalian biochemistry. 7th editions. Auckland: Mc-Graw-Hill Inc.

Smith, J.F., Hagyscg, K.T., Brunswick, L.F.C., and Kelly, R.W. 1979. Coumestans in lucernce and ovulation in ewes. N Z J Agric Res 22: 411-416.

Songkaew, D., and Smitsari, Y. 1985. Effects of white gwow (*Pueraria mirifica*) on mid and late pregnancy in rats. 11th Congress on Science and Technology of Thailand 11: 334 (in Thai).

Soules, M.R., McLachlan, R.I., Ek, M., Dahl, K.D., Cohen, N.L., and Bremner, W.J. 1989. Luteal phase deficiency: characterization of reproductive hormones over the menstrual cycle. J Clin Endocrinol Metab 69: 804-820.

Stock, J.L., Coderre, J.A., and Mallette, L.E. 1985. Effects of a short course of estrogen on mineral metabolism in postmenopausal women. J Clin Endocrinol Metab 61: 596-600.

Sufi, S.B., Donaldson, A., and Jeffcoate, S.L. 1986. WHO matched reagent program method manual. 14th ed. London: WHO collaborating center for immunoassay.

Sukhavachana, D. 1941. Oestrogenic principle of *Butea Superba*. Preliminary Report 24: 83-92 (in Thai).

Tahara, S., Ingham, J.L., and Dziedzic, S.Z. 1987. Structure elucidation of kwakhurin, a new prenylated isoflavone from *Pueraria mirifica* roots. Ibid 42: 510-518.

Tang, B.Y., and Adams, N.R. 1980. Effect of equol on estrogen receptors and on synthesis of DNA and protein in the immature rat uterus. J Endocrinol 85: 291-297.

Taya, K., Watanabe, G., and Sasamoto, S. 1985. Radioimmunoassay for

progesterone, testosterone and estradiol 17 β using 125-iodohistamine radioligands. Jpn J Anim Reprod 31: 186-197.

Tham, D.M., Gardner, C.D., and Haskell, W.L. 1998. Potenital health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. J Clin Endocrinol Metab 83: 2223-2235.

Toivola, P.T.K., Bridson, W.E., and Robinson, J.A. 1978. Effect of luteinizing hormone, follicle-stimulating hormone and testosterone in adult male rhesus monkey. Endocrinology 108: 1815-1821.

Tonetta, S.A., and Dizerega, G.S. 1989. Intragonadal regulation of follicular maturation. Endocr Rev 10: 205-229.

Trisomboon, H., Malaivijitnond S., Taya, K., Watanabe, G, and Suzuki, J. 2002. Potential role of *Pueraria mirifica* on reproductive hormones in aged female cynomolgus monkeys. Forth Intercongress Symposium of the Asia and Oceania Society for Comparative Endocrinology 4: O-19.

Trisomboon, H., Malaivijitnond S., Watanabe, G., and Taya, K. 2004. Estrogenic effects of *Pueraria mirifica* on the menstrual cycle and hormones related ovarian functions in cyclic female cynomolgus monkeys. J Pharmacol Sci 94: 51-59.

Trisomboon, H., Malaivijitnond, S., Watanabe, G., and Taya, K. 2002. Long-term effects of *Pueraria mirifica* on gonadotropins levels in adult female cyomolgus monkeys. 7th Biological Science Graudate Congress 7: 43.

Tsutsumi, N. 1995. Effects of coumestrol on bone metabolism on organ culture. Biol Pharm Bull 18: 1012-1015.

Uesugi, T., Fukui Y., and Yamori, Y. 2002. Beneficial effects of soybean isoflavone

supplementation on bone metabolism and serum lipids in postmenopausal Japanese women: A four-week study. J Am Coll Nutr 21: 97-102.

Varma, T.R., Everard, D., and Hole, D. 1985. Effect of natural estrogen on the serum level of follicle-stimulating hormone (FSH), estradiol and estrone in post-menopausal women an its effect on endometrium. Acta Obstet Gynecol Scand 64: 105-109.

Vincent, A., and Fitzpatrick, L.A. 2000. Soy isoflavones: Are they useful in menopause?. Mayo Clin Proc 75: 1174-1184.

Vincent, A., Riggs, B.L., Atkinson, E.J., Oberg, A.L., and Khosla, S. 2003. Effect of estrogen replacement therapy on parathyroid hormone secretion in elderly postmenopausal women. Menopause 10: 165-171.

Voet, D., Voet, J.G., and Pratt, C. 1999. Fundamentals of Biochemistry. New York: John Wiley and Sons.

Wakai, K., et al. 1999. Dietary intake and source of isoflavones among Japanese. Nutri Cancer 33: 139-145.

Wanadorn, W. 1933. A reputed rejuvenator. J. Siam Soc Nat Hist Suppl. 9: 145-147 (in Thai).

Watanabe, S., et al. 1998. Pharmacokinetics of soybean isoflavones in plasma, urine and feces men after ingestion of 60 g baked soybean powder (Kinako). J Nutr 128: 1710-1715.

Weihua, Z., et al. 2000. Estrogen receptor (ER) β, a modulator of ERα in the uterus. Proc Natl Acad Sci USA 97: 5936-5941.

Whitehead, S.A., Cross, J.E., Burden, C., and Lacey, M. 2002. Acute and Chronic effects of genistein, tyrphostin and lavendustin A on steroid synthesis in

luteinized human granulose cells. Hum Reprod 17: 589-594.

Whitten, P.L., Lewis, C., and Naftolin, F. 1993. A phytoestrogen diet induces the premature anovulatory syndrome in lactationally exposed female rats. Biol Reprod 49: 1117-1121.

Whitten, P.L., Lewis, C., Russell, E., and Naftolin, F. 1995. Phytoestrogen influences on the development of behavior and gonadotropin function. Proc Soc Exp Biol Med 208: 82-86.

Wilcox, G., Wahlqvist, M.L., Burger, H.G., and Medley, G. 1990. Oestrogenic effects of plant foods in postmenopausal women. Br Med J 301: 905-906.

Wildt, L., Hausler, A., Hutchison, J.S., Marshall, G., and Knobil, E. 1981. Estradiol as a gonadotropin releasing hormone in the rhesus monkey. Endocrinology 108: 2011-2013.

Winner, K.K., Yanovski, J.A., Sarani, B., and Cutler, G.B. 1998. A randomized, cross-over trial of once-daily versus twice-daily parathyroid hormone 1-34 in treatment of hypoparathyroidism. J Clin Endocrinol Metab 83: 3480-3486.

Woller, M.J., Everson-Binotto, G., Nichols, E., Acheson, A., Keen, K.L., Bowers, C.Y., and Terasawa, E.I. 2002. Aging-related changes in release of growth hormone and luteinizing hormone in female rhesus monkeys. J Clin Endocrinol Metab 87: 5160-5167.

Wong, C., Lai, T., Hilly, J.M., Stewart, C.E.H., and Farndon, J.R. 2002. Selective estrogen receptor modulators inhibit the effects of insulin-like growth factors in hyperparathyroidism. Surgery 132: 998-1007.

Wu, A.H., Stanczyk, F.Z., Hendrich, S., Murphy, P.A., Zhang, C., Wan, P., and Pike, M.C. 2000. Effects of soy foods on ovarian function in premenopausal women. Br J Cancer 82: 1879-1886.

Wuttke, W., et al. 2003. Phytoestrogens: endocrine disrupters or replacement for hormone replacement therapy? Marturitas 44(suppl.1): S9-S20.

Xu, S., Harris, H.S., Wang, W., Murphy, P.A., and Hendrich, S. 1995. Bioavailablitiy of soybean isoflavones depends upon gut microflora in women. J Nutr 125: 2307-2315.

Yamaguchi, M., and Gao, Y.H. 1998. Inhibitory effect of genistein on bone resorption in tissue culture. Biochem Pharmacol 55: 71-76.

Yamori, Y., et al. 2002. Soybean isoflavones reduce postmenopausal bone resorption in female Japanese immigrants in Brazil: A Ten-week study. J Am Coll Nutr 21: 560-563.

Appendices

J Pharmacol Sci 94, 51 – 59 (2004)

Journal of Pharmacological Sciences

Full Paper

Estrogenic Effects of *Pueraria mirifica* on the Menstrual Cycle and Hormone-Related Ovarian Functions in Cyclic Female Cynomolgus Monkeys

Hataitip Trisomboon[1,2], Suchinda Malaivijitnond[2,*], Gen Watanabe[3,4], and Kazuyoshi Taya[3,4]

[1]*Biological Science Ph.D. Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Primate Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
[3]*Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan*
[4]*Department of Basic Veterinary Science, The United Graduated School of Veterinary Science, Gifu University, Gifu 501-1193, Japan*

Received September 11, 2003; Accepted November 27, 2003

Abstract. This study investigated the estrogenic effect of *Pueraria mirifica* (*P. mirifica*) on menstrual cycle length and hormone-related ovarian function. Nine normal cyclic monkeys (*Macaca fascicularis*) were separated into 3 groups; each group was force fed with a single dose of 10, 100, and 1,000 mg of *P. mirifica*. The experimental schedule was separated into the pre-treatment and post-treatment periods. Blood samples were collected on days 3, 9 – 14, 19, 24, 29, and every 10 days until the next menstruation for one and two menstrual cycles during two consecutive periods and assayed for serum levels of gonadotropins and ovarian hormones. The result showed a significant increase in lengths of the follicular phase and total menstrual cycle in monkeys treated with 1,000 mg of *P. mirifica*, but no change in menstrual cycle length in monkeys treated with 10 and 100 mg of *P. mirifica*. Serum levels of follicle stimulating hormone, luteinizing hormone, estradiol, progesterone, or immunoreactive-inhibin did not change during the first and second menstrual cycles of the post-treatment period for all monkey groups. Our findings demonstrate that although changes in hormonal levels could not be observed in this study, a single dose of 1,000 mg of *P. mirifica* can disturb ovarian function and menstrual cycle in monkeys.

Keywords: *Pueraria mirifica*, phytoestrogen, monkey, reproductive hormone, menstrual cycle

Introduction

Phytoestrogens, which are structurally and functionally similar to 17β-estradiol, are naturally occurring phytochemicals found in plants and plant products (1). The principle classes of phytoestrogens are isoflavones (including daidzein and genistein), coumestans (including coumestrol), and lignans (including esterodiol and enterolactone) that are mainly found in soy and soy food (2 – 5). Estrogenic effects of phytoestrogens were reported on hormonal and reproductive disturbances in both animals (6 – 9) and humans (10 – 12) to include causing infertility in sheep (13), the reduction of ovulation rate in mice (14), and disruption of reproductive hormones and ovarian function in cyclic women (15 – 18). In addition, epidemiological studies have shown that pre- and postmenopausal Japanese and Chinese women who consumed high amounts of isoflavones from soy had a decrease in serum levels of estrone and estradiol (19 – 21).

Puearaia mirifica (*P. mirifica*) is an indigenous Thai herb of the family Leguminoceae. Its tuberous roots also contain phytoestrogenic substances including miroestrol (22), puerarin (23), deoxymiroestrol, kwakhurin (24, 25), and other phytoestrogens that belong to the isoflavone and coumestrol class (26 – 28). Miroestrol compounds isolated from the roots of *P. mirifica* prevented the implantation of blastocysts, promoted uterine weight, vaginal growth, increased

*Corresponding author. FAX: +66-22185386
E-mail: Suchinda.M@chula.ac.th

vaginal fluid in normal female rats (22), and produced cornification of the vaginal epithelium in ovariectomized-adrenalectomized rats (29, 30), but did not stimulate the secretion of endogenous estrogen by the ovaries or the adrenal gland (29). Miroestrol also exhibited mammogenic potency in both ovariectomized rats and mice by restoring the mammary duct growth as estradiol did (22). The potency of subcutaneouly injected miroestrol is about 0.7 times that of estradiol and twice as potent as estrone (31).

In recent years, *P. mirifica* has been widely used in pre- and postmenopausal women. They believe that phytoestrogens contained in the plant, especially isoflavones, support female characteristics including breast and skin appearances as well as improve bone structure and the cardiovascular system. However, *P. mirifica* may disturb reproductive function and menstrual cycle in women.

The aim of this study was to examine the endocrine-modulating effect of *P. mirifica* on menstrual cycle length and hormones related ovarian function in normal cyclic monkeys. Female cynomolgus monkeys (*Macaca fascicularis*) were used as the alternative model to study reproductive hormones and function because the monkeys have physiological systems including hormonal pattern, ovarian cycle, and reproductive function that are similar to those of humans (32, 33). Moreover, to study the effects of *P. mirifica* containing phytoestrogens on changes of serum levels of hormones in humans is very difficult due to uncontrolled diet and follow-up factors.

Materials and Methods

Animals

Seventeen adult female cynomolgus monkeys (*Macaca fascicularis*) with regular menstrual cycles for at least 4 consecutive months, 26 – 37 days in length and weighing 4 – 6.5 kg prior to the study, were used. The first day of menstrual bleeding was considered as day 1 of the menstrual cycle. The monkeys were housed in individual cages at the Primate Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. Lighting conditions of the animal room were controlled (12:12 h light to dark cycle). Temperature (25 – 30°C) and humidity (37 – 41%) fluctuated slightly depending on the season. The monkeys were fed daily with monkey chow (Pokaphan Animal Feed Co., Ltd., Bangkok, Thailand) in the morning (09:00 – 10:00 h) and supplemented with fresh fruits in the afternoon (14:00 – 15:00 h). The experimental protocol was approved in accordance with the guide for the care and use of laboratory animals prepared by Faculty of Science, Chulalongkorn University.

Experimental design

Nine female monkeys were divided into three groups. Each group (n = 3) was force-fed with the suspension of *P. mirifica* at doses of 10, 100, and 1000 mg/5 ml of distilled water/individual, at 08:00 – 08:30 h. The schedule was separated into the pre-treatment and post-treatment periods. The pre-treatment was performed on one menstrual cycle and the post-treatment was performed after a single forced-feeding of *P. mirifica* within 2 menstrual cycles. Day 1 of menstrual bleeding was used as a reference for the first day of a period. During these periods, 3-ml blood samples were collected from the femoral vein without anesthetization between 08:00-09:00 h on day 3 (the early follicular phase); days 9, 10, 11, 12, 13 (the late follicular phase); days 14, 19 (the early luteal phase); days 24, 29 (the late luteal phase); and every 10 days until the next menstruation. Blood samples were immediately centrifuged at 4°C, 1,700 × *g* for 20 min. The serum was then separated and stored at –20°C until follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, and immunoreactive (ir)-inhibin were assayed. Occurrence of menstrual bleeding was daily checked by vaginal swabbing. The suspension of *P. mirifica* (10, 100, and 1,000 mg) was prepared from powder of the tuberous roots and suspended into 5-ml distilled water and then kept in a dark bottle at 4°C until feeding time.

Hormonal analysis

Concentrations of serum FSH and LH were measured by a heterologous radioimmunoassay (RIA) system. Iodination preparations were rat NIDDK-rat FSH-I-5 and rat LH-I-5. The antisera were anti-ovine FSH (NIDDK-H-31) (34) and anti-ovine LH (YM#18) (35). Antiserum against ovine LH (YM#18) was kindly provided by Dr. Y. Mori (University of Tokyo, Tokyo). The results are expressed in terms of NIDDK rat FSH-RP-2 and rat LH-RP-2. The intra- and inter-assay coefficients of variations were 7.8% and 8.5% for FSH and 7.8% and 10.3% for LH, respectively.

Concentrations of estradiol and progesterone were measured by using the established method of the World Health Organization (WHO) (36). The intra- and inter-assay coefficients of variation were 6.15% and 10.96% for estradiol and 5.98% and 11.01% for progesterone, respectively.

Serum concentrations of ir-inhibin were measured by a double-antibody RIA, as described previously (37). The antiserum used was raised in rabbits against bovine inhibin (TNDH-1). Purified bovine 32-kDA inhibin

was used as the standard. The intra- and inter-assay coefficients of variation were 8.8% and 14.4%, respectively.

Statistical analyses

All data including changes of menstrual cycle length and serum levels of hormones were expressed as the mean ± S.E.M. The significance of the differences between the mean was evaluated by the paired *t*-test. $P<0.05$ was considered to be statistically significant.

Control levels of hormones in normal menstrual cycles

According to the interval of blood collection schedule, the prominent peaks of LH and FSH levels during the pre-treatment period could not be caught in 4 out of 9 monkeys (nos. 609, 624, 621, and 626). However, the ovulation was confirmed by the increase of progesterone and ir-inhibin. To evaluate changes of serum levels of gonadotropins and ovarian hormones in the nine monkeys after *P mirifica* treatment, serum levels of those hormones were compared with those of eight monkeys showing normal menstrual cycle, hormonal pattern, and prominent peak LH level from the same colony. To increase the animal numbers in this group, the hormonal levels during the pre-treatment period of 5 monkeys treated with *P. mirifica* (nos. 619, 627, 526, 604, and 104) showing prominent LH level were combined to this control group. Thus, the total number of monkeys in this group is thirteen.

Results

Changes in menstrual cycle length of monkeys treated with 10, 100, and 1,000 mg of P. mirifica

Nine monkeys had normal menstrual cycle length during the pre-treatment period for 30.56 ± 1.30 days, as shown in Table 1. After *P. mirifica*-treatment, lengths of the first or second menstrual cycles of monkeys treated with 10 and 100 mg of *P. mirifica* were not different from the menstrual cycle length at the pre-treatment period. Lengths of the first and second menstrual cycles were 30.67 ± 2.91 and 33.33 ± 3.84 days for monkeys treated with 10 mg of *P. mirifica* and 30.67 ± 2.96 and 35.67 ± 6.33 days for monkeys treated with 100 mg of *P. mirifica*, respectively. Lengths of the first and second menstrual cycles were significantly extended to 42.00 ± 4.04 ($P = 0.004$) and 39.67 ± 0.67 ($P = 0.003$) days, respectively, in monkeys treated with 1,000 mg of *P. mirifica*.

Serum levels of gonadotropins and ovarian hormones in normal cycling monkeys

Serum profiles of gonadotropins (FSH and LH), and ovarian hormones (estradiol, progesterone, and ir-inhibin) in 13 normal cyclic monkeys during the menstrual cycle are shown in Fig. 1 and Table 2. Changes in these hormonal profiles during the menstrual cycle were adjusted according to the day of peak levels of serum LH, defined as an ovulation day (day 0), and separated into 2 phases: the late follicular phase and the early luteal phase. As shown in the Fig. 1, peak levels of serum FSH and LH were 1.82 ± 0.34 and 8.27 ± 0.86 ng/ml on the same day (day 0). The increase of peak serum estradiol levels appears to coincide with the mid-cycle peak levels of FSH and LH. Serum progesterone and ir-inhibin levels remain low during the late follicular phase and then become slightly elevated during the early luteal phase, indicating that cyclic ovulation occurred in this menstrual cycle.

Changes in serum gonadotropins and ovarian hormones in the monkeys treated with 10, 100, and 1,000 mg of P. mirifica.

As shown in Figs. 2, 3, and 4, there were no changes in serum levels of FSH, LH, estradiol, progesterone, or ir-inhibin throughout the first and second menstrual cycles in monkeys treated with 10, 100, and 1,000 mg of *P. mirifica*, respectively. All monkeys exhibited peak levels of serum FSH and LH in the late follicular phase of the first and second menstrual cycles after *P. mirifica* treatment, concurrent with high levels of serum estradiol. Serum progesterone and ir-inhibin levels were low throughout the early follicular phase and gradually increased in the early luteal phase of the

Table 1. Menstrual cycle length of monkeys treated with 10, 100, and 1,000 mg of *P. mirifica*

Treatment groups	Lengths of menstrual cycle after *P. mirifica* treatment (days)	
	The first menstrual cycle	The second menstrual cycle
10 mg	30.67 ± 2.91 ($P = 0.97$)	33.33 ± 3.84 ($P = 0.39$)
100 mg	30.67 ± 2.96 ($P = 0.97$)	35.67 ± 6.33 ($P = 0.23$)
1,000 mg	42.00 ± 4.04* ($P = 0.004$)	39.67 ± 0.67* ($P = 0.003$)

Mean length of menstrual cycle during the pre-treatment period of nine cyclic female monkeys was 30.56 ± 1.30 days. Asterisks represent a significant difference ($P<0.05$).

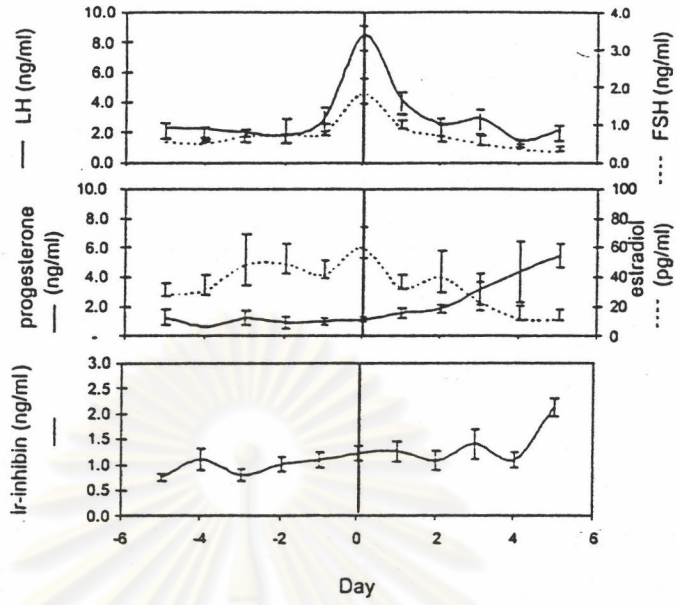

Fig. 1. Serum levels of gonadotropins (FSH and LH) and ovarian hormones (estradiol, progesterone, and ir-inhibin) of normal cyclic monkeys.

Table 2. Mean concentrations of serum gonadotropins and ovarian hormones during the late follicular phase and the early luteal phase in normal cyclic monkeys

	LH (ng/ml)	FSH (ng/ml)	Estradiol (pg/ml)	Progesterone (ng/ml)	Ir-inhibin (ng/ml)
Day of peak levels	0	0	0	5	5
Late follicular phase					
highest levels	8.27 ± 0.86	1.82 ± 0.34	59.89 ± 10.66	0.89 ± 0.53	1.07 ± 0.13
lowest levels	1.60 ± 0.33	0.51 ± 0.03	27.54 ± 4.31	0.27 ± 0.06	0.61 ± 0.08
Early Luteal phase					
highest levels	3.93 ± 0.72	0.94 ± 0.11	39.55 ± 14.22	5.09 ± 0.85	1.98 ± 0.17
lowest levels	1.29 ± 0.20	0.28 ± 0.05	11.99 ± 4.98	1.19 ± 0.3	0.93 ± 0.19

The day of LH surge (Day 0) was used as a reference point for the separation between the follicular phase and the luteal phase. Results are expressed as mean ± S.E.M. (n = 13).

first and the second menstrual cycles. However, at the highest dose (1,000 mg of *P. mirifica*), peak levels of serum LH were delayed from day 10 during the pre-treatment period to days 34 and 29 of the first and second menstrual cycles for monkey no. 621, from day 12 during the pre-treatment period to days 24 and 19 of the first and second menstrual cycles for monkey no. 104, and from day 11 during the pre-treatment period to days 24 and 19 of the first and second menstrual cycles for monkey no. 626 (Fig. 4).

Discussion

The present study provides the first evidence that *P. mirifica* containing phytoestrogens has a profound, dose-dependent effect on the menstrual cycle length. The lengths of the follicular phase and the entire first and second menstrual cycles in monkeys treated with the highest dose (1,000 mg of *P. mirifica*) increased significantly; meanwhile, there were no changes in the lengths of the follicular phase, luteal phase, or total menstrual cycle in monkeys treated with the lowest and

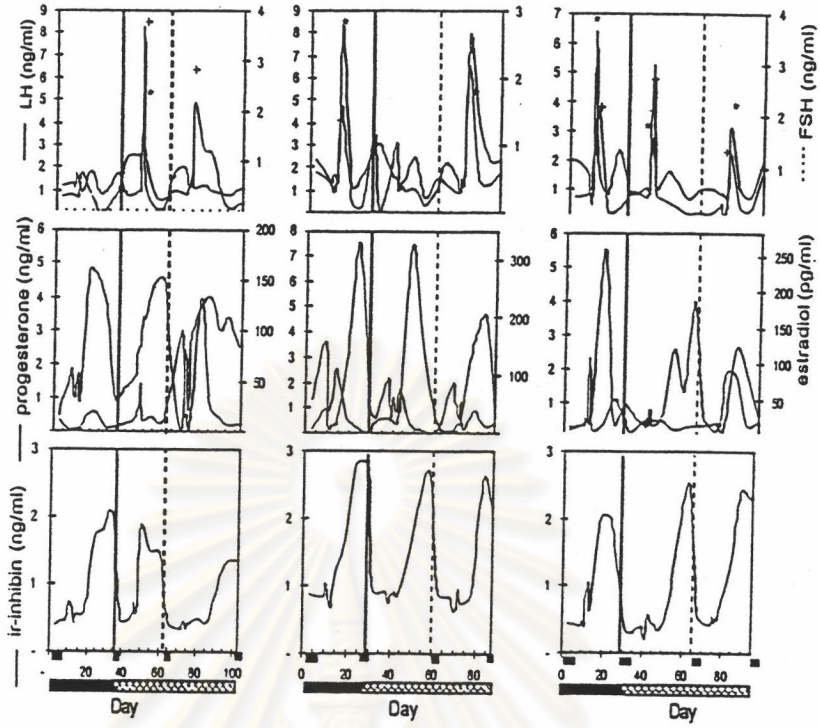

Fig. 2. Changes in serum levels of gonadotropins (FSH and LH) and ovarian hormones (estradiol, progesterone, and ir-inhibin) of monkey nos. 609 (left panel), 619 (middle panel), and 627 (right panel) treated with 10 mg of *P. mirifica*. Horizontal bars represent the day of menses. Day 1 represents the day of menses in the pre-treatment period. The pre-treatment and post-treatment periods are separated by a vertical solid line. Two menstrual cycles during the post-treatment period are separated by a vertical dotted line. The symbols of asterisk and plus show peak levels of LH and FSH, respectively.

medium doses (10 and 100 mg of *P. mirifica*). Additionally, our study did not observe changes in profile levels of serum FSH, LH, estradiol, progesterone, or ir-inhibin during the menstrual cycle.

It has been reported that the tuberous roots of *P. mirifica* contained a higher amount of isoflavones (169.1 mg/100 gram of dry powder) (38) compared to soyabean using the HPLC technique (1). There are many published reports showing that isoflavones from soy are the major component that has an effect on the reproductive system (15 – 17). Thus, it seems to be that isoflavones in *P. mirifica* are the major component to influence the menstruation in this study. Although some reports showed no effect from daily consumption of isoflavones in length of the follicular phase, the luteal phase, or total menstrual cycle in premenopausal women (17, 39, 40), other reports support our finding of a phytoestrogenic effect on menstrual cycle length. Follicular phase length increased in premenopausal women who consumed isoflavones from soy daily (15), and luteal phase length increased in premenopausal women who ingested lignans from flax seed daily (11).

Effect of phytoestrogens from soy on reproductive hormones in premenopausal women has also been demonstrated. There was no change (40) or significant decrease in serum levels of FSH and LH in premenopausal women who consumed daily dietary phytoestrogens throughout their menstrual cycle (16, 17, 41). Serum levels of estradiol and progesterone showed no change (17) or decreased (39, 40).

Phytoestrogens have also been shown to inhibit GnRH-induced LH release. Intravenous administration of coumestrol to ovariectomized rats resulted in reduction in GnRH pulse frequency, as well as reduction in LH pulse frequency and amplitude (42). Estradiol administration also has an effect on reduction in both pulsatile GnRH and LH secretion (42, 43) and caused decreases in serum LH and FSH levels (44). The inhibitory effect of coumestrol on LH pulse frequency was greater than that of estradiol (42). This evidence

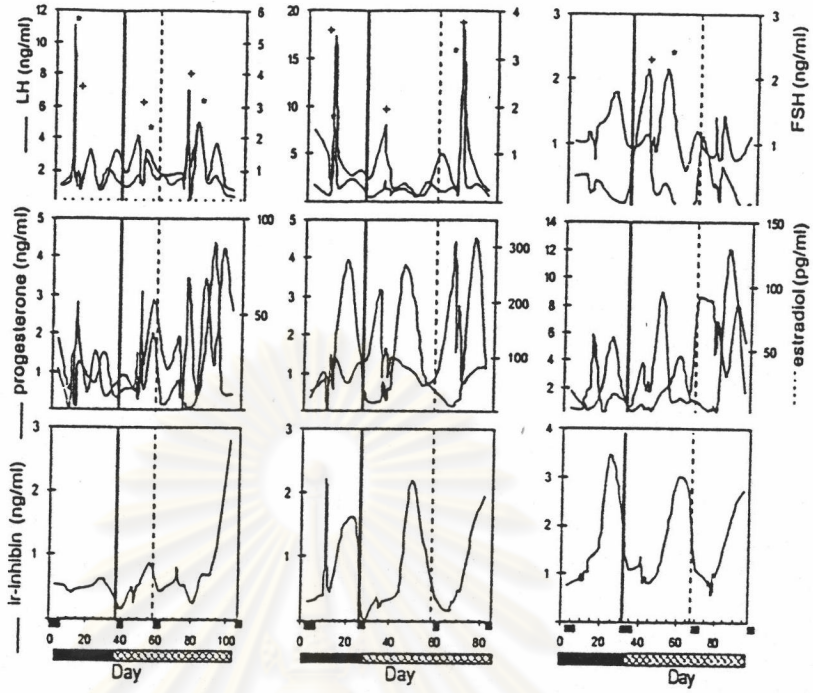

Fig. 3. Changes in serum levels of gonadotropins (FSH and LH) and ovarian hormones (estradiol, progesterone, and ir-inhibin) of monkey nos. 526 (left panel), 601 (middle panel), and 624 (right panel) treated with 100 mg of *P. mirifica*. Day 1 represents the day of menses in the pre-treatment period. The meanings of horizontal bars, vertical solid and vertical dotted lines, and symbols are shown in Fig. 2.

indicates that both phytoestrogens and estradiol have profound effect on decreasing GnRH-induced LH secretion from the pituitary gland. An alteration of pulsatile secretion of serum gonadotropins, leading to decreased of gonadotropin levels, results in disorder of the follicular growth and ovulation found in women with functional hypothalamic amenorrhea (45, 46) and luteal phase deficiency (47). There is a high correlation between pulse amplitude and frequency of LH and LH levels (48).

However, our study could not detect changes in serum levels of either gonadotropins or ovarian hormones in monkeys because after intake of *P. mirifica*, phytoestrogenic substances, including genistein and daidzein, may convert to a phytoestrogen metabolite and excreted in the urine (1). The slow increase in plasma concentrations of the glycosidic forms of the isoflavones is consistent with the facilitation of absorption by hydrolysis in the small and large intestines (49). There have been reports showing that after a single dose of soy in the diet, both genistein and daidzein were excreted in the urine as conjugated metabolite by 15% and 47% in men, respectively, and by 24% and 66% in women, respectively (50). Pharmacokinetic studies show that after a single dose of genistein and daidzein intake, measurable quantities of free genistein and free daidzein are present in the circulation with half-life ($t_{1/2}$) of 3.2 and 4.2 h for free genistein and free daidzein in men, respectively. The elimination half-life values for total genistein and total daidzein in men were 9.2 and 8.2 h, respectively (51). The elimination rates of isoflavones from the circulation are different and affected by sex. Lu and Anderson (50) showed that the elimination half-life values for genistein, daidzein, and equol were 7, 4, and 9 h in women and 4, 3, and 5 h in men, respectively. Another study showed that serum concentration of genistein and daidzein were highest at 5.5 and 7.4 h in premenopausal women (52). These investigations suggest that phytoestrogens are rapidly cleared from the circulation in both males and females.

In this study, it is very difficult to ascribe the reproductive hormonal changes to the acute effect of phytoestrogens in *P. mirifica*. The monkeys were fed with a single dose of *P. mirifica* on day 1 of the men-

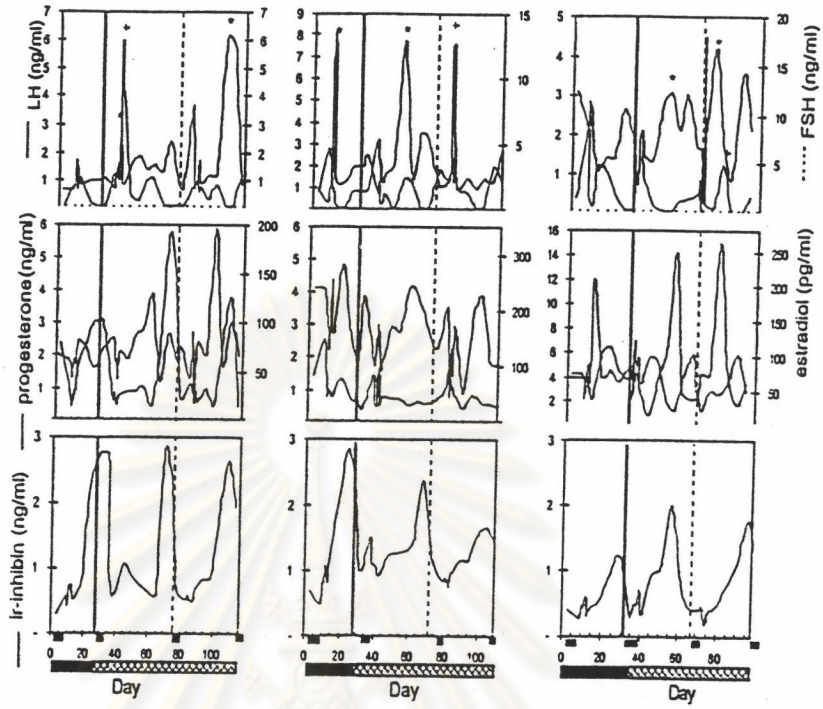

Fig. 4. Changes in serum levels of gonadotropins (FSH and LH) and ovarian hormones (estradiol, progesterone, and ir-inhibin) of monkey nos. 621 (left panel), 104 (middle panel), and 626 (right panel) treated with 1,000 mg of *P. mirifica*. Day 1 represents the day of menses in the pre-treatment period. The meanings of horizontal bars, vertical solid and vertical dotted lines, and symbols are shown in Fig. 2.

strual cycle and blood sample collection began on day 3. It is possible that phytoestrogens are completely removed from the blood circulation within 24 h. Accordingly, at 48 h after feeding time, we did not observe any phytoestrogenic effects on changes in hormonal levels in serum. Moreover, in this study, changes in serum levels of hormones were detected on day 3; days 9 – 14, 19, 24, 29; and every 10 days until menstruation. So, we could not detect whether there are changes in pulsatile secretion of these hormones. However, our result showed the prolongation of menstrual cycle length in the monkeys treated with 1,000 mg of *P. mirifica*. It can be assumed that at the highest dose, phytoestrogens may reduce pulse amplitude and frequency of gonadotropins, especially LH, to support follicular growth and ovulation, resulting in increased length of the follicular phase and total menstrual cycle of the monkeys.

Normal cycling women who were administrated with GnRH antagonist reduced the pulse amplitude and frequency of LH. Serum levels of FSH, LH, estradiol, and ir-inhibin decrease during the menstrual cycle. These changes are consistent with an increase in length of the follicular phase and total menstrual cycle (53, 54). The results of this report support our hypothesis described in the previous paragraph.

In conclusion, the result of the study suggests that a single dose of 1,000 mg of *P. mirifica* disturbs ovarian function and menstrual cycle in normal cyclic monkeys. *P. mirifica* may have positive effects on female characteristics; however, use of this plant or its products in normal cyclic women may have an effect on ovarian function and may induce menstrual cycle disruption. Its lowest dose should be recommended for cyclic women.

Acknowledgments

This work was supported by The Thailand Research Fund (TRF) contract no. PHD/0014/2544 for Royal Golden Jubilee Program, (RGJ network) and no. BGJ /15/2544, Chulalongkorn University Research Grant and a Grant-in-Aid for Scientific Research (The 21st Century Center-of-Excellence-Program, E-I) from the Ministry of Education, Culture, Sports, Science and

Technology of Japan. We are grateful to Dr. A.F. Parlow and the National Institute of Diabetes and Digestives and Kidney Diseases (NIDDK), CA, USA for providing RIA materials for LH and FSH; Dr. T .Mori, University of Tokyo (Tokyo) for providing antisera to ovine LH (YM#18). *P. mirifica* was a generous gift from Dr. W. Cherdshewasart, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. We would like to deeply thank Thomas F. Bley for proofreading.

References

1 Murkies L, Wilcox G, Davis SR. Phytoestrogens. J Clin Endocrinol Metab. 1998;83:297–303.
2 Wakai K, Egami L, Kato K, et al. Dietary intake and source of isoflavones among Japanese. Nutri Cancer. 1999;33:139–145.
3 Horn-Ross PL, Barnes S, Lee M, et al. Assessing phytoestrogens exposure in epidemiologic studies: development of a database (United States). Cancer Causes Control. 2000;11:289–298.
4 Liggins J, Bluck LJ, Runswick S, Atkinson C, Coward WA, Bingham SA. Daidzein and genistein contents of vegetables. Br J Nutr. 2000;84:717–725.
5 Boker LK, Schouw YTV, De Kleijn MJJD, Jacques PF, Grobbee DE, Peeters PHM. Intake of dietary phytoestrogens by Dutch women. Nutr Epidemiol. 2002;132:1319–1328.
6 Whitten PL, Lewis C, Naftolin F. A phytoestrogen diet induces the premature anovulatory syndrome in lactationally exposed female rats. Biol Reprod. 1993;49:1117–1121.
7 Whitten PL, Lewis C, Russell E, Naftolin F. Phytoestrogen influences on the development of behavior and gonadotropin function. Proc Soc Exp Biol Med. 1995;208:82–86.
8 Medlock KL, Branham WS, Sheehan DM. Effects of coumestrol and equol on the developing reproductive tract of the rat. Proc Soc Exp Biol Med. 1995;208:67–71.
9 Burton JL, Wells M. The effect of phytoestrogens on the female genital tract. J Clin Pathol. 2002;55:401–407.
10 Wilcox G, Wahlqvist ML, Burger HG, Medley G. Oestrogenic effects of plant foods in postmenopausal women. Br Med J. 1990;301:905–906.
11 Phipps WR, Martini M, Lampe J, Slavin JL, Kurzer M. Effect of flax seed ingestion on the menstrual cycle. J Clin Endocrinol Metab. 1993;77:1215–1219.
12 Whitehead SA, Cross JE, Burden C, Lacey M. Acute and chronic effects of genistein, tyrphostin and lavendustin A on steroid synthesis in luteinized human granulose cells. Hum Reprod. 2002;17:589–594.
13 Bennetts HW, Underwood EJ, Shier FL. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Aust Vet J. 1946;22:2–12.
14 Fredricks GK, Kincaid RL, Bondioli KR, Wright RW. Ovulation rates and embryo degeneracy in female mice fed the phytoestrogen, coumestrol. Proc Soc Exp Biol Med. 1981;167:237–241.
15 Cassidy A, Bingham S, Setchell KD. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr. 1994;60:333–340.
16 Cassidy A, Bingham S, Setchell KDR. Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. Br J Nutr. 1995;74:587–601.
17 Duncan AM, Berz BE, Xu X, Nagel T, Phipps W, Kurzer MS. Soy isoflavones exert modest hormonal effects in premenopausal women. J Clin Endocrinol Metab. 1999;84:192–197.
18 Kurzer MS. Hormonal effects of soy isoflavones: studies in premenopausal and postmenopausal women. J Nutr. 2000;130:660S–661S.
19 Bernstein L, Yuan JM, Ross RK, et al. Serum hormone levels in pre-menopausal Chinese women in Shanghai and white women in Los Angeles: results from two breast cancer case-control studies. Cancer Causes Control. 1990;1:51–58.
20 Shimizu H, Ross RK, Bernstein L, Pike MC, Henderson BE. Serum oestrogen levels in postmenopausal women: comparison of American whites and Japanese in Japan. Br J Cancer. 1990;62:451–453.
21 Nagata C, Takatsuka N, Inaba S, Kawakami N, Shimizu H. Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. J Natl Cancer Inst. 1998;90:1830–1835.
22 Pope GS, Grundy HM, Jones HEH, Tait SAS. The estrogenic substance (miroestrol) from the tuberous roots of *Pueraria mirifica*. J Endocrinol. 1958;17:15–16.
23 Ingham JL, Markham KR, Dziedzic SZ, Pope GS. Puerarin 6-*O*-*β*-apiofuranoside, A C-glycosylisoflavone o-glycodise from *Pueraria mirifica*. Phytochemsitry. 1986;25:1772–1775.
24 Chansakaow S, Ishikawa T, Seki H, Sekine K, Okada M, Chaichantipyuth C. Identification of deoxymiroestrol as the actual rejuvenating principle of "Kwao keur" *Pueraria mirifica*. The known miroestrol may be an artifact. J Nat Prod. 2000;63:173–175.
25 Chansakaow S, Ishikawa T, Sekine K, et al. Isoflavonids from *Pueraria mirifica* and their estrogenic activity. Plant Med. 2000;66:572–575.
26 Ingham JL, Tahara S, Dziedzic SZ. A chemical investigation of *Pueraria mirifica*. Z Naturforsch. 1986;41c:403–408.
27 Ingham JL, Tahara S, Dziedzic SZ. Coumestans from the roots of *Pueraria mirifica*. Z Naturforsch. 1988;43c:5–10.
28 Ingham JL, Tahara S, Dziedzic SZ. Minor isoflavones from the roots of *Pueraria mirifica*. Z Naturforsch. 1989;44c:724–726.
29 Jones HEH, Pope GS. A study of the action of miroestrol and other oestrogens on the reproductive tract of the immature female mouse. J Endocrinol. 1960;20:229–235.
30 Cantero A, Sancha JL, Flores JM, Rodriguez A, Gonzalez T. Histopathological changes in the reproductive organs of manchego ewes grazing on lucerne. Zentralbl Veterinarmed [A]. 1996;43:325–330.
31 Benson GK, Cowie AT, Hosking ZD. Mammogenic activity of miroestrol. J Endocrinol. 1961;21:401–409.
32 Hotchkiss J, Dierschke DJ, Butler WR, Fritz GR, Knobil E. Relation between levels of circulating ovarian steroids and pituitary gonadotropin content during the menstrual cycle of the rhesus monkey. Biol Reprod. 1982;26:241–248.
33 Harrison MR, Phillippi PP, Swan KF, Henson MC. Effect of genistein on steroid hormone production in the pregnant rhesus monkey. Proc Soc Exp Biol Med. 1999;222:78–86.
34 Toivola PTK, Bridson WE, Robinson JA. Effect of luteinizing hormone, follicle-stimulating hormone and testosterone in adult male rhesus monkey. Endocrinology. 1978;108:1815–1821.
35 Hodgen GD, Wilks JW, Vaitukaitis JL, Chen H-C, Papkoff H,

Ross GT. A new radioimmunoassay for follicle-stimulating hormone in macaques: ovulatory menstrual cycles. Endocrinology. 1976;99:137–145.

36 Sufi SB, Donaldson A, Jeffcoate SL. WHO matched reagent program method manual, 14th ed. London: WHO collaborating center for immunoassay. 1986.

37 Hamada T, Watanabe G, Kokuho T, et al. Radioimmunoassay of inhibin in various mammals. J Endocrinol. 1989;122:697–704.

38 Muangman V, Cherdshewasart W. Clinical trials of the phytoestrogen-rich herb, *Pueraria mirifica* as a crude drug in the treatment of symptoms in menopausal women. Siriraj Hosp Gaz. 2001;53:300–309.

39 Lu LJ, Anderson KE, Grady JJ, Nagamani M. Effects of soya consumption for one month on steroid hormones in premenopausal women: implications for breast cancer risk reduction. Cancer Epidemiol Biomarkers Prev. 1996;5:63–70.

40 Lu LJ, Anderson KE, Grady JJ, Kohen F, Nagamani M. Decrease ovarian hormones during a soya diet: implications for breast cancer prevention. Cancer Res. 2000;60:4112–4121.

41 Nicholls J, Lasley BL, Nakajima ST, Setchell KDR, Schneeman BO. Effects of soy consumption on gonadotropin secretion and acute pituitary responses to gonadotropin-releasing hormone in women. J Nutr. 2002;132:708–714.

42 McGarvey C, Cates PS, Brooks NA, et al. Phytoestrogens and gonadotropin-releasing hormone pulse generator activity and pituitary luteinizing hormone release in the rat. Endocrinology. 2001;142:1201–1208.

43 Wuttke W, Jarry H, Becker T, et al. Phytoestrogens: endocrine disrupters or replacement for hormone replacement therapy? Marturitas. 2003;44 suppl 1:S9–S20.

44 Wildt L, Hausler A, Hutchison JS, Marshall G, Knobil E. Estradiol as a gonadotropin releasing hormone in the rhesus monkey. Endocrinology. 1981;108:2011–2013.

45 Berga SL, Mortola JF, Girton L, Suhs B, Laughlin G, Pham P. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab. 1989;68:301–308.

46 Scheweiger U, Laessle RG, Tuschl RJ, Broocks A, Krusche T, Pirke KM. Decreased follicular phase gonadotropin secretion is associated with impaired estradiol and progesterone secretion during the follicular and luteal phases in normally menstruation women. J Clin Endocrinol Metab. 1989;68:888–892.

47 Soules MR, McLachlan RI, Ek M, Dahl KD, Cohen NL, Bremner WJ. Luteal phase deficiency: characterization of reproductive hormones over the menstrual cycle. J Clin Endocrinol Metab. 1989;69:804–820.

48 Bennet A, Lacaze JC, Caron P, Berrada R, Barbe P, Louvet JP. Correlations between mean LH levels and LH pulse characteristics: differences between normal and anovulatory women. Clin Endocrinol (Oxf). 1991;35:431–437.

49 King RA, Bursill DB. Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. Am J Clin Nutr. 1998;67:867–872.

50 Lu LJ, Anderson KE. Sex and long-term soy diet affected the metabolism and excretion of soy isoflavones in humans. Am J Clin Nutr. 1998;68:1500s–1504s.

51 Busby MJ, Jeffcoat RA, Bloedon LT, et al. Clinical characteristics and pharmacolinetics of purified soy isoflavones: single-dose administration to health men. Am J Clin Nutr. 2002; 75:126–136.

52 Setchell KDR, Faughnan MS, Avades T, et al. Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women. Am J Clin Nutr. 2003;77:411–419.

53 Mais V, Kazer RR, Cetel NS, Rivier J, Vale W, Yen SS. The dependency of folliculogenesis and corpus luteum function on pulsatile gonadotropin secretion in cycling women using a gonadotropin-releasing hormone antagonist as a probe. J Clin Endocrinol Metab. 1986;62:1250–1255.

54 Kettle LM, Rosetf SJ, Chiu TC, et al. Follicular arrest during the midfollicular phase of the menstrual cycle: a gonadotropin-releasing hormone antogonist imposed follicular-follicular transition. J Clin Endocrinol Metab. 1991;73:644–649.

Biography

Mrs.Hataitip Trisomboon was born on January 21, 1971. She graduated with Bachelor degree of Science in Biology from Faculty of Science, Srinakharinwirot University and master degree of Science in Zoology (Reproductive Physiology) from Faculty of Science, Chulalongkorn University. She has worked at Department of Physiology, Faculty of Medicine, Srinakharinwirot University. Her Ph.D. study in Reproductive Physiology, Biological Science Program, Faculty of Science, Chulalongkorn University, was funded by the Royal Golden Jubilee Ph.D. Program (RGJ) and by the Basic Research Grant for Royal Golden Jubilee Ph.D. Program (BGJ) of the Thailand Research Fund (TRF).

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