

References

1. Bennetts, H.W., and Underwood, E.J. "The Oestrogenic Effects of subterranean Clover (*Trifolium subterranean*)". Specialist Conference in Agriculture - Australia (1949).
2. Bartlett, S. et al. "Estrogen in grass and their possible effects on milk secretion". Nature 162, 1948.
3. Andrews, F.N. "The effect of oral and subcutaneous estrogen and androgen administration on growth and carcass quality of lambs". J. Animal Sci. 15, (1956) : 575-585.
4. Castello, C.H. and Lynn, E.V. "Estrogenic substances from plants I. Glycyrrhiza". J. Amer. Pharm. Ass. 39, (1950) : 177-180.
5. Bradbury, R.B. and White, D.E. "Estrogens and related substances in plants". Vitamins Hormones. 12, (1954) : 207-233.
6. Fransworth, N.R. et al. "Potential value of Plants as sources of New Antifertility Agent II". J. Pharm. Sci. 64(5), (1975) : 717-754.
7. Verdeal, K. and Ryan, D.S. "Naturally-Occuring Estrogens in Plant Food stuffs - A Review". J. Food Protection. 42(7), (1979) : 577-583.

8. Chuntragool, S. "Investigation of Natural Products Steroid Hormones : Characterization of steroids Found in Krachai, Dipli, Chan, Ginger, Kha, Kamin, Plai, Takona, Kamlang Suekrong, Quaow and Papaya". J. Sci. Faculty of Chiangmai. 7(1), (1980) : 1-15.
9. Marrian, G.F. "Chemical assay of urinary oestrogens". J. Endocrinology. 5, (1947), lxxi-lxxv.
10. Emmens, C.W. Estrogens in Methods in Hormone Research (Dorfman, R.I. ed.) vol. II Bioassay, pp. 59-108, Academic Press, New York and London, 1962.
11. Heftmann, E. and Mosetig, E. in Biochemistry of Steroids, pp. 153-169, Renold Publishing Corporation, New York, 1960.
12. Greenblatt, R.B. "Estrogen therapy for post-menopausal females". New England J. Med. 272, (1965) : 305-308.
13. Goodman, L. and Gillman, A. in The Pharmacological Basis of Therapeutics. 5th ed. pp. 1431-1434, Macmillan, New York, 1975.
14. Yen Samuel, S.C. and Jaffe, R.B. in Reproductive Endocrinology. pp. 458-463, Philadelphia : WB Saunders company, 1978.
15. Dorfman, R.I. "Bioassay of Steroid Hormones". Physiol. Rev. 34, (1954) : 138-166.
16. Allen, E. and Diosy, E.A. "An Ovarian hormone preliminary report on its localization, extraction and partial purification and action in test animals. J. Am. Med. Assoc. 81 (1923) : 819-821.

17. Biggers, J.D. Plant Phenols possessing oestrogenic activity in
The Pharmacology of Plant Phenolics. (Fair bairn, J.W. ed.)
pp. 51-69, Academic Press Ltd., London, 1959.
18. Bulbring, E., and Burn, J.J. "The estimation of estrin and of
male hormone in oily solution". J. Physiol 85 (1935) :
320-333.
19. Evans, J.S. et al "The mouse uterine weight method ofr the assay
of estrogens". Endocrinology 24, (1941) : 747-752.
20. Lauson, J.D. et al. "The Immature Rat Uterus in the Assay of
Estrogenic Substances and a comparison of Estradiol,
estrone and estriol". Endocrinology 24, (1939) : 35-44.
21. Butenandt, A. and Jacobi, J. "The female sexual hormone. The
preparation of crystalline plant tokokinin (thelykinin)
and its identification with the α -follicular hormone".
Z. Physiol. Chem., 218, (1933) : 104-112.
22. Kopcewicz, J. "Estrogens in Developing Bean)Phaseolus Vulgaris)
Plants". Phytochemistry, 10, (1971) : 1423-1427.
23. Skarzynski, B. "An estrogenic substance from plant material"
Nature 131, (1933) : 766.
24. Heftmann, E. et al. "Identification of estrone in pomegranate
seeds". Phytochemistry 5(6), (1966) : 1337-1339.
25. Bickoff, EmM. et al. "Relative potencies of several estrogen-
like compounds found in forages". J. Agr. Food Chem.
10, (1962) : 410-412.

26. Braden. A.W. et al. "The Estrogenic Activity and Metabolism of certain Isoflavones in Sheep". Aust. J. Agr. Res. 18, (1967) : 335-348.
27. Michael, A.P. "Fennel and Anise as Estrogenic Agents". J. Ethanopharmacology 2, (1980) : 337-344.
28. Amin, E.S. et al. "Isolation of Estrone from Moghat Roots and from Pollen grains of Egyptian Date Palm". Phytochemistry 8, (1969) : 295-297.
29. Marini-Bettolo, G.B. "Plants in Traditional Medicine". J. Ethanopharmacology 1, (1979) : 303-309.
30. Freund, M. and Davis, J.E. "Disappearance Rate of Spermatozoa from the Ejaculate Following Vasectomy". Fertil steril 20, (1969) : 163.
31. Soonawalla, F.P. "Reversal of Male Sterilozation". IPPF Medical Bulletin 12 (6), (1978) : 3-4.
32. Stanly, C.C. "Birth Control Techonology : Today and Tomorrow". Draper Fund Report No. 9. 1980.
33. Setchell, B.P. et al. "Inhibin in The Testis (John, A.D. and Gomes, W.R. eds.) vol. 4 pp. 605-621, Academic Press New York, San Francisco London 1977.
34. Sahni, G. and Dasgupta, P.R. "Antiserum Against Inhibin : Effect of Inhibin and anti-Inhibin serum on the Fertility and Compensatory Gonadal Hypertrophy in Rats. Andrologia 14(5), (1982) : 403-408.

35. Nieschlag, E. and Wickings, E.J. "Chemical Methods for Male Fertility Control". Contraception 23 (1), (1981) : 1-10.
36. Greep, R.O., et al. The Male Reproductive System in Reproduction and Human Welfare : A Challenge to Research pp. 5-277, MIT Press, Cambridge, Mass, 1976.
37. Nicander, L. "A Cytologically Specialized, Highly Androgen-dependent Region in the Epididymal Head, Related to Structural Maturation of Spermatozoa". Int. J. Androl. (suppl 3), (1981) : 66-67.
38. Gomes, W.R. Pharmacological Agents and Male Fertility in the testis (Johnson, A.D. and Gomes, W.R. eds.) vol 4 pp. 605-621. Academic Press, New York, San Francisco, London, 1977.
39. Reyes, A. and Chavarria, M.E. "Interference with Epididymal Physiology as Possible site of Male contraception". Arch. Andrology 7(2), (1981) : 159-168.
40. Kretser, DmM. "Fertility Regulation in the Male : Recent Developments" in Symposium on Recent advances in Fertility. (Fen, c.c. and Griffin, D. eds.) pp. 112-121, Geneva, 1981.
41. Fawcett, D.W. Prospects for Fertility Control in the Male in Hormonal Contraceptives, Estrogens and Human welfare (Diamond, M.C. and Korenbrot, c.c. eds.) pp. 57-75, Academic Press, New York, San Francisco, London, 1977.

42. Potashnik, G., et al. "Suppressive Effect of 1, 2-Dibromo-3-Chloropropane on Human Spermatogenesis" Fertil steril 30, (1978) : 444-447.
43. Torkelson, T.R., et al "Toxicologic Investigations of 1, 2-Dibromo-3-Chloropropane" Toxicol Appl Pharmacol 3, (1961) : 545-554.
44. Powers, M.B., et al "Carcinogenicity of Ethylene Dibromide (EDB) and 1, 2-Dibromo-3-Chloropropane (DBCP) after Oral Administration in Rats and Mice". Toxicol Appl Pharmacol 33, (1975) : 171-172.
45. Bennett, J.P. in Chemical contraception pp. 161, New York, Columbia University Press, 1974.
46. Heller, C.G. et al "Suppression of Spermatogenesis and Chronic Toxicity in Men by a New Series of Bis (Dichloroacetyl) Diamines". Toxicol Appl Pharmacol 3, (1961) : 1-11.
47. Prasad, M.R.N. "Control of Fertility in the Male Pharmacology and the Future of Man". in Proc. 5th Int. Congr. Pharmacology, San Francisco 1972 (Karger, Basel eds). vol 1 : 208-220, 1973.
48. Wong, P.Y.D. and Yeung, C.H. : "Inhibition - chlorohydrin of Fluid Reabsorption in the Rat Cauda Epididymis" J. Reprod. Fertil. 51, (1977) : 469-471.

49. Paz, G.F. and Homonnai, T.Z. "A Direct Effect of -chloro-
hydrin on Rat Epididymal Spermatozoa". Int. J. Androl
5, (1982):308-316.
50. Lobl, T.J. et al. Pharmacologic Agents Producing Infertility
by Direct Action on the Male Reproductive Tract in
Research Frontiers in Fertility Regulation (Zatuchni,
G.I., et al. eds.) pp. 146-168, Harper & Row, Publisher
Hagerstown, 1980.
51. Ford, W.C.L. and Waites, G.M.H. "Chlorinated Sugars : A Bio-
Chemical Approach to the Control of Male Fertility"
Int. J. Androl Suppl 2, (1978):541-562.
52. Cremer, J.F. and Cunningham, V.J. "Effects of Some Chlorinated
Sugar Derivatives on Hexose transport System of the
Blood/Brain Barrier". Biochem J. 180, (1979):677-679.
53. Paulsen, C.A. Regulation of Male Fertility in Frontiers in
Reproduction and Fertility Control pp. 458-465, Part II
MIT Press, Cambridge, Mass., 1977.
54. Hoffer, A.P. and Whistler, R.L. "Diabetogenic Action of 5
Thio-D Glucopyranose in Rats" Biochemistry 7, (1968) :
4479-4483.
55. Bremner, W.J. and De Kretser, D.M. "The Prospects for New
Reversible Male Contraceptives" N. Engl J. Med
295, (1976), 1111-1117.
56. National Coordinating Group on Male Antifertility Agents
"Gossypol A New Antifertility Agent for Males"
Chin Med J. (Engl) 4, (1978) : 417-428.

57. Dai, R.X. et al "A Study of Antifertility of Cotton Seed"
Acta Biol Exp Sinica 11, (1978) : 1-10.
58. Dai, R.X. and Dong, R.H. "Studies on the Antifertility of Gossypol 1. An Experimental Analysis by Epididymal Ligature". Acta Biol Exp Sinica 11, (1978) : 15-22.
59. Poso, H. et al. "Gossypol, the Chinese Male Antifertility Agent, is A Powerful Inhibition of Human Spermatozoal Metabolism". Lancet 1, (1980) : 885-886.
60. Johnsen, O. et al. Gossypol; A Potent Inhibitor of Human Sperm Acrosomal Proteinase" Int. J. Androl. 5, (1982) : 636-640.
61. Diezfalusy, E. "The Chinese Priority Programme for Male Fertility Control - Will Gossypol Provide the Male Pill? In Neischlag, E. et al, ed "Chemical Methods for Male Fertility Control. Contraception 23(1), (1981) : 1-10.
62. Jones, L.A. and Smith. F.H. "Effect of Bound Gossypol and Amino acid Supplementation of Glandless Cotton Seed Meal on the Growth of Weanling Rats. J. Anim. Sci 44, (1977) : 401-409.
63. Jackson, H. "Antispermatogetic Agents" British Medical Bulletin 26, (1970) : 79-86.
64. Soejarto, D.D., et al "Fertility Regulating Agents from Plants". Bull WHO 56(3), (1978) : 343-352.
65. Dixit, V.P. et al. "Effects of Malvaviscus conzattii Flower Extract on the Testicular Function of Dog (Cannis familiaris)" Ind. J. Exp Biol 16, (1978) : 245-249.

66. Joshi, B.C. et al. "Antifertility Effects of Chronically Administered *Malvaviscus conzattii* Flower Extract on Male Albino Mice" Planta Med 41(3), (1981) : 274-280.
67. Kholkute, S.D. "Effect of *Hibicus rosasinensis* on Spermatogenesis and Accessory Reproductive Organs in Rats". Planta Med 31,(1977):127-135.
68. Pakrashi, A. and Pakrasi, P.L. "Antispermatogetic Effect of the Extract of *Aristolochia indica* on Male Mice". Ind J. Exp. Biol 15,(1977):256-259.
69. Kasinathan, S. et al. "Antifertility Effect of *Ocimum sanctum* L.". Ind. J. Exp. Biol. 10,(1972):23-25.
70. Seth, S.D. et al. "Antispermatogetic Effect of *Ocimum sanctum*". Ind. J. Exp. Biol 19,(1981):975-976.
71. Das, R.P. "Effect of Papaya Seed on the Genital Organ & Fertility of Male Rats". Ind. J. Exp. Biol. 18, (1980) : 408-409.
72. Dixit, V.P. and Gupta, R.S. "Antispermatogetic/antiandrogenic Properties of Salasocine ($C_{27}H_{43}O_2N$) Obtained from *Solanum xanthocarpum berris* on the Male Genital Tract of Dog (*Canisfamiliaris*). A Histophysiological Approach". Int. J. Androl. 5,(1982) : 259-307.

73. Sharma, V.N. et al. "Some observations on hypoglycaemic activity of Momordica charantia" Ind. J. Med. Res. 48(4), (1960) : 471-475.
74. Dixit, V.P. et al. "Effects of Momordica charantia L. Fruit Extract on the Testicular Function of Dog". Planta Med. 34,(1978) : 280-286.
75. Council of Sci. and Ind. Res., New Delhi : The Wealth of India vol. VII, pp. 81, (1966).
76. Jain, M.L. and Jain S.R. "Therapeutic Utility of Ocimum basilicum var. album" Planta Med 22,(1972):66-70.
77. Brondegaard, V.J. "Contraceptive Plant Drugs" Planta Med. 23, (1973) : 167-172.
78. Visutakul, P., et al. "Effect of Koenchai or Chinese Celery (Apium graveolens) on Spermatogenesis". J. Med. Ass. Thailand 62(4),(1979):164-173.
79. Visutakul, P., et al. "Effects of various Extracts of Koenchai or Chinese celery (Apium graveolens) on Spermatoginesis in Rat and Rabbit" Personnel Communication.
80. Hanks, J.H. and R.E. Wallace "Relation of oxygen and Temperature in the Preservation of Tissues by Refrigeration". Proc Soc Expt Biol Med 71(1949): 196-200.

81. WHO protocol : MB-50/51 A Method for Examining the Effect of Plant Extracts Administered Orally on the Fertility of Male Rats., 1982.
82. The World Health Organization. Laboratory Manual on Examination of Human Semen and Semen-Cervical Mucus Interaction., 1980.

(1) Epididymal Effects (15 day dosing) Male rats are dosed with extract for fifteen days. On days 14 and 15 each male is housed with 2 females in proestrus selected in accordance with the MB30 protocol. The males are autopsied, their testes and epididymides weighed and epididymal sperm examined. The females are autopsied on day 16 of gestation. The number of pregnant animals, the number of implantation sites, the number of normal and abnormal fetuses and the number of corpora lutea of pregnancy will be recorded and reported exactly as for MB30.

(2) Epididymal and Testicular Effects (60 day dosing). Male rats are dosed with extract for 60 days. If required, they may be mated at days 14 and 15 (as above) and the males autopsied on day 30 if the females are not pregnant. This would reduce the amount of extract required and advance the date of autopsy of the males consequent on a positive MB30. If the MB30 is negative, the males would continue to be doses to day 60, when they are mated (day 59 and 60) autopsied; their testes and epididymides weighed and epididymal sperm examined. The females are autopsied on day 16 in gestation. The number of pregnant animals, the number of implantation sites, the number of normal and abnormal fetuses and the number of corpora lutea of pregnancy will be recorded and reported exactly as for MB30.

B. Animals

Proven fertile Sprague Dawley male rats (10 weeks old, 250-350 g) and virgin females (eight weeks old, 180-200 g) should be housed, not more than four per cage according to cage size.

A large group of females (at least 80) should be smeared to establish their estrous cycles (as per MB30 protocol).

C. Dosing

Males are randomly assigned to experimental and control groups, each group containing six animals. The extract should be administered orally by flexible stomach tube or metal feeding needle immediately after weighing the animals. The animals should be dosed according to their daily weights. Extracts should be administered in the highest possible dose; the limiting factor is the viscosity of the extract solution in a volume not exceeding 1 ml. Dosage should be reported in g of extract per kilogram body weight. The dosage should be made up daily, or every other day, the remainder being kept in the refrigerator. Dosing should continue for 15-60 days according to requirements. The testis volume should be measured at the beginning of dosing and at autopsy.

NOTE : Where appropriate, aliquots of the extract may be frozen at -20°C and an aliquot thawed each day for dosing. If this procedure is adopted, aliquots should be flash frozen using liquid nitrogen or an ethanol-dry ice mixture.

D. Pairing

(1) Epididymal Effects (15 day dosing) On the evening of day 14 of dosing, the male should be placed with two females in proestrus. On the morning of day 15 the male should be removed and given a final dose. On the evening of day 15 of dosing the male is placed with two further females in proestrus. On the following morning, the male is autopsied. The females housed individually or in pairs are given no treatment and are autopsied on day 16th of gestation, according to the MB30 protocol.

Activity of sperm (?) Experimental

Control

(2) Female - The report and procedures outlined here assume a 90% pregnancy rate in the control animals. Levels 1-4 indicate the importance of results in order of priority. Date should be included for both control and treated groups in each case. As indicated in the flow sheet, MB30 + 40 is the line of first priority.

<u>Result</u>	<u>Procedure</u>
<u>Level 1</u>	
<u>Number of animals pregnant</u> <u>Number of animals treated</u>	30% reduction; retest at higher dose.
	40% reduction; result positive, proceed to MB40/41.
<u>Level 2</u>	
<u>Number of implantation sites</u> <u>per pregnant animals</u>	40% reduction; retest at higher dose.
	60% reduction; proceed to MB40/41.
<u>Level 3</u>	
<u>Number of normal fetuses</u> <u>per pregnant animal</u>	60% abnormal; retest at higher dose.
	80% abnormal; proceed to MB40/41.

NOTES - As for MB30.

Biography

Name : Miss Siripen Srichant
Date of birth : July 17, 1954.
Place of birth : Ayuthaya, Thailand
Education : B.Sc. (in Pharm.) Mahidol University
Office : Thailand Institute of Scientific and
Technological Research (TISTR).

