Some thoughts on development of chemically based male contraceptives

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ALTHOUGH intensive research into the development of a hormone-based male contraceptive is being pursued over the past few decades, a potentially viable product which satisfies the cardinal principles of safety, uniform and total efficacy, and complete reversibility is yet to be met with success. In addition, for the potential male contraceptive method to be acceptable by men it is essential to ensure that libido, an androgen-dependent phenomenon, is not impaired or adversely affected.

In the quest for development of such methods, attention has been primarily and largely focussed on interference with the regulation of spermatogenesis and sperm production by blocking the action of specific hormones along the hypothalamo-pituitary-testicular axis. At the moment, understanding of the regulation of spermatogenesis in the primate (including man) has clearly underscored the importance of both the gonadotropins – follicle-stimulating hormone (FSH) and luteinizing hormone (LH) – and androgens (mainly testosterone [T]). It has been observed that though both FSH and T act *via* Sertoli cell functions, they appear to primarily influence different steps of germ cell transformation during spermatogenesis in the adult mammal. Thus, while FSH has been shown to have a significant regulatory effect on spermatogonial proliferation[1–5], T appears to be closely associated with the key event of meiosis which is responsible for the production of round spermatids and sperms[6]. Besides this, while both FSH and T regulate the production of primary spermatocytes[2,6], a need for FSH in the transformation and maturation of round to elongate spermatids representing spermiogenesis has been observed[7–9]. A graphic representation of the various events these two hormones exert their influence on during spermatogenesis is illustrated in Figure 1.

The secretion of FSH and LH from pituitary is regulated by the hypothalamic gonadotropin-releasing hormone (GnRH); in addition, the circulating levels of T or even aromatizable androgens can also exert a feedback inhibition of their secretion. The major thrust areas of research in male contraception revolve around achieving an effective and complete suppression of pituitary FSH and LH (therefore T) secretion by using GnRH agonists/antagonists, a variety of T analogues and progestins, or by immunoneutralization of FSH or LH action on target cells.

GnRH agonists and antagonists

A three-year study on the efficacy of constant release of Buserelin via Alzet mini-osmotic pumps was undertaken under the auspices of the WHO by Ravindranath *et al.*[10] in adult male bonnet monkeys. The results clearly showed that acute oligozoospermia/azoospermia is achieved within 70 days of initiation of treatment and this situation can be maintained for three years by replacement of the Alzet mini-osmotic pump (with Buserelin) once every 21 days. Recovery of normal testicular function and sperm production was achieved within 3–4 months of stopping the treatment. Though Buserelin has been tested in men in short-term studies[11], practicality of this approach has posed problems since the compound is expensive and development of an appropriate delivery system to ensure maintenance of constant release of the compound over protracted period is necessary. In addition, a few new generation peptides are also currently being tested for their contraceptive use (see below).

The agonists act by a slow process of pituitary desensitization to GnRH action. However, the reduction achieved in pituitary LH secretion is not total. In

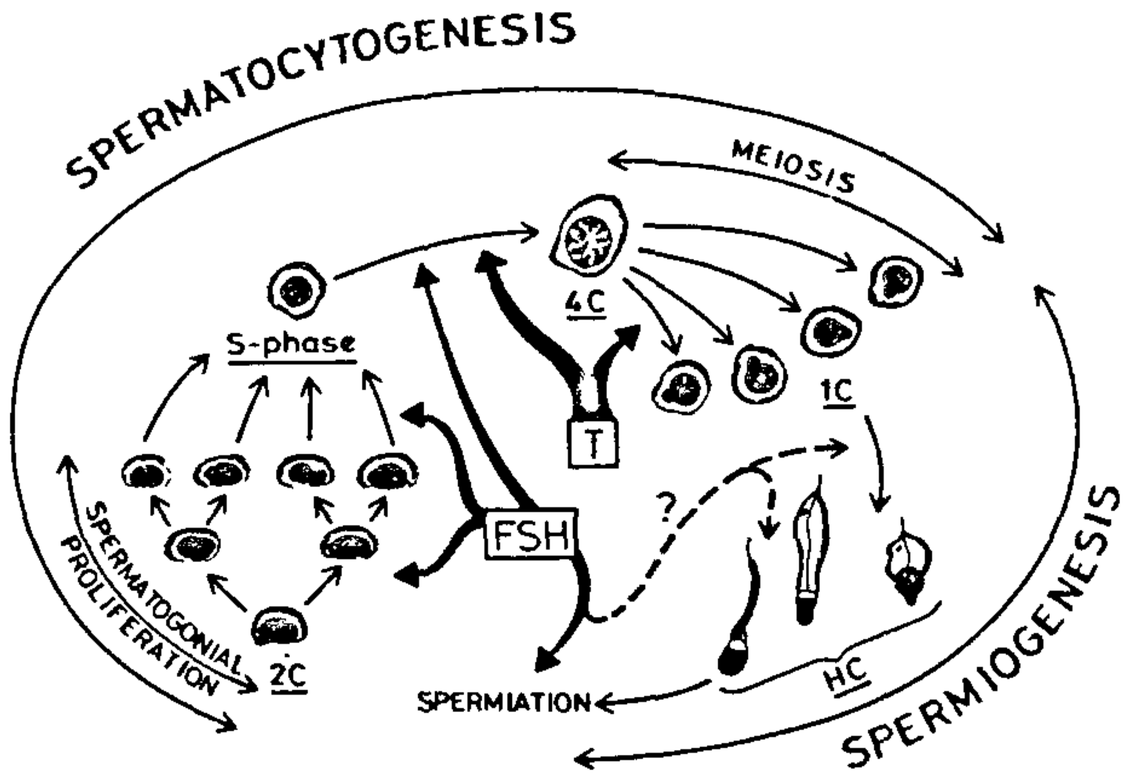

Figure 1. Diagrammatic representation of the possible primary actions of FSH and T on germ cell transformation steps during spermatogenesis. 2C, spermatogonia; 4C, primary spermatocytes, 1C, round spermatids; HC, elongating spermatids. (Modified from Figure 1 of Reference 8)

contrast, the antagonists (e.g. Antide, Nal-Glu) have a decided advantage in having a longer half-life and their ability to bring about an immediate and near-total suppression of gonadotropin secretion[12]. Analogues of GnRH have been tested in the monkey[10,13–16] and human volunteers[17–24] for their potential contraceptive effects. Newer generation antagonists are still emerging (e.g. Cetrorelix, Detirelix, Ganirelix), but the products as of now are expensive and a careful pre-clinical screening is essential to weed out compounds which produce toxic side effects. Administration of either the GnRH agonist or antagonist alone will lead to the loss of libido due to a block in testicular T production. Consequently T needs to be supplemented exogenously to maintain the normal androgen-dependent sexual and anabolic functions.

Use of T and its analogues in feed-back suppression of gonadotropin secretion

A variety of androgens including the natural hormone T and its esters, T enanthate or T buciclate, have been tested for their efficacy to block gonadotropin secretion and the resultant effects on sperm production in both the non-human primate and men[25–29]. These steroids have been administered intramuscularly/subcutaneously either as a depot preparation or as a micro-encapsulated form in a dose range of 50–600 mg per week/month. Administration of androgens in this manner has dual advantage; firstly, they suppress pituitary FSH and LH secretion (and T production) thus severely affecting spermatogenesis and, secondly, they also meet the requirement for exogenous androgen supplementation to maintain libido and accessory gland functions. Although this form of exogenous androgen treatment has been observed to be completely effective in rendering Indonesian men azoospermic[30], in the western hemisphere, a significant percentage of men under such a contraceptive trial have been consistently observed to become only oligozoospermic[31]. The observation of such a geo-ethnic difference has sprung a surprise to researchers thus emphasizing the need for further, closer scrutiny in this direction. Although this method of male contraception appears highly encouraging it is yet to be accepted without reservation because of: (a) still incomplete knowledge on the possible effects of long-term administration of high concentrations of T (being an anabolic steroid) on overall health and general body metabolism[32–34] and (b) the specific effects this may have on normal functioning of the accessory reproductive glands[35,36].

Many of the on-trial contraceptive methods require exogenous T supplementation as the endogenous T production remains suppressed. As far as we are aware no clear cut data are available with regard to the precise concentration of endogenous T required to maintain normal libido and accessory sex gland functions. This concentration of serum T must be, presumably, much less than the intratesticular T concentration that is required to promote optimal spermatogenesis. Currently, supplementation with exogenous androgens requires large, frequent doses that result in supraphysiological concentrations in circulation. However, the recent demonstration by Sundaram *et al.*[37] of the superior benefits of a synthetic androgen (7-α-methyl-nortestosterone, MENT) opens up new vistas in the field of male contraception. MENT has been shown to be the first androgen with a health benefit compared to T. It can be used at a relatively low concentration (being 10× more potent than T), possibly as a subdermal implant lasting for longer duration than the currently used androgens and, more importantly, it does not undergo the typical 5-α-reduction (like T) in the prostate thus having no deleterious effects on this accessory gland. The potential of MENT as a substitute to T particularly in combination with GnRH analogues or progestins (see below) is quite promising.

Progestational compounds

Steroids having progestational activity like the pregnane derivatives (relatives of progesterone – for e.g. medroxy progesterone acetate [MPA]) and the 19-nortestosterones (T-related – for e.g., norethisterone [NET]) are also known to be effective in blocking pituitary gonadotropin secretion[38,39]. Most studies on the efficacy of progestogens have centered around female contraception[40–42]. Clinical testing of these compounds for their male contraceptive potential has not been viewed encouragingly, possibly because of their inherent progestational activity when given in larger doses leading to gynecomastia and such other side-effects[43]. Among these, the compounds having relatively minimal progestational activity but with a maximal ability to suppress pituitary gonadotropin secretion are NET and norethynodrel[39]. Whereas NET at an oral dose of 25 mg/day to human male volunteers has been reported to be effective in blocking T production by 70% (ref. 44), we have observed that a similar effect can be achieved in men by giving microdoses of NET intranasally (unpublished observations). In adult male bonnet monkeys, intranasal administration of microdoses of NET on a daily basis has been shown to suppress T production and effectively impair spermatogenesis and sperm production[45–47]. In addition, constant release of microdoses of either NET or Norethynodrel via Alzet mini-osmotic pump can also suppress T production by > 90% (unpublished observations). The progestational activity associated with these drugs can be, perhaps, significantly reduced when given in microdoses by alternate (to parenteral) modes without compromising their pharmacological efficacy. In addition to progestational compounds, intranasal admini-

stration of steroids (progesterone, estrogen) has also been shown to block T production and affect spermatogenesis in monkeys[45]. This fact is of particular interest since recently, Bhatnagar *et al.*[48] observed that a block in estrogen synthesis in men by administration of non-steroidal aromatase inhibitors led to a significant increase in serum LH, FSH and testosterone levels. By demonstrating that T is unable to maintain LH and FSH levels within the normal physiological limits when its conversion to estrogen is blocked, this study implies that estrogen but not T is the true feedback inhibitor of gonadotropin secretion in man.

Combination approaches

Several recent studies have clearly shown that administration of a combination of exogenous androgen with GnRH analogues[49–52] with progestins[53–56] can be more pronounced in its end-effect on spermatogenesis resulting in near- to complete azoospermia in a greater per cent of human volunteers. Further, these results provide an excellent opportunity to effectively regulate the dose of exogenous androgen supplementation to a bare minimum (for libido/accessory reproductive gland functions), thus circumventing the possible side-effects associated with long-term use of high androgen concentrations. The use of MENT as the supplementary androgen in such situations may provide valuable information in this direction. However, it needs to be determined whether this approach can fill in the differences observed between geo-ethnic groups in relation to the desired end-effect (azoospermia).

The vaccine approach

It is possible to block the actions of FSH and LH by using either FSH, LH or LHRH as a vaccine. The efficacy of these vaccines has been successfully tested in several mammalian species[6,57–59]. Whereas vaccination against LH or LHRH leads to a suppression of endogenous T production thus necessitating exogenous T supplementation to maintain normal libido and accessory gland functions, the FSH-based vaccine does not require T supplementation as endogenous T production is not affected.

The FSH-based vaccine which uses ovine (o)FSH as the immunogen has been thoroughly tested in the monkey for its efficacy to bring about infertility[8,58,60–62]. The entire concept has also been confirmed by independent studies from three different laboratories[63]. One aspect of the study which puzzled investigators early on was that immunization with oFSH results in infertility although the monkeys largely exhibited oligozoospermia. Detailed analysis of the quality of the few sperms voided, however, showed that in addition to the poor motility and viability of the sperms, a marked inhibition in activity of two key acrosomal enzymes (hyaluronidase and acrosin) required for cumulus dispersion and egg penetration was apparent[8]. This perhaps could be the reason why sperms from FSH immunized monkeys are unable to either attach to intact monkey eggs[60] or penetrate zona-denuded hamster eggs[61]. Recovery from the vaccine effect is feasible since stopping of booster injections leads to restoration of normal spermatogenesis and fertility in 9 out of 10 monkeys[58]. This vaccine approach as currently conceived requires an initial schedule of one immunization each at 20 days apart followed by boosters once in 90–100 days.

Following the successful, mandatory pre-clinical toxicology studies in two species of animals at 1X and 5X the dose proposed to be given in the human, Phase-I Clinical Trials were initiated. The recently-concluded phase I clinical trial showed that the oFSH vaccine does not result in any overt toxicity and, at the dose and schedule tested, is apparently safe (Bajaj, J. S., DBT Task Force report, 1994). Analysis of sera from the human volunteers showed that the vaccine had produced antibodies capable of binding to hFSH as well as blocking hFSH action (Moudgal *et al.*, unpublished observations). The affinity of binding of these antibodies to hFSH was also of a high order. Further improvement in this vaccine approach is essential with respect to (a) the substitution of oFSH with oFSHβ subunit, (b) a better formulation resulting in higher and prolonged antibody titer in circulation, and (c) the use of recombinant-derived instead of pituitary-isolated product of FSH or its β subunit.

The oLH derived vaccine shows high potency in bringing about sharp and sustained reduction in serum T levels leading to acute oligozoospermia/azoospemia both in rabbits and monkeys[6]. Vaccination with oLH, unlike with oFSH, results in a significant reduction in testicular size. The ovine gonadotropin (FSH and LH)-based vaccines do not require any conjugation with a second protein (e.g. as in the case of hCGβ[64]) since they are naturally immunogenic and elicit a response when formulated with Alugel, an adjuvant cleared for human use. Immunization with LHRH conjugated to tetanus or diphtheria toxoid or other carrier proteins results in a significant reduction in both FSH and LH levels as well as T production, and the efficacy of this vaccine has been tested in rat, rabbit, monkey and in human volunteers having advanced prostatic cancer[65–67]. However, immunization with either LH or LHRH will have to be accompanied by exogenous T supplementation.

The vaccine approach to male contraception is highly attractive as it calls for injection of the immunogen as boosters, at present only once in 90–100 days as observed in monkeys. However, it takes nearly 2–6

months to achieve the desired positive effects of the vaccine and hence the use of a suitable barrier method of contraception (like the condom) is necessary in the interim period to prevent unwanted conception. Infertility brought about by the vaccine approach should, under normal circumstances, be reversible. Though there has been some apprehension that gonadotropin vaccines might lead to autoimmune diseases, recognition of self-antigen, etc., immunopathology and clinical toxicology studies have shown with regard to oFSH vaccine that this fear is largely unfounded[68].

Gonadotropin receptor-based approach

The possibility of using intact or peptide fragments of FSH and LH receptors, either independently or together with the gonadotropins, offers a new approach for the development of additional male contraceptive methods. Selected fragments of the extracellular domain of the receptor are preferred to the holoreceptor, since the latter leads to production of antibodies that exhibit both agonistic and antagonistic activities[69]. Several investigators have successfully synthesized the desired fragments of gonadotropin receptors either by chemical means[70–76] or by recombinant DNA technology (J. F. Catteral and M. R. Sairam, personal communications) and it remains to be seen how successful these attempts will be in producing a viable product of contraceptive potential. In general, it may turn out that the receptor fragment is a much better candidate contraceptive vaccine as the turnover of the receptors (relative to the gonadotropins) is much slower. Further, it may not be far-fetched to presume that a low antibody titer would suffice to block the receptor sites for longer considering the fact that the Sertoli and Leydig cells in the testis are limited in number in the adult mammal.

Conclusions

Even though research into the development of a viable male contraceptive has been progressing in fits and starts, it is hoped that the information accrued so far would pave the way for a smooth, efficient and faster analysis of the potential methods on hand leading to the availability of newer options for effective male fertility regulation in the near future. It is possible that we will have more than one method suitable for different socio-economic/ethnic groups. In addition, parallel attempts are also underway at several institutions to devise effective methods of controlling the fertility of domestic and other animals. Active participation of and collaboration between pharmaceutical industries and the basic research scientists, being initiated or already in progress at many institutions/research centres, may hasten the entire process. Finally, as Waites[31] purports – '.... the subject of Andrology needs to be strengthened throughout the world so that scientists in developing countries can participate fully in this work'.

1. Russell, L. D., Corbin, T. J., Borg, K. E., de Franca, L. R., Grasso, P. and Bartke, A, *Endocrinology*, 1993, **133**, 2062–2079.
2 Aravindan, G. R., Gopalakrishnan, K., Ravindranath, N. and Moudgal, N R., *J. Endocrinol*, 1993, **137**, 485–495.
3. Suresh, R., Medhamurthy, R. and Moudgal, N. R., 14th Annual Meeting of American Society for Reproductive Immunology, 1994, Abstr. # p. 32.
4. Marshall, G. R., Zorub, D. S. and Plant, T. M., 76th Annual Meeting of the Endocrine Society, 1994, Abstr. # 807, p. 402.
5. Zirkin, B R., Awoniyi, C., Griswold, M. D, Russell, L. D. and Sharpe, M., *J. Androl*, 1994, **15**, 273–276.
6 Suresh, R., Jeyakumar, M., Aravindan, G. R. and Moudgal, N. R., XIIth North American Testis Workshop: Function of Somatic Cells in the Testis, 1993, Abstr. # II–46, p 79
7 Cameron, D E. and Muffly, K. E., *J. Cell Sci.*, 1991, **100**, 523–533.
8. Moudgal, N R. and Aravindan, G. R, in *Immunology of Reproduction* (ed. Naz, R. K.), CRC Press, Florida, 1993, pp. 251–277.
9. Muffly, K. E, Nazion, S. J. and Cameron, D. F., *Biol. Reprod.*, 1994, **51**, 158–166.
10. Ravindranath, N., Ramesh, V., Krishnamurthy, H. N, Rao, A. J. and Moudgal, N. R., *Fertil. Steril.*, 1992, **57**, 671–676.
11. Michel, E., Bents, H., Akhtar, F. B., Honigl, W., Sandow, J. and Nieschlag, E, *J. Androl. Suppl.*, 1985, Abstr., p. 37.
12. Vickery, B. M., *J. Steroid Biochem*, 1985, **23**, 779–791.
13. Marshall, G. R, Akhtar, F. B., Weinbauer, G. F. and Nieschlag, E., *J. Endocrinol.*, 1986, **110**, 145–150.
14. Hodgen, G. D., in *Recent Progress on GnRH and Gonadal Peptides* (eds Bouchard, P., Haour, F., Franchimont, P. and Schatz, B), Elsevier, Amsterdam, 1990, pp. 169–180.
15. Weinbauer, G. E. and Nieschlag, E., in *Horizons in Endocrinology* (eds Maggi, M. and Geenen, V.), Raven Press, New York, 1991, pp. 165–172.
16. Weinbauer, G. F., Limberger, A, Behre, H. M. and Nieschlag, E., *J. Endocrinol*, 1994, **142**, 485–495.
17. Bajusz, S., Csernus, V. J., Janaky, S., Bokser, L., Fekete, M and Schally, A. V., *Int. J. Pept. Protein Res.*, 1988, **32**, 425–435.
18. Jockenhovel, F., Bhasin, S., Steiner, B. S, Rivier, J. E., Vale, W. W. and Swerdloff, R. S., *J. Clin. Endocrinol. Metab*, 1988, **66**, 1065–1070.
19. Pavlou, S. N., Wakefield, G., Schlechter, N. L., Lindner, J, Souza, K. H., Kamilaris, T C, Kondidaris, S, Rivier, J E, Vale, W. W. and Toglia, M., *J. Clin Endocrinol. Metab.*, 1989, **68**, 446–453.
20. Bagatell, C. J., Mclachlan, R. I., de Krester, D M., Burger, H. G, Vale, W. W., Rivier, J. and Bremner, W. J, *J. Clin Endocrinol. Metab.*, 1989, **69**, 43–48.
21. Salameh, W., Bhasin, S., Steiner, B, McAdams, L. A, Peterson, M. and Swerdloff, R S, *Fertil. Steril.*, 1991, **55**, 156–164.
22 Behre, H. M, Klein, B., Steinmeyer, E and Nieschlag, E., *J. Clin. Endocrinol. Metab*, 1992, **75**, 393–398
23. Behre, H. M, Bockers, A., Schlingheider, A and Nieschlag, E, *Clin. Endocrinol*, 1994, **40**, 241–248.
24. Gonzalez-Barcena, D, Buenfil, M. V, Procel, F. G, Guerra-Arguero, L., Cornejo, I. C., Comaru-Schally, A M and Schally, A. V., *Eur J. Endrocrinol*, 1994, **131**, 286–292
25. Matsumato, M, *Fertil. Steril.*, 1988, **50**, 324–328
26. WHO Task Force on Methods for the Regulation of Male Fertility, *Lancet*, 1990, **336**, 955–959
27. Wallace, E M, Aitkin, R. J and Wu, F C, *Int J Androl*, 1992, **15**, 416–424.
28 Behre, H M and Nieschlag, E, *J Clin Endrocrinol Metab*, 1992, **75**, 1204–1210

29 Handelsman, D J, Conway, A J and Boylan, L M., *J Clin. Endocrinol Metab*, 1992, 75, 1326–1332
30 Arsyad, K M, *Int J Androl*, 1993, **16**, 355–361.
31 Waites, G M H, *Br Med Bull*, 1993, **49**, 210–221
32. Wu, F C, *Baillieres Clin Endocrinol Metab*, 1992, **6**, 373–403
33 Anderson, R A, Bancroft, J. and Wu, F. C, *J. Clin. Endocrinol Metab*, 1992, 75, 1503–1507
34 Young, N R, Baker, H W, Liu, G. and Seeman, E., *J. Clin Endocrinol Metab*, 1993, 77, 1028–1032.
35 Wallace, E M, Pye, S D, Wild, S. R. and Wu, F. C., *Int J Androl*, 1993, **16**, 35–40
36 Behre, H M, Bohmeyer, J. and Nieschlag, E., *Clin. Endocrinol*, 1994, **40**, 341–349.
37 Sundaram, K, Kumar, N. and Bardin, C. W., *Ann. Med.*, 1993, **25**, 199–205.
38 Jones, G S, in *Progesterone and Progestins* (eds Bardin, C. W., Milgram, E and Mauvais-Jarvis, P.), Raven Press, New York, 1983, pp 189–202
39 Batzer, F R, *J Reprod Med*, 1986, **31**, 848–864.
40 Zatuchni, G. L., Goldsmith, A., Shelton, J. D and Sciarra, J. J (eds), *Long acting Contraceptive Delivery Systems*, Harper and Row, Philadelphia, 1983, pp. 1–656
41 Grubb, G. S, *Curr. Opin. Obstet. Gynecol*, 1991, **3**, 491–495
42 Pasquale, S A, *Curr Opin. Obstet. Gynecol*, 1992, **4**, 531–535.
43 Heller, C. G, Laidlaw, W. M, Harven, H. T and Nelson, W. O., *Ann N Y Acad Sci*, 1958, **71**, 649–655.
44 Johansson, E. D B. and Nygren, K-G, *Contraception*, 1973, **8**, 219–226.
45 Anand Kumar, T. C., Sehgal, A, David, G. F. X., Bajaj, J. S and Prasad, M R. N., *Biol Reprod*, 1980, **22**, 935–940.
46 Moudgal, N R., Rao, A. J., Murthy, G. S R. C., Ravindranath, N, Srinath, B. R., Kotagi, S G. and Anand Kumar, T C, *Fertil Steril*, 1985, **44**, 120–124.
47 Suresh, R and Moudgal, N. R, *24th Annual Conference of Endocrine Society of India*, Abstr, 1993, p. 108.
48. Bhatnagar, A S., Miller, P. H., Schenkel, L., Trunet, P. F., Beh, I and Schieweck, K., *J Steroid Biochem. Molec. Biol*, 1992, **41**, 437–443
49. Bremner, W. J., Bagatell, C. J and Steiner, R. A., *J. Clin Endocrinol. Metab*, 1991, **73**, 465–469
50. Pavlou, S. N, Brewer, K., Farley, M. G, Lindner, J., Bastias, M C., Rogers, B J, Swift, L. L., Rivier, J E., Vale, W. W., Conn, P. M *et al*, *J Clin. Endocrinol. Metab*, 1991, **73**, 1360–1369.
51. Tom, L., Bhasin, S., Salameh, W, Steiner, B., Peterson, M, Sokol, R. Z., Rivier, J, Vale, W. W. and Swerdloff, R. S., *J Clin Endocrinol. Metab*, 1992, **75**, 476–483.
52 Bagatell, C. J, Matsumoto, A M., Christensen, R. B, Rivier, J. E and Bremner, W. J, *J. Clin Endocrinol. Metab.*, 1993, 77, 427–432
53 Lobel, B, Olivo, J F., Guille, F. and Lannou, D., *Acta. Urol Belg*, 1989, **57**, 117–124.
54 Wu, F. C. and Aitkin, R. J., *Fertil. Steril.*, 1989, **51**, 691–698
55 Pangkahila, W, *Int J Androl*, 1991, **14**, 248–256.
56. WHO Task Force on Methods for the Regulation of Male Fertility, *Fertil. Steril.*, 1993, **60**, 1062–1068
57. Thau, R. B, *Scand J Immunol*, 1992, **36**, 127–130
58. Moudgal, N R, Ravindranath, N, Murthy, G. S, Dighe, R R, Aravindan, G. R. and Martin, F., *J. Reprod Fertil*, 1992, **96**, 91–102.
59. Moudgal, N. R. and Suresh, R, in *Birth Control Vaccines* (ed. Talwar, G. P), Landes Co, Austin, 1994, pp 89–102
60. Raj, H M G, Kotagi, S. G, Letellier, R, Nath, N, Raj, H. G, in *Perspectives in Primate Reproductive Biology* (eds. Moudgal, N. R, Yoshinaga, K, Rao, A. J and Adiga, P R), Wiley Eastern, New Delhi, 1991, pp. 307–316.
61. Srivastava, A and Das, R. P, *Indian J Exp. Biol*, 1992, **30**, 574–577.
62. Sharma, R. K. and Das, R. P., *Indian J Exp Biol*, 1992, **30**, 976–981.
63. Moudgal, N R, Ravindranath, N, Murthy, G. S., Dighe, R R and Aravindan, G. R., in *Perspectives in Primate Reproductive Biology* (eds. Moudgal, N R, Yoshinaga, K, Rao, A. J. and Adiga, P. R), Wiley Eastern, New Delhi, 1991, pp. 297–306
64 Talwar, G. P., Singh, O, Pal, R, Chatterjee, N, Sahai, P, Dhall, K., Kaun, J, Das, S K, Suri, S, Buckshee, K, Saraya, L. and Saxena, B. N, *Proc. Natl. Acad Sci*, 1994, **91**, 8532–8536
65. Talwar, G. P., Singh, O, Pal, R and Chatterjee, N, *Int J Immunopharmacol.*, 1992, **14**, 511–514
66 Ladd, A., Prabhu, G., Tsong, Y -Y., Probst, T, Chung, W. and Thau, R. B, *Am. J. Reprod Immunol*, 1988, **17**, 121–127.
67. Ladd, A, Tsong, Y.-Y., Lok, J. and Thau, R. B, *Am. J Reprod Immunol*, 1990, **22**, 56–63
68 Sehgal, N, Ravindranath, N. and Moudgal, N. R, in *Perspective in Primate Reproductive Biology* (eds Moudgal, N. R, Yoshinaga, R, Rao, A J. and Adiga, P. R), Wiley Eastern, New Delhi, 1991, pp 317–324
69. Jeyakumar, M, Ph D Thesis, Indian Institute of Science, Bangalore, 1993, pp 1–145
70. Charlesworth, M C., McGormick, D J, Madden, B J and Ryan, R. J., *J. Biol Chem*, 1987, **262**, 13409–13416
71. Schneyer, A. L., Sluss, P M., Hutson, J S. *et al*, *Biochemistry*, 1988, **27**, 666–671.
72. Coloma, T. A S, Dattatreyamurthy, B and Reichert, Jr, L. E, *Biochemistry*, 1990, **29**, 1194–1200.
73 Coloma, T A S. and Reichert Jr, L E, *J. Biol. Chem*, 1990, **265**, 5037–5042.
74 Noort, H M, Puijk, W. C, Plasman, M M. *et al*, *Proc Natl. Acad. Sci*, 1992, **89**, 3922–3926
75. Roche, P C., Ryan, R J and McCormick, D J, *Endocrinology*, 1992, **131**, 268–274
76. Dattatreyamurthy, B and Reichert, Jr, L. E., *Endocrinology*, 1993, **133**, 1593–1601.

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