Male Infertility -- New Etiologies and Effective Empirical Therapy

James E. Griffin, M.D.

Department of Internal Medicine

Medicine Grand Rounds

UT Southwestern

November 9, 1995

A little over 10 years ago I reviewed the subject of male infertility for these rounds. Today I want to provide a focussed discussion of some postulated or proven sites of defects in regulation of spermatogenesis or critical genes that lead to male infertility and give you a sense of the excitement of the promise of a new empirical therapy.

OVERVIEW OF THE HYPOTHALAMIC-PITUITARY TESTICULAR AXIS AND THE PROBLEM OF MALE INFERTILITY

The Hypothalamic-Pituitary-Testicular Axis

Neurons in the hypothalamus secrete luteinizing hormone-releasing hormone (LHRH, also called gonadotropin-releasing hormone or GnRH). LHRH is transported to the pituitary by a portal vascular system and interacts with cell surface receptors on pituitary gonadotrophs to stimulate the release of LH and FSH (Fig. 1) (1).

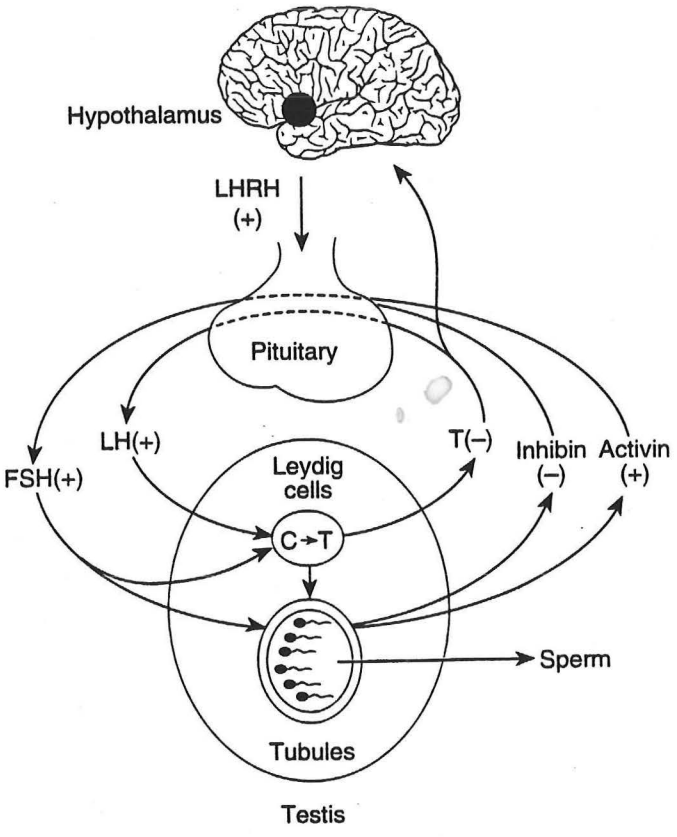


FIGURE 1. Regulation of testosterone and sperm production by LH and FSH. (C, cholesterol; T, testosterone.)

Figure 1

LH and FSH are secreted in the same cells in the pituitary. LH interacts with specific cell surface receptors on Leydig cells resulting in the stimulation of testosterone secretion. FSH binds to receptors on Sertoli cells in the seminiferous tubules and may play a role in steroidogenesis by inducing Leydig cell maturation. In response to locally produced testosterone and effects of FSH on Sertoli cells, spermatogenesis is induced and maintained. Two peptide hormones, inhibin and activin are secreted by the seminiferous tubules.

The secretion of LHRH is episodic resulting in intermittent secretion of LH. Pulsatile secretion of FSH also occurs but is of smaller amplitude. The control of gonadotropin secretion operates primarily by negative feedback. Testosterone and its metabolites act on the CNS to slow the hypothalamic pulse generator and decrease the frequency of LH pulsatile release. Testosterone also appears to have a negative effect feedback on LH secretion at the pituitary level. Inhibin production is enhanced by FSH and androgen and selectively inhibits FSH secretion by the gonadotrophs. Activin, a related peptide hormone, enhances FSH secretion. Testosterone and its metabolites may also have an inhibitory effect on FSH secretion.

1. Griffin JE. 1996 Male reproductive function. In: Griffin JE, Ojeda SR (eds), Textbook of endocrine physiology, 3rd ed,. Oxford University Press: New York; in press.

Causes of Male Infertility

Approximately 15% of couples attempting their first pregnancy are unsuccessful. Couples who have been unable to achieve a pregnancy after one year of unprotected intercourse are usually considered to have primary infertility. In approximately one third of such cases, a significant abnormality impairing fertility is thought to be in the man alone, and in an additional one third, problems in both the man and the woman contribute to decreased fertility. Thus as many as 10% of otherwise healthy men have infertility or at least subfertility with a given partner.

Defective Leydig cell function usually causes infertility because spermatogenesis depends on normal androgen formation and action. However, spermatogenesis may be selectively impaired. Abnormalities of testicular function causing infertility can thus be divided into those with underandrogenization and those with normal virilization (Table I) (2). The level of the defect can also be categorized as hypothalamic-pituitary, testicular, or sperm transport. Certain factors or conditions, such as hyperprolactinemia, radiation, cyclophosphamide, environmental toxins, autoimmunity, paraplegia, and androgen resistance can cause either isolated infertility or a combined defect in testicular function (Table I).

Table I. Abnormalities Causing Male Infertility.

Site of Defect	Infertility with Underandrogenization	Infertility with Normal Virilization
Hypothalamic-pituitary	Panhypopituitarism	
	Isolated gonadotropin deficiency	Isolated FSH deficiency
	Cushing's syndrome	Congenital adrenal hyperplasia
	Hyperprolactinemia	Hyperprolactinemia
	Hemochromatosis	Androgen administration
Testicular	Developmental and structural defects	
	Klinefelter's syndrome	Germinal cell aplasia
	XX male	Cryptorchidism
		Varicocele
		Immotile cilia syndrome
	Acquired defects	
	Viral orchitis	Mycoplasma infection
	Trauma	
	Radiation	Radiation
	Drugs (spironolactone, alcohol, ketoconazole, cyclophosphamide)	Drugs (cyclophosphamide, sulfasalazine)
		Environmental toxins
	Autoimmunity	Autoimmunity
	Granulomatous disease	
	Associated with systemic diseases	
	Renal failure	Febrile illness
	Liver disease	Celia disease
	Sickle cell disease	
	Neurologic diseases (myotonic dystrophy, paraplegia)	Neurologic disease (paraplegia)
	Androgen resistance	Androgen resistance
Sperm transport		Obstruction of epididymis or vas deferens (cystic fibrosis, diethylstilbestrol exposure, congenital absence)

(From Ref. 2)

Relative Frequency of Apparent Causes of Male Infertility

Although this categorization implies that we know a great deal about male infertility, attempts to identify the etiology of the infertility in well studied groups of consecutive men who present with infertility suggests that we do not (Table II) (3, 4).

Table II. Relative Frequency of Causes and Associated Conditions in Men Who Present with Infertility.

Cause or Condition	% in Study of Greenberg et al (3) (n = 425)	% in Study of Baker et al (4) (n = 1041)
Hypogonadotropic hypogonadism	0.9	0.6
Klinefelter's syndrome	1.6	1.9
Cryptorchidism	6.1	6.4
Varicocele	37.4	40.3
Immotile sperm	0.5	0.6
Viral orchitis	1.9	1.6
Radiation/chemotherapy	--	0.5
Obstruction of epididymis or vas deferens	6.1	4.1
Androgen resistance	--	0.1
Coital disorders	4.0	0.5
Idiopathic disorders	41.5*	43.4Δ

*Includes miscellaneous semen abnormalities 10.2%, and undiagnosed primary testicular failure, 5.9%
Δ Includes possible obstruction, 4.5%

However, these estimates are likely overly optimistic in defining causation. Because at best only about half of the almost 40% of infertile men with a varicocele might achieve fertility after varicocele repair, it is probably more appropriate to consider that 60% of infertile men have idiopathic infertility.

2. Griffin JE, Wilson JD. 1992 Disorders of the testes and male reproductive tract. In: Wilson JD, Foster DW (eds). Williams textbook of endocrinology, 8th ed. WB Saunders: Philadelphia; 799-852.

3. Greenberg SH, Lipshultz LI, Wein, AJ. 1978 Experience with 425 subfertile male patients. J Urol. 119:507-510.

4. Baker HWG, Burger HG, deKretser DM, Hudson B. 1986 Relative incidence of etiologic disorders in male infertility. In: Santen RJ, Swerdloff RS, (eds). Male reproductive dysfunction: diagnosis and management of hypogonadism, infertility, and impotence. New York: Marcel Dekker; 342-372.

Lack of Efficacy of "Standard" Therapies of Idiopathic Male Infertility

The management of male infertility is usually unsatisfactory. Disorders for which there are logical or effective treatment include genital tract obstruction, sperm autoimmunity, gonadotropin deficiency, coital disorders, and reversible toxin exposure account for only 10 to 20% of men seen for infertility (5). Although these disorders are treatable, pregnancies are infrequent with genital tract obstruction and sperm autoimmunity. Azoospermia in men with other causes has been largely considered untreatable. This group may account for about one quarter of men seen for infertility in Western societies (4). Although a diagnosis of primary seminiferous tubule failure with germ cell aplasia, germ cell arrest, or severe hypospermatogenesis may be rendered on biopsy in half of these men, in the majority of cases the cause is unknown.

The other two-thirds or three quarters of men seen for infertility have some partial reduction in semen parameters and subfertility of variable degree dependent on relative fertility of the female partner. Particularly in this group of men, empirical therapy in the past has been tried with many later disproven claims. Most reports fail to take into account the spontaneous fertility rate in untreated men (25% in 1 year) (6). Treatment independent pregnancy among infertile couples occurs in all forms of human infertility (male and female factors), and the possibility of its occurrence makes it necessary for all therapies to be evaluated in randomized clinical trials (7). When several different forms of empirical therapy, including testosterone rebound, nonaromatizable androgen (mesterolone), gonadotropin, antiestrogen (clomiphene), antibiotics, bromocriptine, varicocele repair, artificial insemination, and no therapy were evaluated and compared in one large clinical retrospective analysis of oligospermic men at one center in the 1970s, no improvement in the relative pregnancy rate was demonstrated for any empirical therapy compared with no therapy (Table I) (8).

Table III. Treatments of Uncertain Value for Male Infertility of Undetermined Etiology

Treatment	No. of Courses	Pregnancies *		Relative Pregnancy Rate
		No.	Expected	
Testosterone rebound	33	6	7.93	0.76
Mesterolone	49	7	12.93	0.54
hCG	10	2	2.15	0.83
Clomiphene	50	5	10.66	0.47
Antibacterial agents	95	25	18.83	1.33
Bromocriptine	13	3	3.15	0.95
Varicocelectomy	201	70	57.37	1.22
AIH	61	10	11.76	0.85
None	583	102	105.22	0.97

*Melbourne experience, pregnancy rates calculated by life table analysis.
Log rank $X^2 = 11.37$; p = NS. AIH, artificial insemination with husband's semen. (From ref. 8)

A more recent analysis accepting only randomized control trials for male subfertility evaluated 72 trials by meta-analyses concluded that the quality of most trials was poor and that their inclusion made suspect the accuracy of any conclusion. In a few instances, such as bromocriptine and androgen therapy, meta-analysis did allow a firm conclusion that they were ineffective.

5. Baker HWG. 1995 Male infertility. In de Groot LJ, ed. Endocrinology 3rd ed., Philadelphia: W.B. Saunders; 2404-2433.

6. Sherins RJ, Brightwell D, Sternthal PM. 1977 Longitudinal analysis of semen of fertile and infertile men. In: Toen P, Nankin HR, eds. The testis in normal and infertile men. New York: Raven; 473-488.

7. Collins JA, Written W, James LB, Wilson EH. 1983 Treatment-independent pregnancy among infertile couples. N Engl J Med. 309:1201-1209.

8. Baker HWG. 1994 Male infertility of undermined etiology. In: Bardin CW ed. Current therapy in endocrinology and metabolism, 5th ed. St. Louis: Mosby; 314-318.

9. O'Donovan PA, Vandekerckhove P, Lilford RJ, Hughes E. 1993 Treatment of male infertility: is it effective? Review and meta-analyses of published randomized controlled trials. Hum Reprod. 8:1209-1222.

EMERGING VIEW OF THE REGULATION OF SPERMATOGENESIS - LOCAL CONTROL SYSTEMS WITHIN THE TESTIS

Structural Organization of the Seminiferous Epithelium--Sites of Action of FSH and Androgen

The development of germ cells from the initial spermatogonia through spermatocytes, spermatids to a mature spermatogonia takes place with cells embedded within the Sertoli cell cytoplasm in the seminiferous tubules. The seminiferous tubules are surrounded by peritubular myoid cells and Leydig cells in the interstitial space. Cell-cell interactions within the testis can take place between the two components (tubular and interstitial) as well as between the cells within each component (Fig. 2) (10,11).

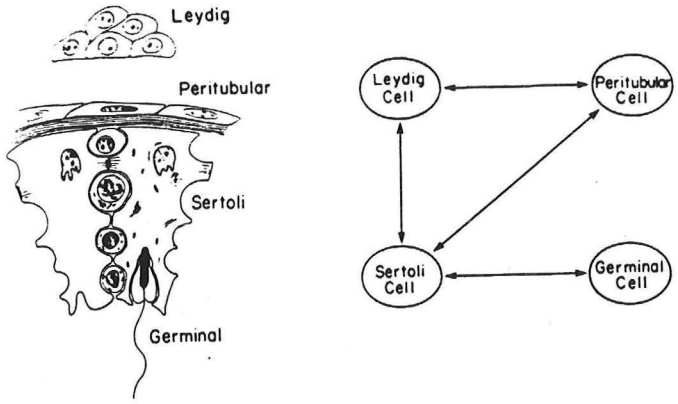


FIG. 2. Cell-cell interactions in the testis.

Fig. 2

The two main hormones of importance for spermatogenesis are FSH and testosterone. FSH receptors are present on Sertoli cells and spermatogonia. Androgen receptors are present in Sertoli cells, Leydig cells, and peritubular cells.

During germ cell development, germ cells show incomplete cytokinesis with a resulting connection by cytoplasmic bridges between differentiating spermatocytes and spermatids (12). This interconnection is thought to facilitate coordinated development of groups of germ cells along the seminiferous epithelium, a so-called wave or synchrony of histologically distinct stages. Recently, two groups have reported stage-dependent expression of androgen receptors in rat Sertoli cells identifying a new level of regulation of androgen action in spermatogenesis (13, 14). The amount of receptor peaked at stage VII of the spermatogenic cycle and was shown to be controlled by androgen in one study (Fig. 3) (14). In both studies the level of androgen receptor immunostaining in peritubular myoid cells was prominent and consistent throughout the cycle.

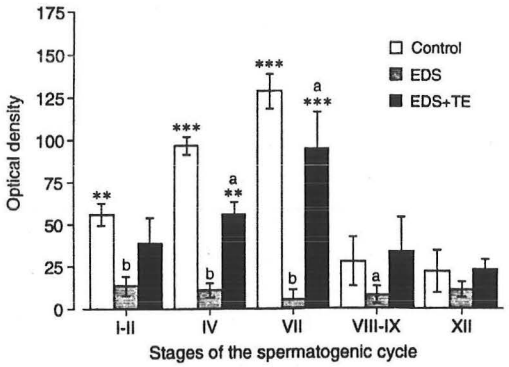


FIG. 3. Quantitative analysis of the intensity of SC nuclear immunostaining for the AR at different stages of the spermatogenic cycle in a control rat or a rat treated 6 days earlier with EDS alone or EDS supplemented with T esters (EDS + TE). Data are the mean ± SD for 5 tubules/stage(s) and were based on the analysis of 10 nuclei/tubule. Tissue sections from each animal were processed in parallel for immunostaining to avoid interexperiment error. Comparable results were obtained in a separate experiment involving tissue from different animals. **, $P < 0.01$; ***, $P < 0.001$ (compared with values for stage XII in the same treatment group). a, $P < 0.05$; b, $P < 0.001$ (compared with respective control values for that stage).

Fig. 3

In man there are thought to be fewer stages than in the rat with a helical arrangement (12), apparently not always easy to demonstrate (15). Variation of androgen receptor expression by spermatogenic cycle stage in man has not been reported.

Very recently one group has reported that androgen may affect Sertoli cells by a rapidly increasing cytosolic calcium by a nongenomic-mediated mechanism (16).

Tight junctions between Sertoli cells at a site between the spermatogonia and primary spermatocytes form a diffusion barrier that divides the testis into two functional compartments, the basal and adluminal (Fig. 4) (12). The basal compartment consists of the Leydig cells, the peritubular cells, and the outer layer of the tubule containing the spermatogonia. The adluminal compartment consists of the inner two-thirds of the tubules including primary spermatocytes and more advanced stages of spermatogenesis.

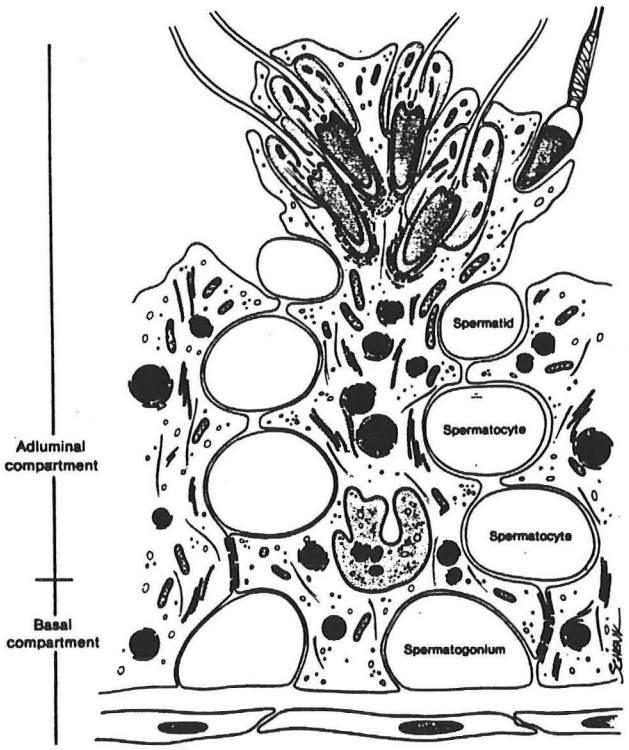


Figure 4. Diagram of the Sertoli cell showing the relation between Sertoli cell cytoplasm and developing spermatocytes.

Figure 4

The primary function of this Sertoli cell barrier is probably to ensure that the proper conditions for germ cell development exist in the tubules. Some molecules enter the tubules readily while others are almost completely excluded. For example, testosterone and glucose appear to have accelerated entry rates while dyes and peptide hormones are generally excluded. Peptide hormones produced or secreted into the tubular lumen are retained there by the barrier and probably do not function as endocrine factors outside the testis. Since the Sertoli cell barrier prevents most substances from entering the seminiferous tubule and directly influencing germ cell development, the testis cannot rely solely on the delivery of circulating hormones, nutrients, and growth factors but must independently produce its own regulatory substances.

10. Skinner MK. 1991 Cell-cell interactions in the testis. Endocrine Rev. 12:45-77.

11. Verhoeven G. 1992 Local control systems within the testis. Bailliere's Clinical Endocrinol Metab. 6:313-333.

12. De Kretser DM, Riskbridger GP, Kerr JB. 1995. Basic endocrinology of the testis. In: de Groot LJ, (ed). Endocrinology, 3rd ed. Philadelphia: WB Saunders; 2307-2335.

13. Vornberger W, Prins G, Musto NA, Suarez-Quian CA. 1994 Androgen receptor distribution in rat testis: New implications for androgen regulation of spermatogenesis. Endocrinology. 134:2307-2316.

14. Bremner WJ, Millar MR, Sharpe RM, Saunders PTK. 1994 Immunohistochemical localization of androgen receptors in the rat testis: Evidence for stage-dependent expression and regulation by androgens. Endocrinology. 135:1227-1234.

15. Johnson L. 1994 A new approach to study the architectural arrangement of spermatogenic stages revealed little evidence of a partial wave along the length of human seminiferous tubules. J Androl. 15:435-441.

16. Gorczynska E, Handelsman DJ. 1995 Androgens rapidly increase the cytosolic calcium concentration in Sertoli cells. Endocrinology. 136:2052-2059.

Three Additional Factors Essential for Spermatogenesis

In addition to FSH and testosterone, three other factors have been shown to play an essential role in modulating spermatogenesis: vitamin A, c-fos, and stem cell factor (17).

Vitamin-A has been known to be important for spermatogenesis for some time. It must be transported to the developing germ cells. Retinoid binding proteins have been localized to both Sertoli cells and germ cells. They facilitate retinoid transport into the nucleus for binding to receptors leading to activation of specific mRNA transcription. Replacement of vitamin A to deficient rats with germ cell arrest restores spermatogenesis in a synchronized stage-specific fashion throughout the seminiferous tubules (18). Retinoic acid receptor-α mRNA is regulated during the spermatogenic cycle showing a 7-fold increase in the level of 3.4-kb mRNA at stages VIII-IX (19). Since stage VIII is where the germ cells development is arrested in vitamin A-deficient rats, this suggests the α mRNA transcription may be necessary before more advanced stages of germ cell will occur. Treatment of vitamin A deficient rats with retinol led to a rapid 3-fold increase in retinoic acid receptor α mRNA levels. The precise regulation of receptor by retinol suggests that its synthesis is required before it can be used to modulate the transcription of retinol-inducible genes. In contrast retinoic acid receptor-β mRNA remained unchanged after injection of retinol.

Retinoic acid receptor α is present in both Sertoli cells and germ cells (20). Study of the effect of testosterone administration to 20-day-old rats indicate that testosterone results in a rapid increase in the steady-state levels of mRNAs for RARα while decreasing the level of mRNA for RARγ (Table IV) (20). It is postulated that testosterone may affect some of the Sertoli cell functions through RAR-mediated mechanism.

Table IV. Relative Abundance of 3.4 kb mRNA Transcripts for RARα and RARγ in 20-day-old rat testes 1 h after Testosterone Administration.

Testosterone	RARα	RARγ
Control	100	100
0.001 mg	115 ± 5[b]	86 ± 2
0.01 mg	134 ± 8[b]	83 ± 7
0.1 mg	147 ± 8[bc]	78 ± 6
1 mg	195 ± 13[bc]	64 ± 20[b]

The peak of each transcript was normalized against the area of actin mRNA and expressed as percent ± SEM of the control animals from their identical experiments. b, $p < 0.05$ vs controls; c, $p < 0.05$ vs .001 mg group (from ref. 20).

17. Pescovitz OH, Srivastava CH, Breyer PR, Monts BA. 1994 Paracrine control of spermatogenesis. Trends Endocrinol Metab. 5:126-131.

18. Morales C, Griswold MD. 1987. Retinol-induced stage synchronization in seminiferous tubules of the rat. Endocrinology. 121:432-434.

19. Kim KH, Griswold MD. 1990 The regulation of retinoic acid receptor mRNA levels during spermatogenesis. Mol Endocrinol. 4:1679-1688.

20. Huang HFS, Li MT, Pogach LM, Qian L. 1994 Messenger ribonucleic acid of rat testicular retinoic acid receptors: Developmental pattern, cellular distribution, and testosterone effect. Biol Reprod. 51:541-550.

The c-fos nuclear proto-oncogene is widely distributed and rapidly induced in cells during mitogenesis. It is a member of a gene family whose products mediate protein-protein interactions. Although c-fos is widely distirbuted it may not be critical for the replication of most cells in the prenatal and more mature mouse. Homozygous mutant mice with germ line mutations in the c-fos locus show significant loss of viability at birth (21). However, approximately 40% survive and grow at normal rates until osteopetrosis begins to develop about 11 days. These mice may live as long as wild type or heterozygous littermates. Among other abnormalities, these mice show abnormal gametogenesis, lymphopenia, and altered behavior. The testes are small, and progression of spermatogonia through meiosis is diminished. Even when spermatozoa are present, matings have been sterile (21). Spermatogenesis appears to be one of the few physiologic processes dependent on normal expression of c-fos.

21. Johnson RS, Spiegelman BM, Papaioannou V. 1992 Pleiotropic effects of a null mutation in the c-fos proto-oncogene. Cell. 71:577-586.

Another paracrine system important for spermatogenesis is the interaction of stem cell factor and its receptor, c-kit. The c-kit proto-oncogene was first described in the evaluation of the dominant white spotting (W) locus of the mouse (22). The characteristic phenotype of mutants at this locus includes white coat color, sterility, and anemia. The c-kit proto-oncogene encodes a tyrosine kinase receptor. The phenotype could be attributed to the failure of stem cell populations to migrate and/or proliferate effectively during development. Stem cell factor was described as a growth factor for early hematopoietic cells and is the major ligand for the c-kit receptor (23, 24). The mouse Sl locus encodes stem cell factor (also known as Steel factor). Homozygous mutants at the Sl locus are similar to the W locus homozygous mutants with deficiency in germ cells, melanocytes, and hematopoietic cells.

The gonadal expression studies of c-kit demonstrated localization in spermatogonia through primary spermatocytes (25). The ligand for c-kit was not found in germ cells but was specifically localized to mouse Sertoli cells (26). The use of monoclonal antibody to c-kit allowed the demonstration of the function significance of c-kit (27). Injection of the antibody in prepubertal mice completely blocked the mitosis of mature type A spermatogonia to the intermediate spermatogonium but not of the primitive type A spermatogonia including the self-renewing testicular stem cells. Subsequently, c-kit has been shown to be in the same family as the platelet-derived growth factor receptor, and stem cell factor has been found in both soluble and membrane-bound forms (28). The overall localization site of action of the c-kit receptor and its ligand are diagramed in Fig. 5 in which slf is an abbreviation for Steel factor, another name for stem cell factor (28).

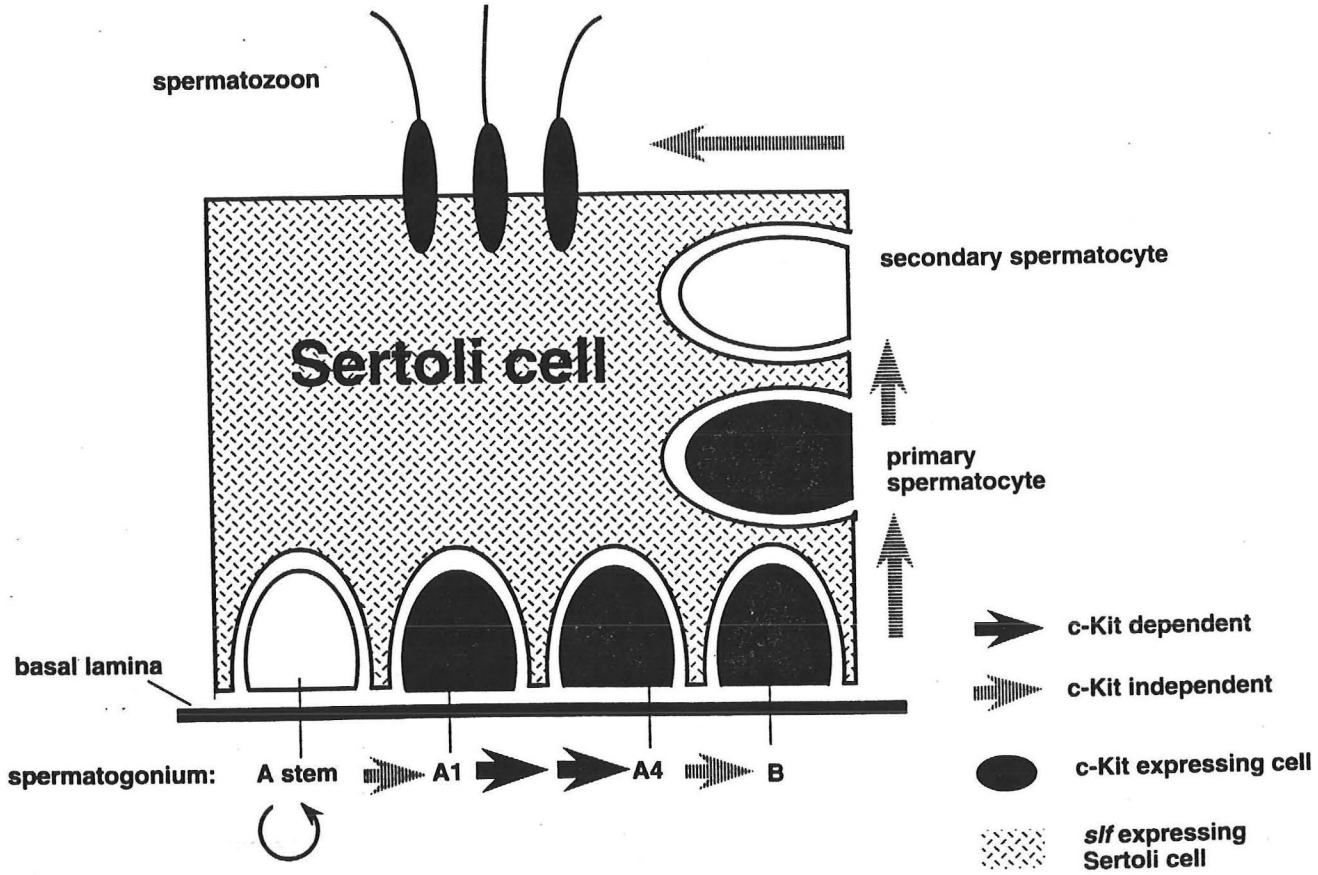


Fig. 5. Scheme of spermatogenesis within the testis. The stages requiring a functional c-Kit receptor are indicated.

Fig. 5

22. Geissler EN, Ryan MA, Housman DE. 1988 The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. Cell. 55:185-192.

23. Zsebo KM, Williams DA, Geissler EN, Broudy VC, Martin FH, Atkins HL, Hsu RY, Birkett NC, Okino KH, Murdock DC, Jacobsen FW, Langley KE, Smith KA, Takeishi T, Cattanach BM, Galli SJ, Suggs SV. 1990 Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell. 63:213-224.

24. Huang E, Nocka K, Beier DR, Chu TY, Buck J, Lahm HW, Wellner D, Leder P, Besmer P. 1990 The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. Cell. 63:225-233.

25. Manova K, Nocka K, Besmer P, Bachvarova RF. 1990 Gonadal expression of c-kit encoded at the W locus of the mouse. Development. 110:1057-1069.

26. Rossi P, Albanesi C, Grimaldi P, Geremia R. 1991 Expression of the mRNA for the ligand of c-kit in mouse Sertoli cells. Biochem Biophys Res Commun. 176:910-914.

27. Yoshinaga K, Nishikawa S, Ogawa M, Hayashi SI, Kunisada T, Fujimoto T, Nishikawa SI. 1991 Role of c-kit in mouse spermatogenesis: identification of spermatogonia as a specific site of c-kit expression and function. Development. 113:689-699.

28. Morrison-Graham K, Takahasi Y. 1993 Steel factor and c-kit receptor: From mutants to a growth factor system. Bio Essays. 15:77-83.

Postulated Intratesticular Paracrine Control Factors and Regulators of Spermatogenesis

It is clear that the Sertoli cell is the major mediator of germ cell development. FSH acts on the Sertoli to increase production of androgen-binding protein (ABP), transferrin, inhibin, aromatase, plasminogen-activators (reviewed in 12). FSH also enhances glucose transport in Sertoli cells and conversion of glucose to lactate. Although Sertoli cells contain androgen receptors, it appears that the androgen-mediated stimulation of the major Sertoli cell proteins such transferrin and inhibin is indirect (10). P-Mod-S is an androgen-induced protein produced by peritubular myoid cells that stimulates both transferrin (Fig. 6) and inhibin production is isolated Sertoli cells (10). P-Mod-S (for Peritubular factor Modifying Sertoli cell function) has been isolated and characterized as consisting of two forms with molecular weight of 56 kD and 59 kD.

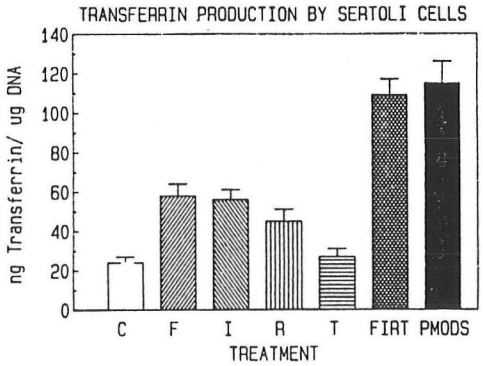


FIG. 6 Transferrin production by Sertoli cells isolated from 20 day-old rats and cultured for 5 days in the absence, control (C), or presence of 100 ng/ml FSH (F), 5 μg/ml insulin (I), 0.35 μM retinol (R), 1 μM testosterone (T), a combination of FSH, insulin, retinol, and testosterone (FIRT), or 20 ng/ml PModS (PMODS). Transferrin levels were determined with a RIA and expressed as (nanograms per μg Sertoli cell DNA) and presented as the mean ± SEM of three different experiments performed in triplicate.

Fig. 6

A listing of some of the known peptide hormones, growth factors, releasing factors, and cytokines which may act on Leydig cells and Sertoli cells in a paracrine or autocrine fashion and their sources gives an idea of the complexity of intratesticular control (Table V) (11).

Table V. Peptide hormones, growth factors, releasing factors, and cytokines which may act on Leydig and Sertoli cells in a paracrine or autocrine fashion.

	Leydig cells		Sertoli cells	
Peptide hormones	Angiotensin II	(L,G)	ACTH	(L)
	Oxytocin	(U)	α-MSH	(L)
	Vasopressin	(S,L)	Opioids	(L,S,G,P)
Growth factors	Inhibin/activin	(S,L)	NGF	(G)
	bFGF	(S,G)	bFGF	(S,G)
	IGF-I	(S,P,L)	IGF-I	(S,P,L)
	TGF α/β	(S,P,L)	TGFα	(S,P,L)
Releasing factors	LHRH	(U)	GHRH	(G)
	CRH	(L, G)		
Cytokines	IL-1	(S,M)		
	TNF	(M)		

Putative intratesticular sources in parentheses: G, germ cells, L, Leydig cells; M, macrophages; P, peritubular cells; S, Sertoli cells; U, unknown (modified from Ref. 11)

Most of these postulated paracrine or autocrine effects are derived from in vitro studies. As stressed by Verhoeven (11) and by Nieschlag (29), one should not jump from studies of isolated cells in immature animals to the conclusion of physiological relevance in the in vivo adult.

29. Spiteri-Grech J, Nieschlag E. 1993 Paracrine factors relevant to the regulation of spermatogenesis - a review. J Reprod Fertil. 98:1-14.

I would like to cite a few specific examples of recent studies relating some of these factors to spermatogenesis.

There is a stage specific expression of the inhibin and activin subunit mRNAs in the rat seminiferous epithelium (Fig. 7) (30, 31) the α and β_B mRNA are co-regulated with FSH receptor and FSH-stimulated cAMP production and are highest at stages XIII-IV. The β_A

mRNA is highest at stages VIII-XI. Thus relative production of inhibin and activin may change during the seminiferous epithelium cycle. In a separate study activin-A was shown to stimulate intermediate spermatogonia and preleptolene spermatocyte DNA synthesis in a dose dependent manner, whereas inhibin-A inhibited DNA synthesis in these cells. Correlation of the activin effects with activin-A receptor expression was possible for the intermediate spermatogonia but not the spermatocyte (30).

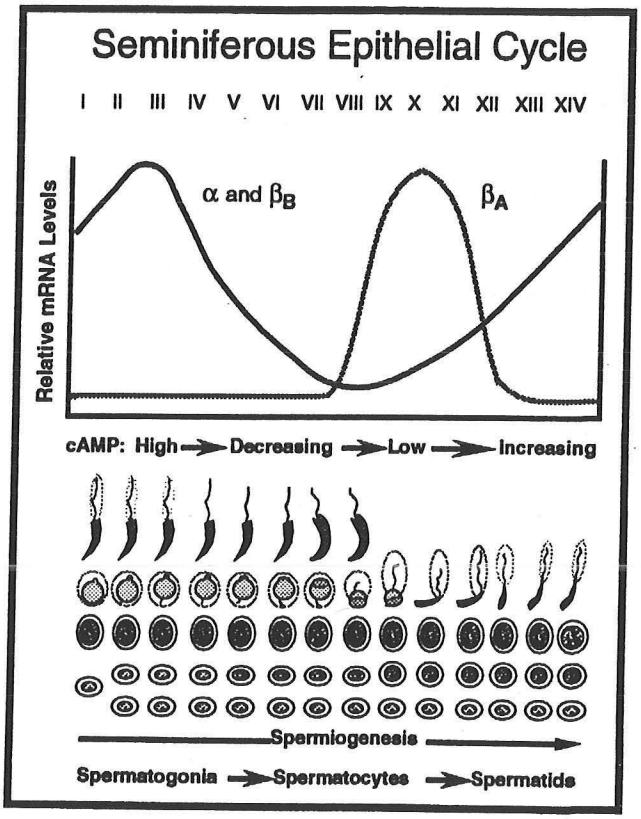


Fig. 7 Is a schematic representation of changes in inhibin and activin α, β_A, and β_B subunit mRNAs during the stages of the rat seminiferous epithelial cycle. Also indicated are changes in gonadotropin-stimulated cAMP levels in Sertoli cells and the cellular changes associated with differentiation of the germ cells. Species differences, particularly with respect to expression in the primate ovary, are discussed in the text. These diagrams indicate changes in subunit mRNA abundance, but they do not reflect the absolute levels of the α, β_A and β_B subunit mRNAs, which differ as discussed in the text.

Fig. 7

Transgenic mice with disruption of the inhibin-specific α subunit develop gonadal stromal cell tumors with eventual cessation of spermatogenesis. Disruption of the β_B subunit (combined inhibin and activin deficiency), interestingly, did not impair male fertility (reviewed in 31) .

30. Kaipia A, Penttilä TL, Shimasaki S, Ling N, Parvinen M, Toppari J. 1992 Expression of inhibin β_A and β_B, follistatin and activin-A receptor messenger ribonucleic acids in the rat seminiferous epithelium. Endocrinology. 131:2703-2710.

31. Mayo KE. 1994 Inhibin and activin. Molecular aspects of regulation and function. Trends Endocrinol Metab. 5:407-415.

32. Hakovirta H, Kaipia A, Söder O, Parvinen M. 1993 Effects of activin-A, inhibin-A, and transforming growth factor-β1 on stage-specific deoxyribonucleic acid synthesis during rat seminiferous epithelial cycle. Endocrinology. 133:1664-1668.

β-Nerve growth factor (NGF) has testosterone-down regulated receptors in Sertoli cells. Analysis of stage-specific expression in the rat seminiferous epithelium showed NGF protein and mRNA at all stages of the cycle. The testosterone-regulated receptor was only found in late stage VII and stage VIII, the sites of onset of meiosis, and was present in the plasma membrane of Sertoli cells (33). NGF was shown to stimulate in vitro DNA synthesis of seminiferous tubule segments with preleptotene spermatocytes at the onset of meiosis while other segments remained nonresponsive (33).

33. Parvinen M, Pelto-Huikko M, Söder O, Schultz R, Kaipia A, Mali P, Toppari J, Hakovirta, Lönnerberg, Ritzén EM, Ebendal T, Olson L, Hökfelt T, Persson H. 1992 Expression of β-Nerve growth factor and its receptor in rat seminiferous epithelium: specific function at the onset of meiosis. J Cell Biol. 117:629-641.

IGF-I is thought to be an important regulator of the seminiferous epithelium. The synthesis and secretion of IGF-I by Sertoli cells in culture has been shown along with its stimulation by FSH in a dose-dependent manner (reviewed in 34). Receptors for IGF-I have been demonstrated on secondary spermatocytes and early spermatids as well as Sertoli cells supporting both a paracrine and autocrine function. IGF-I stimulates ^{3}H-thymidine incorporation into DNA in Sertoli cells as well as lactate production and plasminogen activator secretion. However, it inhibits transferrin production. IGF-I and TGF-α (but not FGF or PDGF) stimulate differentiation of type A spermatogonia in organ culture of adult mouse cryptorchid testes in a dose-dependent manner (35).

34. Spiteri-Grech J, Nieschlag E. 1992 The role of growth hormone and insulin-like growth factor I in the regulation of male reproductive function. Horm Res. 38(suppl 1):22-27.

35. Tajima Y, Watanabe D, Koshimizu U, Matsuzawa T, Nishimune Y. 1995 Insulin-like growth factor-I and transforming growth factor-α stimulate differentiation of type A spermatogonia in organ culture of adult mouse cryptorchid testes. J Androl. 18:8-12.

Rat and human testes produce large amounts of an interleukin-1 (IL-1)-like factor originating from the seminiferous tubules. IL-1α stimulates both mitotic and meiotic DNA synthesis in spermatogonia of hypophysectominized rats in vivo. Along the wave of the seminiferous epithelium IL-1 secretion and content were found to correlate with spermatogonial DNA synthesis (36). A study of isolated segments of seminiferous tubules at various stages in vitro with chemically defined medium indicated that added IL-1α could stimulate DNA synthesis in both mitotic and meiotic phases (37).

36. Söder O, Syed V, Callard GV, Toppari J, Pöllänen, Parvinen M, Fröysa B, Ritzén. 1991 Production and secretion of an interleukin-1-like factor is stage-dependent and correlates with spermatogonial DNA synthesis in the rat seminiferous epithelium. J Androl. 14:223-231.

37. Parvinen M, Söder O, Mali P, Fröysa B, Ritzén EM. 1991 In vitro stimulation of stage-specific deoxyribonucleic acid synthesis in rat seminiferous tubule segments by interleukin-1α. Endocrinology. 129:1614-1620.

EXAMPLES OF SPECIFIC GENE DEFECTS CAUSING HUMAN MALE INFERTILITY

Although Table I lists many conditions associated with male infertility, most of the listed conditions (e.g., Klinefelter's syndrome, cryptorchidism, systemic diseases, etc.) do not define a specific gene which when defective results in defective spermatogenesis. Three examples with recent definition of a genetic mechanism are described below.

The Azoospermia Factor(s) (AZF) on the Y Chromosome

The initial idea that a factor controlling spermatogenesis might reside on the Y chromosome came from a survey of a large number of men with azoospermia (38). Six men with normal male phenotypic development were found to have a deletion of the distal fluorescent portion of the long arm of the Y chromosome as well as part of the nonfluorescent segment lying proximal to it. Based on the reports of other Y chromosome abnormalities in the literature, the authors postulated that the subsequently named azoospermia factor (AZF) must be at the interface between the fluorescent and non-fluorescent material (Fig. 8) (39). The AZF locus has been subsequently mapped to interval 6 of the Y chromosome map and lies within band Yq11.23 (reviewed in 39).

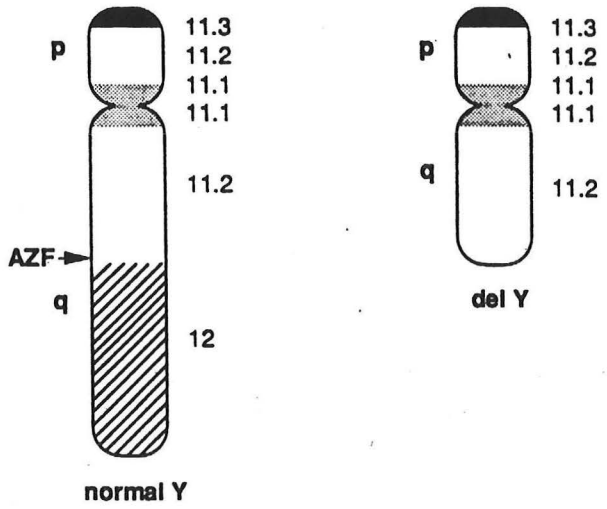


Figure 8 Normal human Y chromosome (left), showing localization of AZF, the 'azoospermia factor', as postulated by Tiepolo and Zuffardi (1976) from phenotype–genotype correlation based on azoospermic men carrying a deleted Yq chromosome (right).

Fig. 8

A search for microdeletions of the Y chromosome interval 6 revealed 4 men of 50 screened with cytologically normal Y chromosomes to have microdeletions within this region (39-41). The men screened typically had oligozoospermia or azoospermia with elevated FSH and testis biopsies (performed in half) showing Sertoli-cell-only syndrome, hypospermatogenesis, or maturation arrest at the spermatocyte or spermatid stage. Within the 14 subintervals of interval 6, one individual showed deletion in proximal sub-interval I whereas the three others showed deletions in distal subintervals XII-XIV. These proximal and distal microdeletions did not overlap, indicating either that AZF was a large locus or that more than one gene or gene family located with Yq11.23 might be involved in spermatogenesis.

A second study of 50 Japanese men with azoospermia and cytogenetically normal Y chromosomes disclosed six men with microdeletions in the same region (42). This combination of these two studies might suggest that as many as 10% of men with unexplained azoospermia have microdeletions in this region of the Y chromosome.

Position cloning was used to isolate a new gene family in this region termed the Y-located RNA Recognition Motif genes (YRRM) (43). This family is part of a super family of RNA binding proteins, the heterogeneous nuclear ribonuclear proteins (HnRNPs) (39). The YRRM genes have a particularly close similarity (60% amino acid conservation) to HnRNPG. This family of RNA binding proteins are as abundant in the nucleus as histones and have been shown to be involved in RNA trafficking between nucleus and cytoplasm (44) and implicated in RNA processing. Unlike many members of the HnRNP superfamily YRRM and HnRNPG have one RNA binding (RRM) domain rather than two. The RRM contains a pair of highly conserved motifs, an octapeptide RNP1 and a hexapeptide RNP2. These parts of the protein are thought to form β-sheets which contact the RNA directly. The YRRM gene family has at least 15 members, more than one of which are transcribed. Transcription of the YRRM genes has not been detected outside the testis. In situ hybridization shows a strong signal on Yq11

and a secondary, but much weaker signal on proximal Yp (39). Gene copies of YRRM are thus principally clustered in a 1-2 Mb region of Yq, interval 5 and 6 with other copies proximal on both the long and short arms.

RNA in situ hybridization to normal adult testes tissue indicated gene expression of the YRRM family is limited to germ cells, primarily spermatogonia and early primary spermatocytes. Insignificant labeling was seen over spermatids and Sertoli cells (39).

Studies of the conservation and sex specificity of the YRRM sequences using paired male and females DNAs from various species shows the YRRM signal is clearly male and that there is amplification of the gene only in humans and gorilla among species tested (Fig. 9) (43).

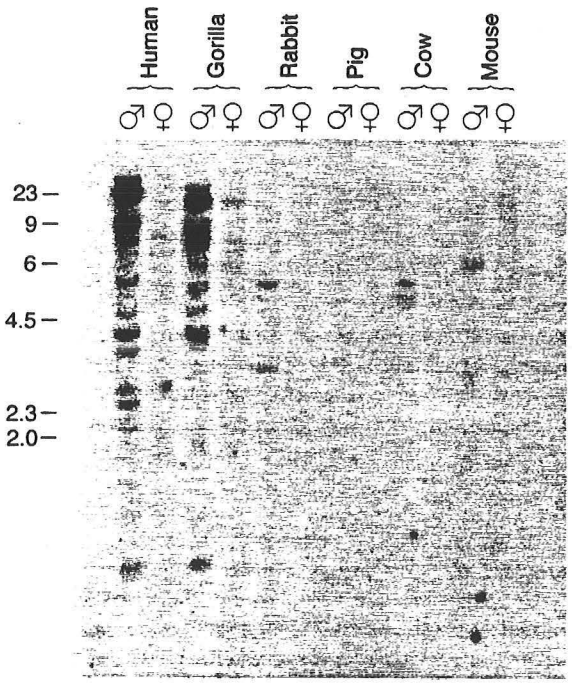


Figure 9 Distribution of *YRRM* Sequences in Male and Female Mammals

Filters were hybridized with ^{32}P-labeled *MK5* cDNA probe at 60°C in 4× SSC and washed at similar stringency. DNA source and sex are shown above each lane. Molecular weight markers (λ/HindIII digest) are shown at left in kilobases.

Fig. 9

Whether this is just a curiosity or indicates that humans are inefficient producers of sperm compared with the mouse and need multiple YRRM genes is not known. It appears that YRRM is a strong candidate for the AZF.

Subsequently the group reporting microdeletion in the Yq11 region in Japanese men (42) reported a more detailed analysis using 15 loci mapping to Yq11.23 and the YRRM probe in 63 men (Fig. 10) (45). They detected microdeletions in 10 men out of the 50 included in the previous study, one (#1493) showed a deletion in addition to the previous six patients and lacked theYRRM locus. Among the 13 new patients, three (#1503, #1958, and #1960) showed deletions. Patient #1960 lacked YRRM, whereas the other two, like five of the originally reported patients, had deletions for 9 loci not involving YRRM.

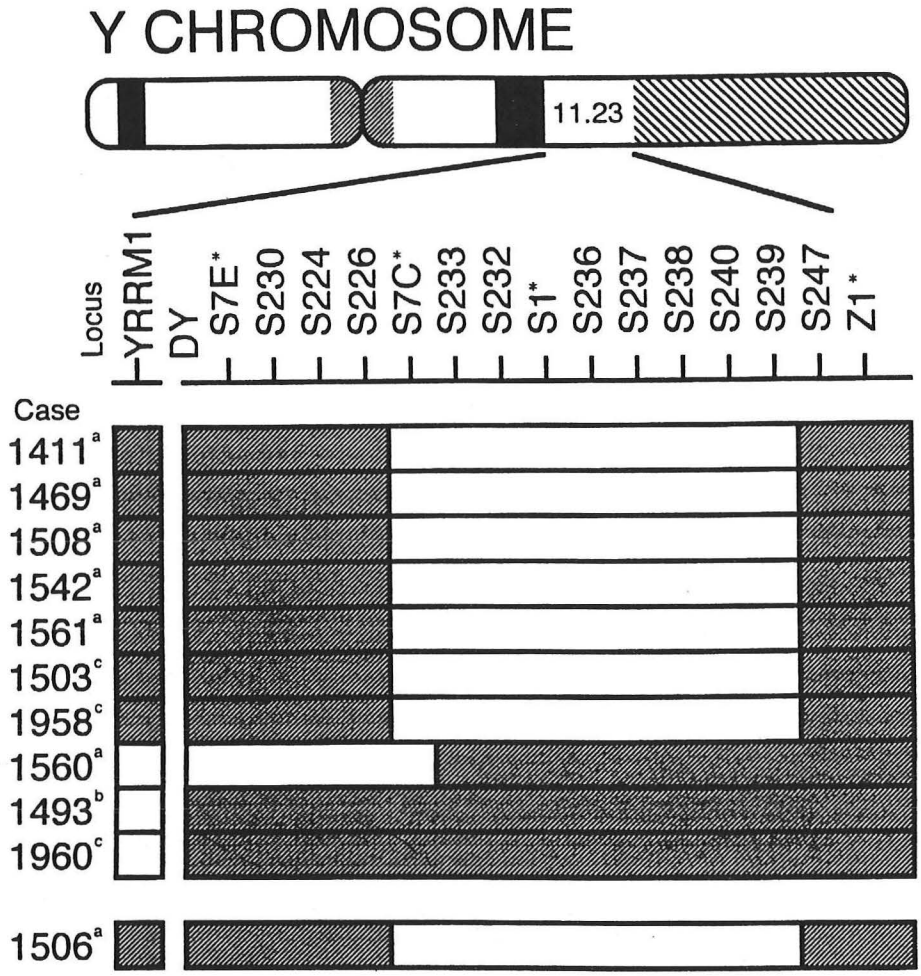


Fig. 10. Summary of DNA analysis in individual patients. Only those lacking at least one locus were included. Every locus was detected by PCR except DYS7C which was examined by Southern hybridization. *Four loci examined in our previous study (7). Hatched bars indicate the presence of loci, while open bars indicate the absence. # 1506 is the father of # 1411. [a]Samples with deletions detected in our previous study (7). [b]Those included in the previous study but no deletion was detected based on the analysis of four loci (*). [c]Individuals in this study.

Fig. 10

Thus there must be at least one additional gene between DYS226 and DYS247, deletion of which causes azoospermia or severe oligozoospermia in addition to the YRRM genes which map outside this region. The publication of a sequenced-tagged sites (STS) map of the human Y chromosome by Vollrath et al (46) should make it possible to rapidly detect Y-microdeletion as in the study of the Japanese men and better define the frequency of this cause of oligo- and azoospermia.

38. Tiepolo L, Zuffardi O. 1976 Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet. 34:119-124.

39. Chandley AC, Cooke HJ. 1994 Human male fertility - Y-linked genes and spermatogenesis. Hum Mol Gen. 3:1449-1452.

40. Ma K, Sharkey A, Kirsch S, Vogt P, Keil R, Hargreave TB, McBeath S, Chandley AC. 1992 Towards the molecular localisation of the AZF locus: mapping of microdeletions in azoospermic men within 14 subintervals of interval 6 of the human Y chromosome. Hum Mol Gen. 1:29-33.

41. Vogt P, Chandley AC, Hargreave TB, Keil R, Ma K, Sharkey A. 1992 Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of AZF, a human spermatogenesis gene. Hum Genet. 89:491-496.

42. Nagafuchi S, Namiki M, Nakahori Y, Kondoh N, Okuyama A, Nakagome Y. 1993 A minute deletion of the Y chromosome in men with azoospermia. J Urol. 150:1155-1157.

43. Ma K, Inglis JD, Sharkey A, Bickmore WA, Hill RE, Prosser EJ, Speed RM, Thomson EJ, Jobling M, Taylor K, Wolfe J, Cooke HJ, Hargreave TB, Chandley AC. 1993 A Y chromosome gene family with RNA-binding protein homology: Candidates for the azoospermia factor AZF controlling human spermatogenesis. Cell. 75:1287-1295.

44. Piñol-Roma S, Dreyfuss G. 1993 hnRNP proteins: localization and transport between the nucleus and the cytoplasm. Trends in Cell Biol. 3:151-155.

45. Kobayashi K, Mizuno K, Hida A, Komaki R, Tomita K, Matsushita I, Namiki M, Iwamoto T, Tamura S, Minowada S, Nakahori Y, Nakagome Y. 1994 PCR analysis of the Y chromosome long arm in azoospermic patients: evidence for a second locus required for spermatogenesis. Hum Mol Genet. 3:1965-1967.

46. Vollrath D, Foote S, Hilton A, Brown LG, Beer-Romero P, Bogan JS, Page DC. 1992 The human Y chromosome: A 43-interval map based on naturally occurring deletions. Science. 258:52-59.

Mutations of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Causing Isolated Congenital Bilateral Absence of the Vas Deferens (CBAVD)

The high prevalence of CBAVD in men with cystic fibrosis has been known for a long time (see ref. in ref. 2). More recently studies have shown that specific mutations in the coding region of the CFTR gene may result in CBAVD without the classical pulmonary disease usually associated with cystic fibrosis (47, 48).

Mutations in the CFTR gene which encodes a cAMP-regulated chloride channel, have been found in cystic fibrosis. Patients with classic form of cystic fibrosis have severe mutations in each copy of the CFTR gene, whereas patients with a less severe phenotype (e.g., mild pulmonary disease without pancreatic insufficiency) have a severe mutation in one copy of CFTR and a mild mutation in the other or mild mutations in both copies.

Patients with CBAVD who have mutations in the CFTR gene usually only have mutations in one copy of the gene. In about one third of patients with CBAVD no CFTR gene mutation has been found (47). This inability to identify two mutations could be explained by mutations in noncoding regions of the gene. Such mutations could effect mRNA generation and protein levels resulting in deficient CFTR for vas deferens development but enough to prevent disease in other organs usually affected by cystic fibrosis.

Studies of CFTR mRNA have identified various mRNA molecules that lack exons 4, 9, or 12. Whether or not CFTR mRNA contains exon 9 (an exon critical for normal function), depends on the variable length of the DNA sequence of thymidines in intron 8 of CFTR (49). This sequence contains five, seven, or nine thymidine (the 5T, 7T, and 9T alleles). As shown in Fig. 11 (49), the 5T allele causes the lowest levels of normal CFTR mRNA.

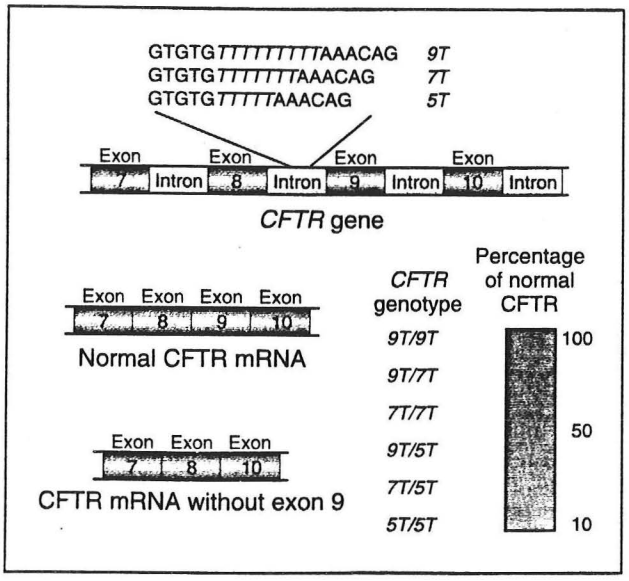


Fig. 11 DNA Variants in Intron 8 of the *CFTR* Gene and Their Effects at the mRNA Level.

The region of the *CFTR* gene that includes exons 7 to 10 is shown at the top. During processing, the sequences not involved with protein synthesis (introns) are eliminated, and the remaining sequences (exons) are spliced to form mature mRNA (center left). The processing of *CFTR* is not completely efficient, because 10 to 92 percent of transcripts lack exon 9 (bottom left), depending on the person's genotype.[13,18] When both *CFTR* genes bear the *5T* allele (the *5T/5T* genotype), the proportion of normal CFTR mRNA is reduced to approximately 8 to 12 percent, indicating that the shorter the sequence of thymines in intron 8, the higher the proportion of CFTR mRNA in which exon 9 is lacking.[18]

Fig. 11

One group has reported an extensive analysis of ultimately 102 unrelated men with azoospermia due to CBAVD (48, 50-52). None had pulmonary or gastrointestinal manifestations of cystic fibrosis. Sweat electrolytes levels were increased in three-fourths of patients (50). Nineteen patients had mutation in both copies of the CFTR, and 54 patients had mutations in only one CFTR allele. In 29 patients after comprehensive screening by

denaturing-gradient gel electrophoresis or single-strand conformation analysis, no mutation in the coding or splice region of CFTR could be detected. In 33% of patients with CBAVD, the 5T allele was associated with the presence of a cystic fibrosis mutation in the other copy of the CFTR gene. Two patients were found to have two 5T alleles. The frequency of heterozygosity for the CFTR 5T allele among patients with CBAVD was 40.2% compared with the 10% incidence in a general population or a population of men with azoospermia but no CBAVD (52). In the 102 men only 22 had neither a CFTR gene mutation nor the 5T allele in one of their copies of CFTR. The particular combination of the two CFTR alleles in a given person (the genotype) is thus thought to result in specific levels of normal CFTR mRNA and a clinical phenotype (Fig. 12) (52).

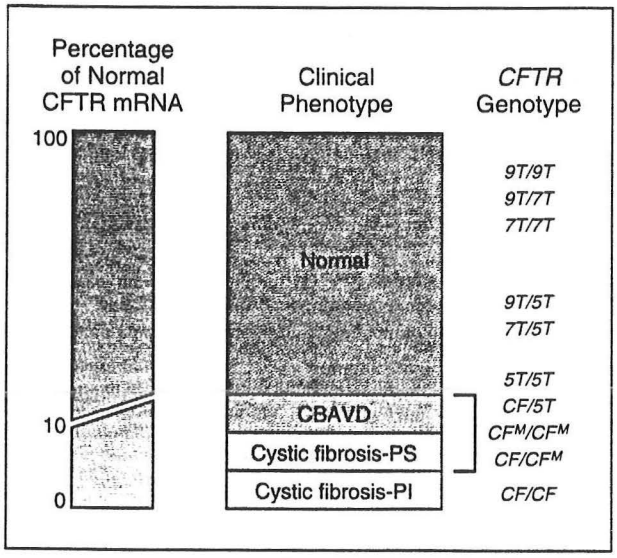


Fig. 12 Comparison of Percentages of Normal CFTR mRNA, Clinical Phenotypes, and *CFTR* Genotypes.

Levels of normal CFTR mRNA depend on the genotype determining the length of the thymine sequence in intron 8 of *CFTR*, the presence of cystic fibrosis mutations, or both. Decreased levels of normal CFTR mRNA may be involved in various clinical phenotypes, ranging from the normal phenotype to the phenotypes of CBAVD, cystic fibrosis with pancreatic sufficiency (PS), and cystic fibrosis with pancreatic insufficiency (PI). Genotypes that correspond to the combination of a cystic fibrosis mutation with a *5T* allele (*CF/5T*) have been found in normal persons, patients with CBAVD, and patients with cystic fibrosis and pancreatic sufficiency. Genotypes combining a severe and a moderate cystic fibrosis mutation (*CF/CFM*) or two moderate mutations (*CFM/CFM*) can be involved in either CBAVD or cystic fibrosis with pancreatic sufficiency (bracket). The delimitation between the normal, CBAVD, and cystic fibrosis phenotypes and their relations with levels of CFTR mRNA is only approximate. The distribution of levels of CFTR mRNA in relation to the presence of the *5T*, *7T*, and *9T* alleles and genotypes is derived from the work of Chu et al.[18]

Fig. 12

The overall data support the concept that the 5T mutation generally causes CBAVD when it is associated with a cystic fibrosis mutation in the other chromosome. The fact that 22% of patients do not have either one CF mutation or 5T suggests that another gene or genes could be responsible for CBAVD. Since 25% of men with congenital unilateral absence of the vas deferens (CUAVD) have the 5T allele, CUAVD could be an incomplete form of CBAVD (52). Thus CBAVD and cystic fibrosis are extreme forms of a spectrum of conditions that have a common molecular basis.

47. Anguiano A, Oates RD, Amos JA, Dean M, Gerrard B, Stewart C, Maher TA, White MB, Milunsky A. 1992 Congenital bilateral absence of the vas deferens. A primarily genital form of cystic fibrosis. JAMA. 267:1794-1797.

48. Culard JF, Desgeorges M, Costa P, Laussel M, Razakatzara G, Navratil H, Demaille J, Claustres M. 1994 Analysis of the whole CFTR coding regions and splice junctions in azoospermic men with congenital bilateral aplasia of epididymis or vas deferens. Hum Genet. 93:467-470.

49. Chu CS, Trapnell BC, Curristin S, Cutting GR, Crystal RG. 1993 Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. Nature Genet. 3:151-156.

50. Casals T, Bassas L, Ruiz-Romero J, Chillón M, Giménez, Ramos MD, Tapia G, Narváez H, Nunes V, Estivill X. 1995 Extensive analysis of 40 infertile patients with congenital absence of the vas deferens: in 50% of cases only one CFTR allele could be detected. Hum Genet. 95:205-211.

51. Mercier B, Verlingue C, Lissens W, Silber SJ, Novelli G, Bonduelle M, Audrézet MP, Férec C. 1995 Is congenital bilateral absence of vas deferens a primary form of cystic fibrosis? Analyses of the CFTR gene in 67 patients. Am J Hum Genet. 56:272-277.

52. Chillón M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, Romey MC, Ruiz-Romero J, Verlingue C, Claustres M, Nunes V, Férec C, Estivill X. 1995 Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N Engl J Med. 332:1475-1480.

A Defect in a Neural-Cell Adhesion Molecule Causes Kallmann's Syndrome

Isolated gonadotropin deficiency is a relatively common cause of absent or incomplete pubertal development (Table I) (2). As indicated in Table I, isolated gonadotropin deficiency (also called idiopathic hypogonadotropic hypogonadism) is typically associated with infertility combined with undervirilization. The hypogonadism is due to deficiency of LHRH. The defect may be of variable severity making the diagnosis more difficult at times. In its more

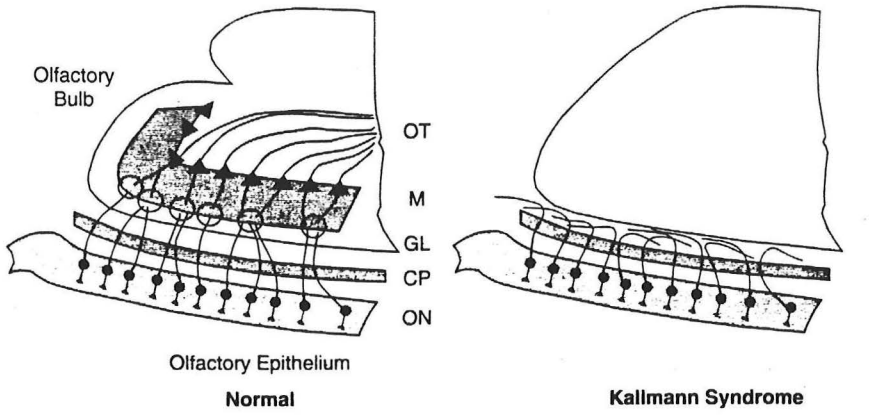
severe forms the hypogonadism is associated with anosmia and is termed Kallmann's syndrome. Kallmann's syndrome is inherited with an excess of male over female patients, suggesting a preponderance of families with X-linked inheritance. However, families with autosomal-dominant and autosomal recessive inheritance have been reported. The anosmia is due to hypoplasia or aplasia of the olfactory bulb and tracts. Other defects may be present in some patients including midline facial defects such as cleft lip and palate, various neurological defects, and abnormalities in development of the kidney.

The neurons that secrete LHRH originate in the olfactory placode and migrate into the brain with the olfactory, terminalis, and vomeronasal nerves. The locus for X-linked Kallmann's syndrome has been assigned to Xp22.3. Position cloning was used to isolate a gene from this region termed the KALIG-1 (Kallmann's syndrome interval gene 1) (53). Analysis of the predicted protein sequence revealed homology with neural-cell adhesion molecules that play an important part in axonal path-finding, and with protein kinases and phosphatases that interact with intracellular matrix and cell adhesion molecules to regulate neural development and morphogenesis (53).

Screening of 77 families with Kallmann's by the group reporting the initial cloning disclosed that one family had a 3300 bp deletion inherited in two sons from their mother entirely confined to the KALIG-I gene (54).

The second group to isolate what they termed the KAL gene also described a highly homologous pseudogene on the X-chromosome (reviewed in 55). Southern analysis of 80 patients disclosed partial deletion of the 5' end of the gene in one patient and deletions including the whole gene in two other patients. In 19 patients with X-linked familial Kallmann's syndrome sequencing of the entire coding region (14 exons) and splice site junctions revealed nine patients with point mutations at separate locations in four exons and one splice site (56). These authors also isolated the chicken KAL homologue and studied its expression in late embryonic development confirming its expression in mitral cells of the olfactory bulbs, the target of olfactory axons (57). The chicken KAL homologue was also expressed in the Purkinje cells of the cerebellar cortex. Subsequently the same authors confirmed KAL expression in the olfactory bulb and cerebellum as well as other areas of the human fetal brain (58).

Thus the proposed model for KAL function and the Kallmann syndrome-pathogenesis is as depicted in Fig. 13 (59).



A proposed model for *KAL* function and Kallmann syndrome pathogenesis. In normal individuals, axons of olfactory neurons (ON) traverse the cribriform plate (CP) to reach the olfactory bulb. Within the glomerular layer (GL) of the bulb, they make synapses with dendrites of mitral cells (M) whose axons will form the olfactory tracts (OT). A tentative model is proposed in which the *KAL* protein (shaded area) is secreted by the mitral cells and is required in the glomerular layer for the establishment and maintenance of proper interactions with olfactory axons. In Kallmann syndrome, *KAL* protein is absent and therefore olfactory axons cannot interact properly with their target, ending their migration between the cribriform plate and the forebrain.

Fig. 13

The KAL protein may be a substrate adhesion molecule mediating interactions between dendrites of mitral cells and olfactory axons. In the absence of KAL, these interactions may not be established, and this would cause a regression of the olfactory bulb after an initial phase of normal development. The migration defect of LHRH neurons would be a secondary effect caused by the lack of contact between the olfactory nerves and the forebrain, and therefore by the absence of a migration route.

The expression of the KAL gene in other sites might correlate with some of the manifestations other than anosmia and hypogonadism seen in some patients with the syndrome. Thus it is possible that Purkinje cell involvement could lead to cerebellar dysfunction, mesonephros/metanephros involvement to unilateral renal aplasia, and facial mesenchyme involvement to cleft palate (59).

53. Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E, Pieretti M, Taillon-Miller P, Brown CJ, Willard HF, Lawrence C, Persico MG, Camerino G, Ballabio A. 1991 A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature. 353:529-536.

54. Bick D, Franco B, Sherins RJ, Heye B, Pike L, Crawford J, Maddalena A, Incerti B, Pragliola A, Meitinger T, Ballabio A. 1992 Brief report: Intragenic deletion of the KALIG-1 gene in Kallmann's syndrome. N Engl J Med. 326:1752-1755.

55. Legouis R, Cohen-Salmon M, Castillo ID, Petit C. 1994 Isolation and characterization of the gene responsible for the X chromosome-linked Kallmann Syndrome. Biomed Pharmacother. 48:241-246.

56. Hardelin JP, Levilliers J, Blanchard S, Carel JC, Leutenegger M, Pinard-Bertelletto JP, Bouloux P, Petit C. 1993 Heterogeneity in the mutations responsible for X chromosome-linked Kallmann syndrome. Hum Mol Genet. 2:373-377.

57. Rugarli EI, Lutz B, Kuratani SC, Wawersik S, Borsani G, Ballabio A, Eichele G. 1993 Expression pattern of the Kallmann syndrome gene in the olfactory system suggests a role in neuronal targeting. Nature Genet. 4:19-26.

58. Lutz B, Kuratani S, Rugarli EI, Wawersik S, Wong C, Bieber FR, Ballabio A, Eichele G. 1994 Expression of the Kallmann syndrome gene in human fetal brain and in the manipulated chick embryo. Hum Mol Genet. 3:1717-1723.

59. Rugarli EI, Ballabio A. 1993 Kallmann syndrome. JAMA. 270:2713-2716.

INTRACYTOPLASMIC SPERM INJECTION AS EMPIRICAL THERAPY FOR ALMOST ALL FORMS OF MALE INFERTILITY

I ended my discussion of male infertility 10 years ago by noting that in vitro fertilization appeared to show promise as a means of achieving fertility for some men because as few as 0.5 million/ml of motile sperm in the ejaculate might be sufficient. The diminished fertilizing capacity of sperm from men with multiple abnormal semen parameters was noted. For mild to moderate defects of semen quality, most current in vitro fertilization programs can obtain 10% or more live births per attempt (60). These rates are 3- to 5-fold higher than the natural pregnancy rates per month in this group of patients. Moreover, most groups do not find a higher pregnancy loss in male factor infertility.

However, standard in vitro fertilization does not provide good results in men with the more severe defects in semen parameters. In the Melbourne experience men with sperm counts less than 5 x 10^6/ml, poor motility and increased abnormal forms had only 35% of oocytes fertilized and cleaved normally (less than half of normal), and over 40% of couples had 0 to 20% of oocytes fertilized (61). In most instances with failure of fertilization, only very few sperm bind to the zona pellucida. Failure of undergoing the acrosome reaction may be a cause for multiple sperm binding to the zona with failure of fertilization (62).

Although a number of variations in standard in vitro fertilization were developed and championed (at least briefly), the breakthrough came with the report by a group in Brussels in 1992 of a method for microinjecting single sperm into the ooplasm to achieve high rates of normal fertilization and pregnancy (63). This technique, termed intracytoplasmic sperm injection or ICSI has rapidly been adopted by many assisted reproduction units (including ours at Southwestern) and greatly improved the outlook for couples with severe male factor infertility.

Prior to the development of ICSI, subzonal inseminization (SUZI) of sperm was tried for severe male factor infertility. Direct comparison of the two techniques showed that ICSI achieved two to three times the fertilization rate as SUZI (64, 65). Fertilization rates of 50 to 70% can be achieved with ICSI, i.e., rates similar to those obtained with normal semen and standard in vitro fertilization. The activation of the oocyte by a calcium ionophore has been reported to result in 90% fertilization rates with ICSI (66).

The fertilization rate is only slightly lower in a group with no motile sperm in the ejaculate but the ongoing pregnancy rate is similar (Table VI) (67).

Table VI Fertilization and Pregnancy Rates Obtained after ICSI in Relation to Total Number of Normal Motile Sperm in the Ejaculate

No. of Motile sperm	No. of Cycles	Fertilization Rate, %	% Ongoing Pregnancies
0	76	58.2*	30.3
39 to 500,000	100	65.5	44.0
>500,000	51	69.6	33.3

*Significantly different than other two groups (from ref. 67).

This same group also compared their ICSI parameter for male factor patients with conventional IVF for couples with fallopian tube pathology treated during the same time period with the same batches of culture media and identical criteria and laboratory methods (Table VII) (67).

Table VII. Outcome of Couples Treated with ICSI and IVF (Tubal Indication) in the Same Time Period

	ICSI	IVF
No. of cycles	227	179
No. of inseminated oocytes	1923	1719
Fertilization rate	59.4%	63.2%
No. of embryos transferred	653	530
No. of replacements	217	173
Ongoing pregnancy rate	37.0%	32.9%

(from ref. 67)

In a smaller study component of another report, a group of 18 male factor patients were treated with both ICSI and routine IVF in a first cycle because of the high likelihood of failed fertilization due to less than 20% of sperm having normal morphology (68). In this group ICSI oocytes had a fertilization rate of 76% compared to 15% for the routine IVF (control) oocytes, and six patients conceived after transfer of ICSI embryos (33%) (68).

The Melbourne group has reported a detailed analysis of the effect of different semen abnormalities (e.g., decreased number, decreased motility, decreased percent normal morphology, and combinations of abnormalities finding a similar normal fertilization rate (61 to 69%) in each group (69). In their hands only the male genital tract obstruction group was significantly different with a higher fertilization rate of 75%. Recall that this genital tract obstruction group is one of the potentially treatable conditions with a low success rate when treated by conventional therapies. This is also true for sperm autoimmunity which can be readily treated by ICSI.

The difference between ICSI and conventional IVF was most evident in the improvement of treatment of men with CBAVD and other causes of obstructive azoospermia. Although the microsurgical aspiration of sperm from the proximal epididymis (MESA) may be quite successful, fertilization rates in conventional IVF had been disappointing. The Brussels group compared effectiveness of the two assisted reproduction methods in comparable groups of men with obstructive azoospermia (Table VIII) (70). ICSI apparently overcomes the lack of sperm maturation.

Table VIII. Comparison of ICSI to Conventional IVF following Microsurgical Aspiration of Sperm from the Epididymis in Similar Patient Populations.

	No. of cycles	No. of mature eggs	Fertilization rate, %	Ongoing pregnancy rate, %
ICSI	17	197	41%	30%
IVF	67	1427	6.9%	4.5%

(from ref. 70)

The success of ICSI has been so great that more than one group has concluded that pregnancy outcome is primarily limited by age of the women and its effect on egg quality and number (Table IX) (71).

Table IX. Fertilization and Pregnancy Rate in Male Factor Infertility Treated by ICSI According to Age of the Woman

Age of the woman	No. of cycles	Eggs per retrieval	Normal fertilization rate, %	Pregnancies/ cycle, %
23 to 29 y	37	15.1	50%	30%
30 to 34 y	78	13.5	46%	31%
35 to 39 y	83	10.1	45%	16%
40 to 48 y	31	6.5	36%	13%

(from ref. 71)

The encouraging results in men with decreased sperm number, decreased normal forms, absent motility, and lack of the maturation normally occurring in the epididymis has left one author to speculate: "What forms of male infertility are left to cure?" (72). ICSI now even appears to offer a solution for men with nonobstructive azoospermia. Such patients include men with Sertoli cell only syndrome, maturation arrest, post-cryptorchidism tubular atrophy, mumps or Klinefelter's syndrome. It is known that an extremely minute amount of sperm production in a grossly deficient testicle might be associated with absolute azoospermia in the ejaculate. When that is the case spermatozoa can be obtained by testicular biopsy (73) or testicular sperm extraction (TESE) (74). Reportedly there have been fertilization and pregnancy rates similar to that of a couple in whom the man has a normal sperm count (74). Successful pregnancy has been achieved using spermatid extraction from Sertoli cells in men with apparent complete absence of spermatogenesis on biopsy with the diagnosis of azoospermia due to Sertoli cell only or maturation arrest (75).

In time ICSI may greatly increase the number of offspring from men with severe infertility. This could increase the chances of transmitting genetically-determined defects of spermatogenesis. Patients with CBAVD likely have a mutation in the CFTR or decreased production of CFTR mRNA and must understand the genetic implications. Since the AZF gene on the Y chromosome may be compatible with oligospermia, screening techniques must be developed for this and other mutations as they are recognized.

The enthusiasm for ICSI should not preclude appropriate evaluation of all men with an abnormal semen analysis. Men with partial isolated gonadotropin deficiency may be missed with resultant failure of rendering them eugonadal and decreasing their risk of osteoporosis. Likewise, failure of recognition of other treatable conditions (hyperprolactinemia, drug effects, hemochromatosis, environmental toxin exposure, liver and kidney disease) would be unfortunate.

60. Bhasin S, de Kretser DM, Baker HWG. 1994 Pathophysiology and natural history of male infertility. J Clin Endocrinol Metab. 79:1525-1529.

61. Baker HWG, Liu DY, Bourne H, Lopata A. 1993. Diagnosis of sperm defects in selecting patients for assisted fertilization. Hum Reprod. 8:1779-1780.

62. Liu DY, Baker HWG. 1994 Acrosome status and morphology of human spermatozoa bound to the zona pellucida and oolemma determined using oocytes that failed to fertilize in vitro. Hum Reprod. 9:673-679.

63. Palermo G, Joris H, Devroey P, Van Steirteghem AC. 1992 Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 340:17-18.

64. Van Steirteghem AC, Liu J, Joris H, Nagy Z, Janssenswillen C, Tournaye H, Derde MP, Van Assche E, Devroey P. 1993 Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. Hum Reprod. 8:1055-1060.

65. Catt J, Ryan J, Pike I, O'Neil C. 1995 Fertilization rates using intracytoplasmic sperm injection are greater than subzonal insemination but are dependent on prior treatment of sperm. Fertil Steril. 64:764-769.

66. Tesarik J, Sousa M. 1995 More than 90% fertilization rates after intracytoplasmic sperm injection and artifical induction of oocyte activation with calcium ionophore. Fertil Steril. 63:343-349.

67. Palermo GD, Cohen J, Alikani M, Adler A, Rosenwaks Z. 1995 Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility. Fertil Steril. 63:1231-1240.

68. Payne D, Flaherty SP, Jeffrey R, Warnes GM, Matthews CD. 1994 Successful treatment of severe male factor infertility in 100 consecutive cycles using intracytoplasmic sperm injection. Hum Reprod. 9:2051-2057.

69. Harari O, Bourne H, McDonald M, Richings N, Speirs AL, Johnston WI, Baker HWG. 1995 Intracytoplasmic sperm injection: a major advance in the management of severe male subfertility. Fertil Steril. 64:360-368.

70. Silber SJ, Nagy ZP, Liu J, Godoy H, Devroey P, Van Steirteghem AC. 1994 Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. Hum Reprod. 9:1705-1709.

71. Sherins RJ, Thorsell LP, Dorfmann A, Dennison-Lagos L, Calvo LP, Krysa L, Coulam CB, Schulman JD. 1995 Intracytoplasmic sperm injection facilitates fertilization even in the most severe forms of male infertility: pregnancy outcome correlates with maternal age and number of eggs available. Fertil Steril. 64:369-375.

72. Silber SJ. 1995 What forms of male infertility are there left to cure? Hum Reprod. 10:503-504.

73. Yemini M, Vanderzwalmen P, Mukaida T, Schoengold S, Birkenfeld A. 1995 Intracytoplasmic sperm injection, fertilization, and embryo transfer after retrieval of spermatozoa by testicular biopsy from an azoospermic male with testicular tubular atrophy. Fertil Steril. 63:1118-1120.

74. Devroey P, Lui J, Nagy Z, Goossens A, Tournay H, Camus M, Van Steirteghem A, Silber SJ. 1995 Pregnancies after testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia. Hum Reprod. 10 (in press).

75. Silber SJ, Van Steirtghem A, Nagy P, Tournaye H, Liu J, Devroey P. 1995. Successful pregnancy in humans using testicular spermatid extraction (TESE) and ICSI for azoospermia due to Sertoli cell only and maturation arrest. Fertil Steril. 64 (suppl): (Abstract) S17-18.