For Det. Sgt. M. Pillay

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ABSTRACT

This investigation reports the effects of diabetes mellitus on some aspects of the male reproductive system. When compared to non-diabetic controls, human diabetics indicated a 35% reduction in gross sperm motility, a 13% decrease in progressive sperm motility and a 49% reduction in rapid sperm motility. Sperm viability also decreased by 35% in this group. Semen carnitine and acid phosphatase levels were elevated by 47% and 13% respectively, whilst the circulating testosterone level decreased by 20%. Semen pH and volume, sperm concentration and sperm morphology did not differ significantly between the diabetic and non-diabetic control patients. Circulating LH, FSH and prolactin, as well as seminal zinc, fructose and citric acid levels were found to be similar in the two groups.

In a human infertility group, the number of morphologically normal sperm was 46% lower in comparison to the control group. Gross sperm motility decreased by 21%, progressive motility by 11% and sperm viability by 9%. Carnitine concentration in semen was 38% higher and fructose levels 27% lower than that of the control group. There were also significant reductions in circulating testosterone and LH levels (16% and 28% respectively). Other parameters investigated, but which were not significantly different from the control, were semen pH and volume, sperm

concentration, seminal zinc, acid phosphatase and citric acid concentrations, and circulating FSH and prolactin levels.

Streptozotocin-induced diabetic rats maintained on insulin for 90 days, were also assessed for testicular function. Extracts from the cauda epididymis indicated a 39% decrease in sperm motility and a 19% reduction in viability. Unlike the human diabetics, a 59% reduction in sperm number per cauda was also noted, together with an 86% rise in morphologically abnormal sperm. Measurements of the accessory organ weights indicated a 44% loss in prostate weight only. The testicular weight and testicular cell size and number did not differ significantly between the two groups. Circulating testosterone levels also remained unchanged. Reduced fecundity in these animals was displayed by the reduction in the number of pups sired.

Collectively these results indicate that diabetes adversely affects the male reproductive system. The profile of the human diabetes group was similar to that of a group of infertile males, indicating a severe loss in reproductive potential in these patients. The results obtained from the streptozotocin-induced diabetic rats complimented the investigation on human diabetics. The animal studies further indicated reduced fecundity and a compromised accessory organ function, as suggested by the weight loss of these organs.

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1. INTRODUCTION

1.1 The Testis

By virtue of its endocrine and exocrine activity, the testis determines nearly every aspect of what is commonly referred to as "maleness". The exocrine activity ensures the production of spermatozoa. The endocrine activity, whilst supporting spermatogenesis, also stimulates and regulates the functioning of the accessory organs.

1.1.1. Structure of the Testis

The compartmentalised testis consists of vascularised interstitial tissue and seminiferous tubules as indicated in the accompanying illustration. In the human testis the seminiferous tubules are arranged in lobules separated by bands of fibrous tissue, whilst in the rat, the tubules lie at random throughout the testis (Setchell, 1982). The interstitial tissue consists of Leydig cells, macrophages and blood and lymph vessels. The seminiferous tubules are comprised of a peritubular layer of myoid cells and a basal membrane bearing Sertoli and germ cells (Tindall et al, 1985). The tubules merge with the rete testis; the vasa efferentia lead into the epididymis which opens into the vas deferens (Fawcett, 1982).

The seminiferous tubule is divided further into an adluminal and a basal compartment by the blood-testis barrier.

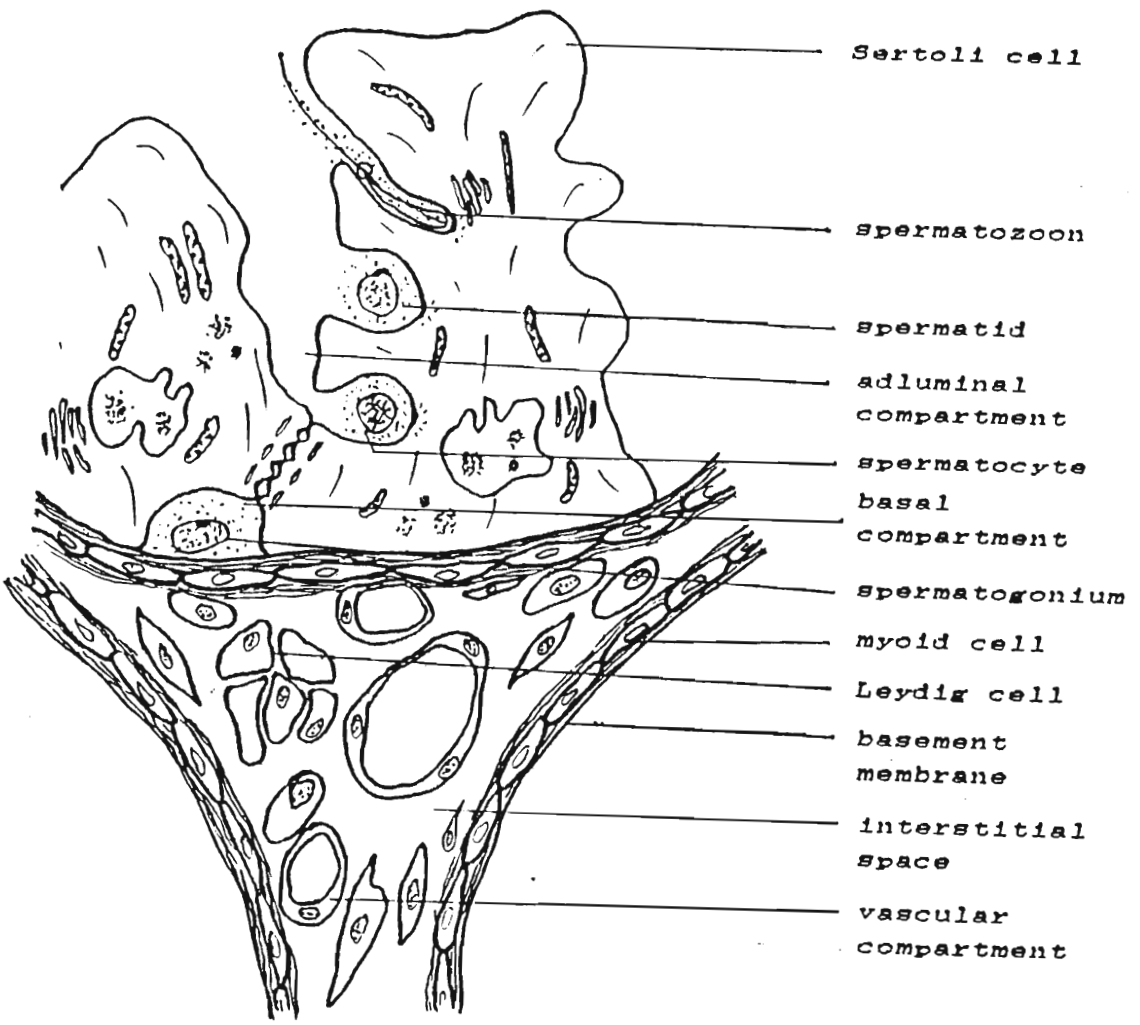

Illustration representing a cross section through part of an adult testis (Adapted from Johnson and Everitt, 1988).

1.1.1.1. The Interstitial Space

As indicated in the accompanying illustration the vascularised interstitial space bears the Leydig cells, the function of which is the synthesis and secretion of androgens primarily required for the stimulation and support of spermatogenesis. The Leydig cells are polyhedral cells occuring in clusters (Amann & Schanbacher, 1983) and occupying 2-37% of the testicular volume, depending on the species (Christensen, 1975). The interstitial fluid which bathes the Leydig cells, blood vessels and outer lining of the seminiferous tubules, transports nutrients and hormones to the seminiferous tubules (Fawcett et al, 1973). The rate of interstitial fluid formation, which essentially controls the rate of entry of most compounds into the testis, is determined by the permeability of the testicular capillaries which in turn is under hormonal control (Sharpe, 1983).

1.1.1.2. The Seminiferous Tubule

In the mammalian testis, the avascular seminiferous tubules appear as coiled loops draining at both ends into the rete testis (Mann & Lutwak-Mann, 1981). The basal membrane, which is lined by stratified epithelium encloses the lumen. The fixed population of the epithelium consists of somatic Sertoli cells which extend from the basal lamina to the luminal surface of the epithelium. The mobile population of germ cells are in intimate contact with the Sertoli cells, which provide physical and nutritional support.

The germ cells continuously change their positional relationship with the Sertoli cell during their differentiation and maturation. The germ cells indent the cytoplasm of the Sertoli cell and are attached to the cell by ectoplasmic specialisations (Amann & Schanbacher, 1983). At any particular time the mammalian testis supports 4 or 5 different stages of germ cell development simultaneously, from spermatogonia to spermatids. Sertoli cells exhibit marked changes in volume and alterations in morphology in accordance with the various stages of spermatogenesis. This suggests a local interaction of Sertoli and germ cells which is maintained to meet the metabolic requirements of spermatogenesis (Kerr, 1988). It seems probable that a particular germ cell compliment, at a given stage, "orders" what is required from the Sertoli cell, and that the germ cell actively communicates with the Sertoli cell (Sharpe, 1986). Sertoli cells, which are in constant contact with all the germinal elements through cytoplasmic processes, may secrete substances that control the rate of development of the various spermatogenic stages (Clermont, 1972).

Seminiferous tubular fluid is secreted by Sertoli cells into the lumen of the tubule and is responsible for the transport of spermatozoa into the epididymis (Waites & Gladwell, 1982). It also contains secretory products to nourish the liberated spermatozoa.

1.1.1.3. The Blood-Testis Barrier

The interstitial compartment is physiologically separated from the seminiferous tubule by a blood-testis barrier which is responsible for selective transport and secretion of substances, thus disallowing the free exchange of water-soluble materials. This barrier is located on, or within the seminiferous tubule, depending on the species. Myoid cells, which are contractile in nature, surround the seminiferous tubules thereby prevening the penetration of substances into the germinal epithelium. However, some of these myoid cell-to-cell junctions are not closed at their points of membrane contact, and this permits the penetration of some substances into the germinal epithelium at certain sites along the length of the tubule (Dym & Fawcett, 1970). The Sertoli cells on the inner surface of the tubules form a second barrier. Multi-layers of junctional complexes encircle the Sertoli cells at their bases, linking each firmly to its neighbour (Johnson & Everitt, 1984). This barrier allows the passage of substances, of certain molecular size, into the testis but not into the lumen of the rete testis.

In humans the blood-testis barrier is formed by Sertoli cell junctions only, unlike that of rodents which consists of the myoid layer of cells together with Sertoli-Sertoli cell complexes (Tindall et al, 1985).

These specialised junctions between neighbouring Sertoli cells isolate the spermatogonia in a separate basal compartment, and other differentiating germ cells in an adluminal compartment (Dym & Fawcett, 1970). This morphological separation is modified at certain stages of the cycle to allow the preleptotene primary spermatocytes to move from the basal to the adluminal compartment. It seems likely that neighbouring Sertoli cells move in beneath the preleptotene primary spermatocytes and "zipper up" the junctional complexes. This action would result in the movement of the differentiating spermatocytes toward the lumen since they are not free to move independently (Dym & Fawcett, 1970).

1.1.2 Function of the Testis

1.1.2.1. Spermatogenesis

Batches of type A spermatogonia, residing in the basal compartment of the seminiferous tubule, undergo mitosis or spermatocytogenesis, to produce clones of type A_1-A_4 daughter cells. To maintain a constant stock of type A spermatogonia, one of the daughter cells of the type A clone ceases to divide during mitosis and returns to the resting phase to undergo division at a later stage. The resting type A spermatogonium re-enters mitotic division after several days of quiescence. Those type A daughter cells which remain mitotically active give rise to intermediate and type B spermatogonia (Johnson & Everitt, 1984). After a set number

of mitotic divisions, characteristic of the species, the type B spermatogonia divide mitotically to produce primary resting spermatocytes that enter the meitotic phase. The resultant preleptotene spermatocytes duplicate their DNA and migrate into the adluminal intratubular compartment. In the adluminal compartment the first meiotic division results in two haploid secondary spermatocytes, which in turn divide to yield early spermatids.

The development of the spermatocyte, from the commencement of meiosis to the detachment of the spermatozoan from the Sertoli cell, is known as spermiogenesis (Setchell, 1982). Spermatogenesis is the collective term for spermatocytogenesis and spermiogenesis.

Spermiogenesis is characterised by the cytoplasmic remodelling of the spermatid (Setchell, 1982). Throughout the process of differentiation, which involves the tail, mid-piece, nucleus and acrosome, the spermatid continues to migrate toward the lumen of the tubule. The completion of spermatid elongation leads to retraction of the Sertoli cell around the spermatids. Release of the spermatozoa from the Sertoli cell into the lumen of the seminiferous tubule is known as spermiation (Setchell, 1982). The mass of cytoplasm, which is retained from the spermatogonial stage and to which the sperm ramains attached, does not shift as the sperm moves toward the lumen. Thin strands of cytoplasm form as the spermatozoa are drawn further and further away from the Sertoli cells. Eventually these strands break leaving a small remnant, known as the cytoplasmic droplet,

around the neck of the sperm. The spermatids are then released into the lumen of the seminiferous tubule. The remaining cytoplasm in the Sertoli cell crypt, known as a residual body, is phagocytosed by the Sertoli cell (Setchell, 1982).

The entire process of spermatogenesis takes 72 days to complete in man and 48 days in the rat (Sharpe, 1986). The "spermatogenic cycle", is the difference in time between the production of two of the same stages of spermatogenesis. In the rat the spermatogenic cycle is 12 days, which implies that 4 successive spermatogenic processes are occuring simultaneously since spermatogenesis runs to completion after 48 days. This succession of stages has a constant duration (Monesi, 1972; Parvinen, 1982).

The sequence of cellular stages along the length of a seminiferous tubule constitutes the "wave" of the seminiferous epithelium (Monesi, 1972). Successive lengths of the tubule would be at earlier stages in the cycle thus forming a wave. One complete wave is the distance between two identical stages in the tubule (Parvinen, 1982).

1.1.2.2. The Hypothalamo-pituitary-testis Axis

The endocrine function of the testis is reflected by circulating levels of gonadotrophins and androgens (Vouk & Sheehan, 1983). The synthesis and secretion of follicle stimulating hormone (FSH) and luteinising hormone (LH) by

the anterior pituitary depends on a gonadotrophin releasing hormone (GnRH) signal from the hypothalamus (de Kretser, 1984). GnRH is released from the hypothalamus into the hypothalamo-pituitary portal system in discrete pulses. Upon reaching the anterior pituitary it causes the pulsatile discharge of the LH and FSH (Amann & Schanbacher,1983). LH secretion results in the stimulation of Leydig cells to produce testosterone. Testosterone in turn regulates the secretion of LH via a negative feedback control (de Kretser, 1984). Elevated levels of the androgen suppress, whilst depressed levels enhance gonadotrophin release. Although testosterone effects FSH secretion, this effect is not as strong as that exerted on LH secretion. Inhibin, which is thought to be secreted by the Sertoli cell, also regulates the secretion of FSH (Jackson & Schnieden, 1982).

Prolactin, which is also synthesised and secreted by the pituitary, is primarily under the control of a release-inhibiting factor called dopamine (Johnson & Everitt, 1984). High concentrations of plasma prolactin result in an increased dopamine turnover which subsequently depresses prolactin secretion. Prolactin influences the number of LH receptors within the testis and enhances testosterone synthesis and secretion (Hussein & Zipf, 1988).

1.1.2.3 Hormonal Control of Spermatogenesis

Testosterone is produced by the Leydig cells, and is selectively transferred to the tubular compartments of the testis (Setchell, 1982). LH stimulates Leydig cell function by acting on the cell membrane to induce adenyl cyclase activity, resulting in cyclic adenosine-5-monophosphate (cAMP) moving into the cytoplasm as the actual effector of testosterone production and secretion. FSH does not stimulate Leydig cell testosterone secretion directly (Sharpe, 1983) but does increase Leydig cell responsiveness to LH.

The functioning of the Sertoli cell is regulated by testosterone and FSH (Means et al, 1980). Biochemical and morphological changes in the Sertoli cells are induced by FSH which enters via the basal aspect of the Sertoli cell (Amann & Schanbacher, 1983). It is also suggested that LH has a direct effect on the maintenance of Sertoli cell morphology (Tindall et al, 1985).

By binding to receptors on the Sertoli cell membrane, FSH initiates spermatogenesis, whilst maintenance of spermatogenesis is controlled by testosterone (Sharpe, 1983). Experiments in normal adult rats suggest that established spermatogenesis is not FSH dependent (Davies, 1981), and although there exists clear evidence in favour of a major role of testosterone in the control of

spermatogenesis, its only known action on the Sertoli cell is the stimulation of androgen binding protein (ABP) secretion.

Within the tubule testosterone binds to androgen receptors on the Sertoli cell membrane thereby stimulating the secretion of ABP (Waites & Gladwell, 1982). The interaction of FSH with the cell membrane also results in an increased secretion of ABP (Fakunding et al, 1976). ABP is secreted into the extra-cellular fluid between the cells of the germinal epithelium. The protein is thought to maintain a high concentration of androgens in the fluid surrounding the maturing spermatozoa and to facilitate spermatogenesis by buffering fluctuations in the concentration of androgens in the extracellular fluid surrounding the germinal epithelium (Davies, 1981).

In the adult rat testis, FSH also stimulates the synthesis of plasminogen activator. This is a secretory protein of Sertoli cell origin, which is probably related to spermiation and the disassociation of Sertoli cell junctional complexes, thereby allowing sperm to enter the adluminal compartment of the seminiferous tubule (Lacriox et al, 1977).

Outside the basement membrane of the seminiferous tubule are myoid and undifferentiated fibroblast-like cells. The positioning of these cells, between the Leydig and

seminiferous tubule may be responsible for mediating some of the effects of Leydig cell secretory products on the Sertoli cell, and vice versa (Sharpe, 1986).

Leydig cells produce testosterone that acts on the Sertoli cells thereby driving spermatogenesis. This suggests that the Sertoli cell should be able to exert some control over the Leydig cell to ensure appropriate supply of androgen. A GnRH-like peptide in Sertoli cell secretions is a probable messenger which binds to receptors on the Leydig cells thus mediating Leydig-Sertoli cell communication (Sharpe, 1984). This factor could probably stimulate the secretion of testosterone by the Leydig cells (Sharpe, 1986). It is inhibitory in the presence of normal physiological levels of LH and stimulatory in the presence of raised levels of LH. This provides growing evidence for local control of entry of substances into the interstitial fluid (Sharpe, 1983) and hence into the seminiferous tubules.

1.2 The Epididymis

The epididymis is a long duct divided into three functional regions which are not distinctally demarcated. Proximally is the caput, distally the cauda and the central portion is known as the corpus epididymis. Sperm maturation takes place in the caput and corpus epididymis whereas the cauda serves chiefly as a storage area, where mature sperm maintain a quiescent condition prior to ejaculation (Brooks, 1983).

Epididymal structure and function is dependent on androgens which reach the epididymis via the rete testis fluid and blood stream. Dihydrotestosterone, rather than testosterone is the predominant androgen within the epididymis (Brooks, 1983).

The epididymis conveys spermatozoa from the vasa efferentia to the vas deferens, from which they are ejaculated. Transport of spermatozoa within the seminiferous tubules is initially brought about by secretions flowing from the testis and then by the ciliary and contractile activity of the luminal wall of the vasa efferentia and epididymis (Bedford, 1975). The epididymis receives its luminal fluid from the testis, and the fluid is altered substantially by the absorptive and secretory activity of the epididymal epithelium.

The acquisition of fertilising ability, during epididymal passage, results from the maturation of different organelles which are necessary for the functional competence of the sperm cell (Cooper et al, 1986). The change in their functional state may be reflected in the following (Bedford, 1975): 1. development of the capacity for sustained progressive motility. 2. modification of metabolic character and structural state of certain tail organelles and sperm nuclear chromatin. 3. alteration in the nature of the surface of the plasma membrane. 4. morphological changes

involving movement and loss of the remnant of spermatid cytoplasm. 5. modification in the acrosome form in some species.

To acquire the capacity for initial vibratory type motility, raised levels of cAMP are required (Brandt et al, 1978). Conversion of this to forward progression is thought to require a protein of epididymal origin referred to as the forward motility protein in bovine sperm (Brandt et al, 1978), or progressive motility sustaining factor in human sperm (Sheth et al, 1981). Rapid progressive motility, that appears in the corpus epididymis and predominates in the cauda and vas deferens, is associated with a reduction in amplitude in the flagellar beat (Bedford, 1975). Reduction in the flexibility of the tail is the result of disulphide bonds (-S-S-) which are formed within the tail during epididymal passage (Bedford et al, 1973).

Sperm DNA also undergoes progressive condensation as the duct is traversed. Variations in the nucleus occur before spermiation, however it is likely that individual differences in the structural quality of the nucleus does occur in the epididymis (Bedford et al, 1973). This is based on the knowledge that nuclear chromatin in eutherian spermatozoa is stabilised by -S-S- crosslinks whilst traversing the epididymis (Calvin & Bedford, 1971). Abnormalities of intact acrosomes in human spermatozoa probably have their origin in the spermatid (Bedford et al,

1973). Failure of nuclear condensation in the epididymis does not have an adverse effect on the normally developed acrosome.

Sperm undergo complex surface changes during maturation in the epididymis. These include the addition of glycoproteins secreted by the epithelium of the epididymis (Eddy et al, 1985). It is likely that the glycoproteins bind to the plasma membrane of the sperm and in so doing produce the zona binding ability of the sperm. The mechanism of protein synthesis appears to be regulated by androgens (Eddy et al, 1985).

In the epididymis the cytoplasmic droplet begins to move away from the neck of the sperm. The droplet is generally lost in ejaculated sperm. Its presence in a significant percentage of sperm may be indicative of pathology of sperm maturation and sometimes infertility (Bedford, 1975). With exception of the cytoplasmic droplet, human spermatozoa do not appear to undergo important morphological changes during their passage through the epididymis (Bedford, et al 1973).

The transit time of the spermatozoa through the caput and corpus epididymis in mammalian species, ranges from 2 to 5,5 days, and this is unaltered by ejaculation frequency (Jackson & Schnieden, 1982). Passage through the cauda epididymis however, is shorter in sexually active males.

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1.3 Diabetes and the Male Reproductive System

The prevalence of sexual disturbances amongst men with diabetes mellitus is fairly common (Schoffling et al, 1963; Fairburn et al, 1982; Handelsman et al, 1985). Amongst other problems diabetic impotence presents itself as a gradual and irreversable decline in sexual function even when the diabetes is apparently well controlled and the patient is not under psychological stress (Fairburn et al, 1982). Retrograde ejaculations are the best described form of an ejaculatory disorder in diabetic men, in which semen is passed backward into the bladder rather than forward along the anterior urethra (Greene & Kelalis, 1968). The competence of the internal sphincter, at the neck of the bladder, is thought to be compromised, either physically or pharmacologically, with the result that it fails to close during the propulsive phase of ejaculation (Fairburn et al, 1982).

Although the cause of impotence in diabetic men is not clear neuropathy and artherosclerosis have been documented as contributing factors (Carlin, 1988). Some authors have suggested that the development of erectile failure may be unrelated to the quality of diabetic control (Kolodny et al, 1974), whilst others dispute this (McCulloch et al, 1980).

Studies on the endocrine and spermatogenic function of the testis in diabetic men are limited (Rodriguez-Rigau, 1980; Faiburn et al, 1982). There are reports of defective

spermatogenesis (Foglia et al, 1963; Schoffling et al, 1963) and testicular atrophy and infertility (Rodriguez-Rigau, 1980). Other authors however, reported few, if any disturbances in spermatogenesis in patients with well controlled diabetes or mild carbohydrate intolerance (Klebanow & MacLeod, 1960). Sperm concentration and morphology were also reported to be uneffected in diabetic men (Handelsman et al, 1985), and histological examination of the testes of impotent diabetics have revealed normal morphology of the interstitial tissue, although hypospermatogenesis was noted (Faerman et al, 1972). This implied that diabetic impotence was not secondary to hypogonadism and therefore patients would probably not improve with exogenous androgens and gonadotrophins. The treatment of impotent diabetic men with GnRH indicated no change in sexual function and erectile potency (Levitt et al, 1980). However, some impotent patients responded favourably to androgen therapy (Schoffling et al,1963; Murray et al, 1987) .

Biopsies performed on diabetic men indicated germ cell depletion in the seminiferous epithelium, malorientation of spermatids and thickening of the seminiferous tubule walls (Cameron et al, 1985). Sertoli cell junctions were structurally altered and their apical cytoplasm displayed degeneration. A reduction in the Leydig cell population was also noted (Schoffling et al, 1963).

Abnormalities in gonadotrophin and androgen levels have also been reported in diabetic men (Schoffling et al, 1963), however this was not confirmed by other studies (McCullock et al, 1980; Handelsman et al, 1985). Ando and co-workers (1979) have reported low plasma testosterone levels in diabetic men. Other reports oppose these findings (Kolodny et al, 1974; Handelsman et al, 1985; McCulloch et al, 1980). Plasma LH and FSH levels were elevated in diabetic men (Anderson et al, 1987; Handelsman et al, 1985), whilst other works indicated normal gonadotrophin levels (Gattucio et al, 1979; McCulloch et al, 1980; Fushimi et al, 1987).

Investigation of prolactin levels in diabetic men has led to the report of a positive correlation between prolactin and zinc levels in blood plasma (Arreola et al, 1986). Low zinc concentration has been associated with impaired androstenedione conversion to testosterone (Paniagua et al, 1982). Since low circulating zinc and prolactin levels have been observed in diabetics, it is assumed that there is an alteration in steroid biosynthesis (Arreola et al, 1986). Zinc is believed to act as a metalloenzyme activator or protective agent supporting certain membrane enzymes, such as 17-α-hydroxysteroid dehydrogenase (Paniagua et al, 1982), responsible for the conversion of androstenedione to testosterone. Prolactin was demonstrated to increase the activity of this particular enzyme (Musto et al, 1972). Therefore low prolactin levels could be associated with low

testosterone levels. Lester and co-workers (1981) however, reported that in insulin and non-insulin dependent diabetics, the serum prolactin concentration was normal.

In some animal studies a loss in turgor of the testis and atrophy of the seminal vesicles and prostate have been reported together with testicular weight changes (Foglia et al, 1963; Paz & Hommonai, 1979a; Paz et al, 1978; Fushimi et al, 1987). Other studies, with streptozotocin-induced diabetic rats however, have not reported changes in testicular weight (Paz et al, 1978; Ford & Hamilton, 1984).

While some streptozotocin-treated rats have displayed normal androgen levels (Fushimi et al, 1989), others have not (Paz & Hamonnai, 1979a; Paz et al, 1978; Kuhn-Velten et al, 1987; Ford & Hamilton, 1984; Anderson et al, 1987; Fushimi et al, 1987). These animals have also reflected severe depression in plasma LH concentration (Paz and Homonnai, 1979a; Paz et al, 1978). The reduction in LH receptors and androgen production was reversable with insulin administration (Paz et al, 1978) but testicular function however, was not restored, nor did fertility improve (Paz & Hommonai, 1979a; 1979b). Testosterone alone stimulated the growth of accessory glands but the addition of insulin was required to restore full size and function to these organs (Paz et al, 1978). Insulin has been shown to enhance the production of ABP (Karl & Griswold, 1980) and improve the binding of testosterone to the prostate and epididymis (Oksanen & Tuochimma, 1975). In organ culture of the ventral prostate,

insulin facilitated the action of testosterone in the incorporation of precursors for RNA and proteins synthesis (Santti & Johnsson, 1973), and in the restoration of the normal histological appearance (Lostroh, 1971) and secretory activity of the organ (Lostroh, 1968). A loss in body weight was also observed in diabetic rats which was partly restored with insulin therapy (Paz & Homonnai, 1979b; Fushimi et al, 1987).

When testing the fecundity of pancreatectomised rats, mated with normal females, it was reported that the number and the weight of the offspring was similar to the control (Foglia et al, 1963). Reproductive function later altered during diabetes causing progressive atrophy of the testis and infertility. Diabetic rats with normal testosterone levels and unaltered sperm motility and number however, did not sire any pups. Neuropathy causing sexual dysfunction seemed a distinct possibility for this reduction in fertility (Anderson et al, 1987).

In the present study, on the effects of diabetes mellitus on male sexual function, the physiological function of the various accessory sex organs in the male was evaluated. This was accommplished by analysing some aspects of the biochemical composition of semen. An introduction to some of these aspects is given below.

Although coagulation and liquifaction of human semen are generally not factors critical to fertility, they serve as functional indicators of the seminal vesicle and prostate

gland. Semen is ejaculated in a liquid form but coagulates immediately, only to re-liquify about 30 minutes later (Montagnon et al, 1982). The sperm do not become fully motile until after liquifaction is achieved, hence the examination of sperm motility after 30 minutes. The main components of the coagulum are basic glycoproteins (Kusamran & Surakarnkul, 1983) which are thought to form complexes with fructose, and these complexes in turn reticulate to form the coagulum. Mixing of the enzymatic part of the ejaculate, which is primarily of prostatic origin, with the last ejaculate fraction contributed by the seminal vesicles, result in liquifaction. Liquifaction is an enzymatic process (Aafjies et al, 1985) during which the fructose molecules are liberated (Kusamran & Surakarnkul, 1983).

Spontaneous agglutination of spermatozoa is thought to present a fertility problem. The mixed antiglobulin reaction (MAR) test is used to detect antibodies that have adhered to the surface of the spermatozoan, and which may be responsible for sperm agglutination reactions (Cerasaro et al, 1985). Since many free swimming spermatozoa can often be seen between the the agglutinates in semen from infertile men with autoagglutination, it is suggested that sperm agglutination as a single factor cannot be responsible for infertility (Jager et al, 1978). The presence of antisperm antibodies is linked to reduced penetration of spermatozoa into cervical mucus. This is due to the fact that free swimming spermatozoa, bearing antibodies, change their progressive motility to a local shaking movement after

contact with the cervical mucus (Kremer & Jager, 1976). This is probably as a result of an antibody reaction of the sperm with the glycoproteins of the cervical mucus (Jager et al, 1978). A positive MAR test is reported to be synonymous with low normal sperm morphology, thus suggesting a damaging effect of the antibodies on normal sperm maturation (Cerasaro et al, 1985). No correlation was found between positive MAR tests and low sperm counts, or poor motility (Cerasaro et al, 1985). Etribi and co-workers (1982) however, correlated the presence of antibodies with poor sperm motility.

The exact relationship between seminal zinc and fertility is not clear. In the male reproductive system, zinc is secreted by the prostate (Coffey, 1988), and has been associated with low sperm motility when no other cause of infertility could be identified (Clademone et al, 1979). Treatment with oral zinc sulphate resulted in a slight increase in seminal zinc concentration, but sperm motility increased significantly with a trend toward an increased sperm count (Clademone et al, 1979). Zinc is also essential for the regulation of metabolic processes in the biosynthesis of RNA and DNA (Netter et al, 1981). In animal studies zinc deprivation has been shown to reduce testicular RNA and DNA, depress spermatogenesis and elevate plasma FSH. Plasma testosterone has been reported to increase with zinc therapy (Netter et al, 1981) as discussed earlier in the introduction. The presence of high levels of zinc have also been known to exert adverse effects on spermatozoal motility and

morphology (Kvist & Eliasson, 1980), on the preservation of intact cellular membranes (Kvist, 1980) and on the stability of sperm nuclear chromatin.

Acid phosphatase belongs to a group of phosphatases which catalyse the hydrolytic cleavage of phosphoric acid esters. In the male reproductive system, acid phosphatase is produced by the prostate gland (Mann & Lutwak-Mann, 1981). The exact function of this enzyme in seminal plasma is unclear, but it is used for the diagnosis of prostatic carcinoma. In rat spermatozoa it is also believed to be one of the acrosomal or subacrosomal enzymes which may participate in capacitation, whilst in the testis acid phosphatase production is associated with the seminiferous tubules and probably the interstitial tissue (Mann & Lutwak-Mann, 1981). Hypophysectomy results in decreased acid phosphatase activity but treatment with LH and FSH or testosterone enables spermatogenesis to progress and restores acid phosphatase to normal levels. In the seminal plasma acid phosphatase is responsible for the enzymatic hydrolysis of phosphorylcholine (Coffey, 1988). This reaction, resulting in free choline and inorganic phosphate, occurs rapidly after ejaculation (Noppinger et al, 1987). The role played by these products is however unclear (Mann & Lutwak-Mann, 1981).

Fructose provides a source of energy for ejaculated spermatozoa (Jungreis et al, 1989). This reducing sugar is produced in the seminal vesicles, the functioning of which

is under androgenic control (Jungreis et al, 1989). Low concentrations or the absence of fructose in semen is indicative of obstruction of the vas deferens or congentital bilateral absence of this duct respectively (Amelar, 1962). In the absence of carbohydrates citric acid is used as a energy source (Mann & Lutwak-Mann, 1981). In the male reproductive system, citric acid is secreted by the prostate (Coffey, 1988). Low levels of citric acid are indicative of prostatic cancer, whilst abnormally high levels suggest a greater contribution of secretory products by the prostate to the seminal plasma, hence seminal vesicle dysfunction (Marberger et al, 1962).

Carnitine is present in high concentration in the epididymal fluid of man (Lewin et al, 1976). It is produced in the liver (Menchini-Fabris et al, 1984) absorbed from the blood stream and secreted into the epididymal lumen by the epididymal epithelium of the caput and corpus (Lewin et al, 1976; Soufir et al, 1984). The accumulation of carnitine in the epididymis is androgen dependent (Brooks, 1983). It is an important energy source during spermatozoal maturation which takes place in the epididymis (Soufir et al, 1984). Sperm utilise lipids for energy and carnitine plays an important role in the transport of long chain fatty acids into the mitochondrion. High seminal carnitine levels have been obtained in semen samples where sperm display good motility and forward progression, implying that low carnitine levels are indicative of epididymal dysfunction (Bornman et al, 1989). Hinton and co-workers (1981) reported

that carnitine stimulated sperm motility, in sperm obtained from the caput epididymis. After 20 minutes this effect was reversed, suggesting an inhibitory action. This supported the suggestion that carnitine may be associated with initiating motility of the sperm in the proximal regions of the epididymis, but is also important in maintaining a quiescent condition in the distal regions of the epididymis, where carnitine is found in higher concentrations (Hinton et al, 1981). An increase in carnitine levels has also been observed with increasing lengths of abstinence (Soufir et al, 1984).

In view of the dispute regarding the androgen and gonadotrophin levels in diabetic male patients, amongst other conflicts, this study was an attempt to clarify some aspects concerning the effects of diabetes on the male reproductive system. Since insulin receptors have been demonstrated in the testis (Saucier et al, 1981), the testis can be regarded as an insulin-dependent organ. Insulin receptors found on the Sertoli cells of the rat testis (Gonzales et al, 1989) are thought to enhance glucose metabolism in these cells, which in turn provide support and nutrition for the differentiating germ cells. Therefore diabetes mellitus would be expected to have some effect on the male reproductive system. To determine what these effects are, the hormonal status of the human diabetic male has been examined in this study. An assessment of the type of sperm produced by these patients has also been noted. The

analysis of some biochemical markers of the male reproductive system, has not been carried out in diabetic patients in the past. This investigation will include the quantitative assessment of some of these substances. The results obtained for the diabetes group of patients were not only compared to a control group but also to an infertility group, thus the comparison of diabetics to the extremes of the male population. This has permitted the determination of the degree to which diabetes affects the male reproductive system.

The poor quality of semen obtained from diabetic men has initiated studies of the effect of experimental diabetes on the testis and reproductive potential of the laboratory rat (Ford & Hamilton, 1984). Streptozotocin, which is a highly specific diabetogen of relatively low toxicity, has been used to induce diabetes in male Wistar rats in this study. This has allowed the investigation of testicular biochemistry, accessory gland examination and fertilising potential of the diabetic animal, which could not be established in the human patients.

2. MATERIALS AND METHODS

2.1 Human patients

Twenty three male diabetic patients from a local diabetes clinic participated in this investigation, whilst the husbands of patients consulting a local gynaecologist for infertility constituted the infertility group. Control subjects were drawn from males with no known history of infertility. The purpose and nature of the study was explained to all subjects, and their informed consent was obtained before their participation in the project. Their medical history was also noted.

2.1.1 Blood and Semen Collection

Initially the capillary blood glucose level of each patient was measured on a Glucometer II (Model 5550) with Glucostix (Ames Division, Miles Laboratories Inc. U.S.A). Blood samples (ca. 20 ml) were then collected from the brachial vein of each patient into heparinised venoject vacuum tubes. The samples were centrifuged at 320 g for 30 min. Plasma was collected and stored at -20°C until required for analysis.

Semen samples were obtained by masturbation, after 3 days of sexual abstinence. The ejaculates were collected in a disposable, sterile, wide-mouthed plastic container with a screw-on lid. Some ejaculates were collected at the laboratory whilst those patients who experienced difficulty in producing the sample at the laboratory collected the specimen at home and brought it to the

laboratory within an hour. These patients transported the sample in a pocket close to the body to avoid external temperature extremes.

2.1.2 Semen Analysis

Upon receipt, the sample was registered and the state of coagulation noted. However this could not be done on ejaculates collected at home. Trial studies on the maintenance of semen indicated that the longevity of the sperm was optimum at 35°C, hence all samples were kept in an incubator at this temperature before and during analysis. Any absence of, or delay in liquifaction was also noted. Other macroscopic recordings included colour, viscosity and quantity of debris in the semen. Thereafter the volume and pH were measured.
The seminal plasma, which was used for the various biochemical tests, was collected after liquifaction and centrifugation at 480g.

2.1.2.1 Assessment of Sperm Motility

One drop of semen was place on a warm (35°C), clean slide and diluted with one drop freshly prepared phosphate buffered saline (PBS, Oxoid Ltd., England). Viscous specimens were forcibly ejected through a 18 gauge needle before assessment (Amelar, 1962). The number of motile and non-motile sperm were counted in 10 random fields of view under the 40 x objective of a light microscope. The percentage motile sperm was calculated as follows:

$$\text{gross motility (\%)} = \frac{\text{number of motile sperm}}{\text{total number of sperm counted (motile + non-motile)}} \times 100$$

Progressive motility was graded on a scale ranging from 1 to 7 as follows:

1 = no motility

2 = motile, but no progressive motility

3 = direction is forward occasionally

4 = forward motility is slow/sluggish

5 = good forward progression

6 = excellent progressive motility

7 = moves forward with quick wave-like action

However, this form of evaluation is highly subjective, therefore a comparison of the data obtained on the progressive motility of sperm in this study with that of other reports would be difficult.

A measure of rapid sperm motility was determined turbidimetrically (Sokoloski et al, 1977; Ford & Harrison, 1985). A length of glass tubing with an internal diameter of 1 mm was glued to the corner of a disposable plastic cuvette, with the lower end of the tube 1 mm above the floor of the cuvette whilst the upper end projected above the top of the cuvette. Freshly prepared PBS (1,8 ml) was added to the cuvette which was placed in a Beckman spectrophotometer (Model 25) at 34°C. The absorbance of the PBS, at 500 nm, was recorded (A_0). A Gilson Minipuls II peristaltic pump was used to deliver 0,25 ml semen onto the floor

of the cuvette via a 21 gauge needle that was inserted into the glass tubing. After 10 min the absorbance was again measured (A_1), since preliminary trials by Ford and Harrison (1985) indicated that all motile sperm migrated up the buffer after this time. A stirring rod was then used to suspend the spermatozoa in the PBS and a final reading (A_2) was taken. The percentage of rapidly motile sperm was calculated from these readings:

$$\text{rapidly motile sperm (\%)} = \frac{A_1 - A_0}{A_2 - A_0} \times 100$$

where A_0 is absorbance of the blank; A_1 is the turbidity induced by rapidly moving sperm and A_2 is the final absorbance after mixing the contents of the cuvette.

2.1.2.2 Mixed Antiglobulin Reaction Test

The mixed antiglobulin reaction (MAR) test was used to detect antisperm antibodies on free swimming spermatozoa (Jager et al, 1978). One drop of group O Rh-positive erythrocytes, sensitized with incomplete anti-D serum (Natal Blood Transfusion Service) was placed on a slide together with 1 drop of semen and 1 drop of undiluted monospecific anti-IgG antiserum (Behring Institute, Germany). The 3 drops were brought into contact concurrently and mixed. This mixture was then covered with a cover-slip and allowed to stand, in a humid petri dish, at room temperature for 10 min before the test was interpreted. Agglutination of the red blood cells serves as an internal control. A positive test was indicated by motile mixed agglutinates which formed between the

erythrocytes and spermatozoa. No interpretation was made unless there was agglutination of the red blood cells and adequate motility of the spermatozoa.

2.1.2.3. Determination of Sperm Viability

Sperm viability was determined by supravital staining (Eliasson, 1977). One drop of semen was mixed with 1 drop of 1% eosin (in distilled water) and allowed to stand for 15 sec on a spotting plate. Thereafter 2 drops of 10% nigrosin (in distilled water) was added to the mixture and allowed to stand for a further 30 sec. An air dried smear of 1 drop of the final mixture on a clean microscope slide was examined. Intact live sperm excluded the stain while those with damaged cell membranes were red in colour. Nigrosin, which was used as a counterstain provided a dark background that allowed easy perception of the white (unstained) live spermatozoa. The number of live sperm was expressed as a percentage of the total number of sperm counted under the 40 x objective of a light microscope.

2.1.2.4 Assessment of Sperm Morphology

Sperm morphology was strictly assessed according to the criteria of Menkveld and co-workers (1990). A drop of fresh semen was smeared onto a clean slide and allowed to air dry. The specimen was then stained according to the Papanicolaou staining procedure (Mortimer, 1985) as follows:

The specimen was hydrated in 80%, 70% and 50% ethanol for 10 sec each. After immersion in distilled water for 10 sec, the slide

was transferred to hematoxylin for 3 min, and then rinsed in running tap water for 3 min. Subsequently the slide was dipped in 0,5% hydrochloric acid and rinsed in running tap water for 5 min before being dipped in 1% lithium carbonate and then rinsed again in running tap water for 5 min. An ascending series of ethanol (50%, 70%, 80% & 90%) was used to dehydrate the specimen which was then stained for 2 min in Orange G 6. The slide was transferred to 95% ethanol (x 2) for 5 sec and then to EA50 for 3 min for further staining. This was followed by further dehydration with 95% ethanol (x 3) and 100% ethanol for 5 sec each. After allowing the slide to stand in an 1:1 solution of ethanol and xylene for 2 min, it was treated with xylene (x 2) for 10 sec and left in an additional xylene solution for 20 min. The specimen was mounted with D.P.X. mounting medium and left to air dry.

One hundred sperm were examined under an oil immersion objective (100 x) of a light microscope. The criteria used to score sperm abnormality included the following: sperm size defects, amorphous, tapered or dulicate heads, abnormal shape and size of the acrosome and nucleus, midpiece and tail defects and immature forms represented by spermatids, primary spermatocytes and cytoplasmic droplets (Menkveld et al, 1990).

2.1.2.5 Determination of Sperm Concentration

To obtain an accurate count of the number of sperm in the semen, the sample was gently mixed after liquifaction to ensure equal

distribution of sperm. Fifty microlitres of the sample was then diluted with 250 µl drops PBS and this mixture was used to flood a haemocytometer. The haemocytometer was then left to stand, in a humid petri dish, to allow the sperm to settle before counting. The sperm were scored under the 40 x objective of a light microscope. Total sperm number, per ml and per ejaculate, was calculated according to the following formula:

Depth of counting chamber = 0,1 mm
Area of small central square = 1 sq. mm
Total number of squares in central area = 400
Since 80 small squares were counted, then area = 0,2 sq. mm

Therefore number of sperm in million per ml =

$$\text{Number of sperm counted} \times \frac{1}{0,02} \times \text{dilution} \times 1000$$

where the dilution was 5 (50 µl of human semen + 250 µl of PBS were mixed and used to flood the counting chamber).
The number of sperm/ml X volume of semen = number of sperm/ejaculate.

2.1.2.6 Microscopy to Detect Infection

To ascertain the existance of an underlying infection, the semen was microscopically examined for the presence of leucocytes. To facilitate visualisation of these cells, 1 drop of 50% albumin was mixed with 1 drop of semen before a smear was made on a slide. The slide was immediately fixed in 50% ethanol, to prevent

crystallisation of the albumin, and then stained according to the Papanicolaou procedure described in 2.1.2.4.

2.1.2.7 Determination of Acid Phosphatase Concentration

The determination of acid phosphatase in seminal plasma was based on the hydrolysis of the substrate, p-nitrophenyl-phosphate (PNPP) to p-nitrophenyl by acid phosphatase, at pH 4,8 and 37°C (Mortimer, 1985). The reaction was stopped by sodium hydroxide and the intensity of the coloured anion was determined photometrically.

Twenty µl of seminal plasma was diluted 25 000 x with double distilled water. Half a ml of 0,01 M PNPP was pipetted into an appropriate number of clean test tubes and incubated in a water bath at 37°C for 5 min, before any other constituents of the assay could be added. Thereafter 0,5 ml of 0,09 M sodium citrate buffer (pH 4,8 adjusted with HCl) was added to each test tube and the contents allowed to stand for 5 min. After mixing, 0,2 ml of the sample, standards or distilled water (blank) was then added to the appropriate tube. The solutions were vortexed and incubated at 37°C for 30 min. Five ml of 0,1 N NaOH was added to stop the reaction. The intensity of the resultant colour was read at 405 nm on a Beckman spectrophotometer (Model 25). Each absorbance value, was read off a standard curve and the result expressed in U/ml. The standard curve (Fig. 1) was drawn from the following standard concentrations: 15, 25, 30, 40, 50 and 60 U/ml, where 1 unit is the enzyme activity that transforms 1 µmol

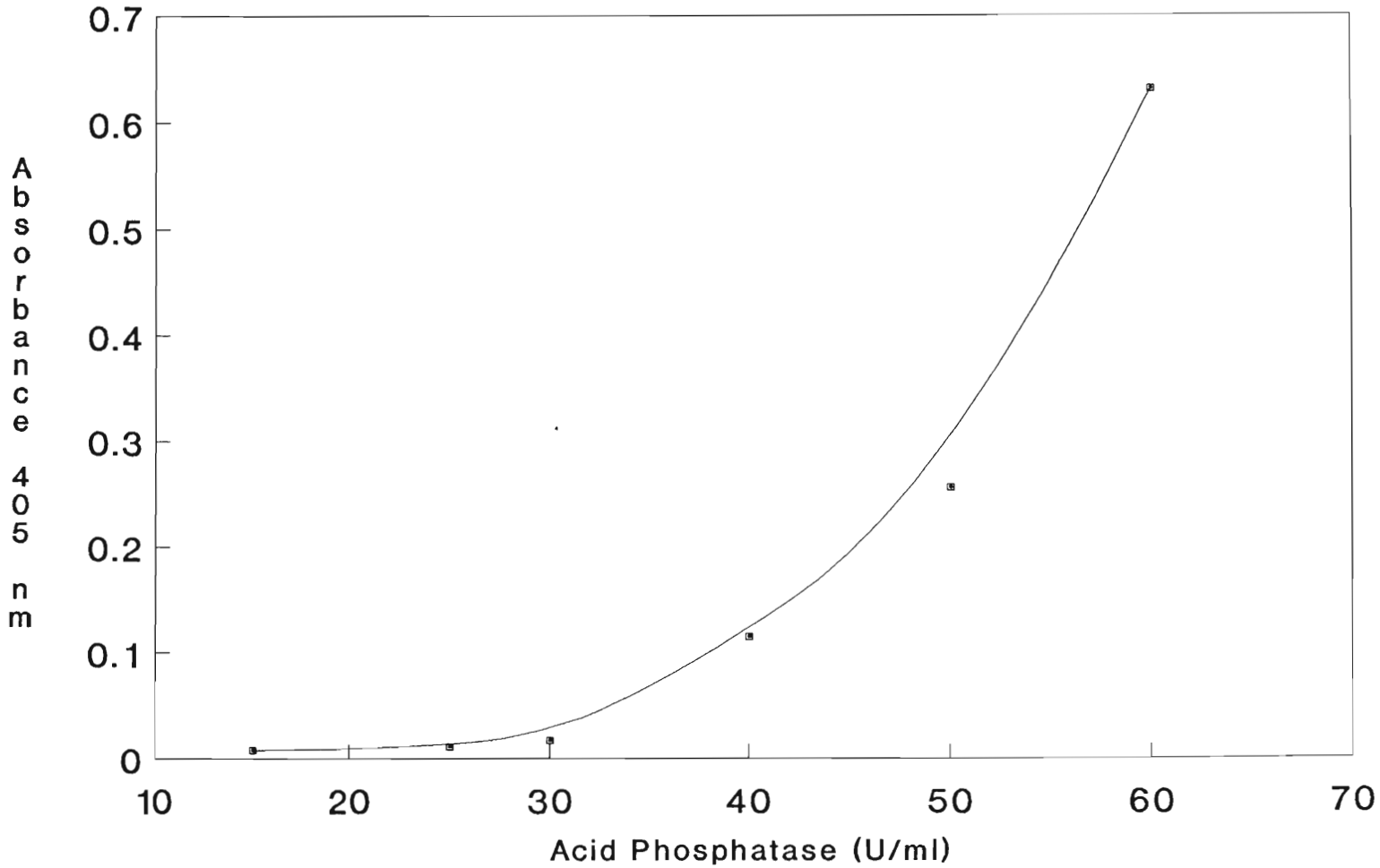

Fig.1 Acid Phosphatase Standard Curve

of substrate in 1 minute.

The intra-assay coefficient of variation for this procedure was 6,7%, whilst the inter-assay value was 7,3%.

2.1.2.8 Determination of Fructose Concentration

The quantity of fructose present in seminal plasma was determined according to the method outlined by Mortimer (1985). One hundred µl of seminal plasma was diluted with 3,9 ml of double distilled water. Two ml of 10% zinc sulphate and 2 ml of 0,1N sodium hydroxide were then added to the same test tube and vortexed. This mixture was incubated at 80°C for 2 min, cooled in water, and centrifuged at 480 g for 10 min. Two ml of the supernatant was added to 2 ml of 0,1% alcoholic resorcinol (in 90% alcohol) and 6 ml of hydrochloric acid. To obtain a blank, 2 ml of saturated benzoic acid was used instead of the supernatant. Standard solutions with the concentrations of 0,56; 1,11; 2,22; 4,17; 8,33 and 16,67 mmol/l were used to draw a standard curve (Fig. 2). The test tubes were vortexed and incubated at 80°C in a water bath for 8 min. After cooling, under running water, the colour intensity was read on a Beckman spectrophotometer (Model 25) at 490 nm. The sample values were determined from the standard curve and were expressed in mmol/l.

The coefficient of variation obtained for this method of fructose analysis was 4,1% (intra-assay) and 5,2% (inter-assay).

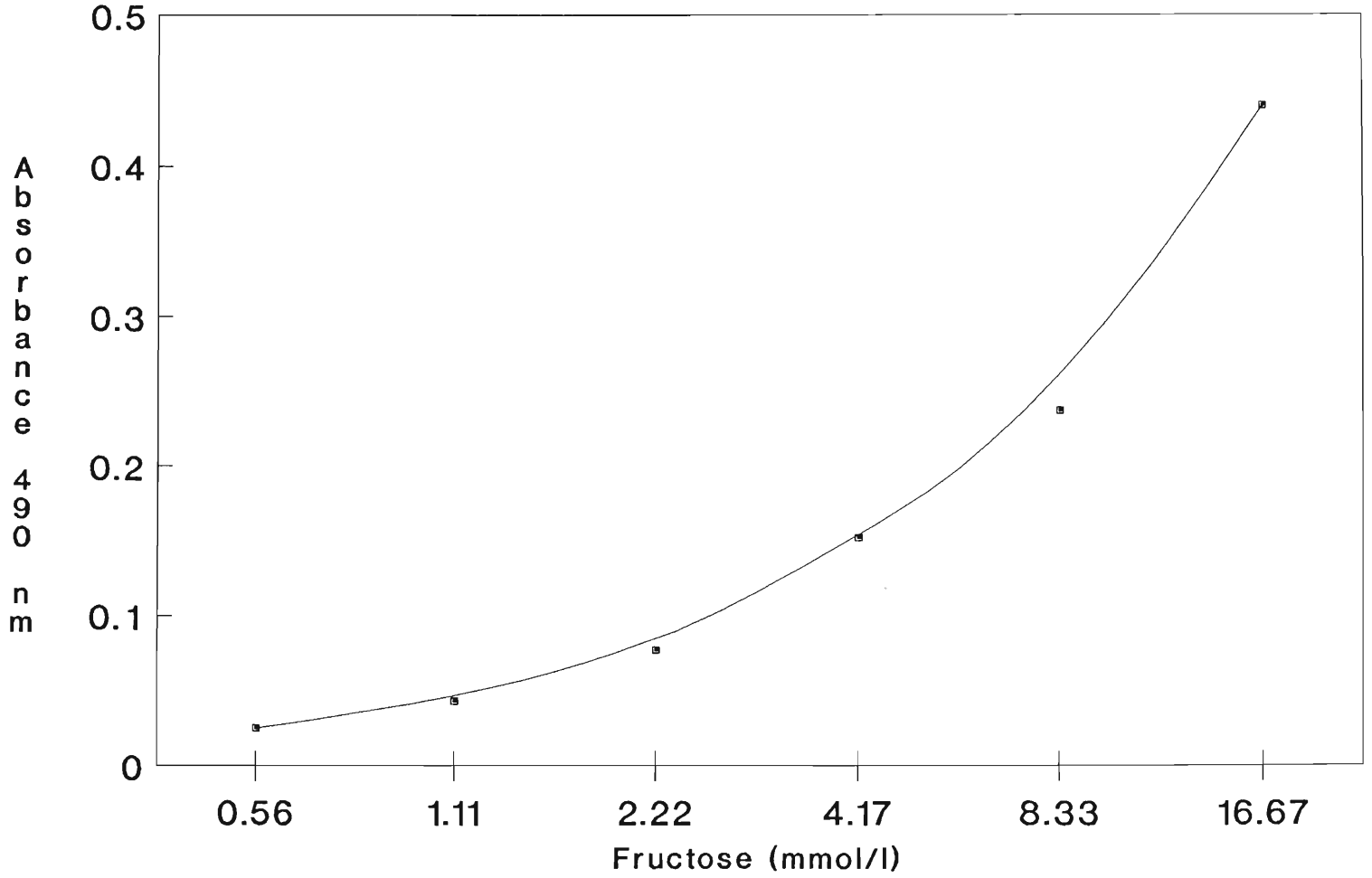

Fig.2 Fructose Standard Curve

2.1.2.9 Determination of Zinc Concentration

The quantity of zinc in seminal plasma was determined with the use of an atomic absorption spectrophotometer (Perkin-Elmer, Model 2380). The seminal plasma was diluted by adding 0,99 ml of distilled water to 0,01 ml of the sample. One ml of 10% tri-chloroacetic acid was added to this diluted sample, mixed and then centrifuged at 480 g for 10 min. The absorbance of the supernatant was determined with an oxidising air-acetylene flame at 213,9 nm with a slit setting of 0,7 nm. The samples were aspirated manually and an average of 5 absorption readings were recorded. The zinc standard values, used to construct a standard curve were 0,00; 0,5 and 1,00 ug/ml. Sample values were determined from the standard curve (Fig.3) and expressed in mmol/l.

The intra-assay coefficient of variation for zinc was 0,80%.

2.1.2.10 Determination of Carnitine Concentration

A commercial test kit (Boehringer Mannheim, Germany) was used for the determination of carnitine levels in human seminal plasma. Carnitine was acetylated to acetyl carnitine by acetyl coenzyme A, in the presence of carnitine acetyl transferase. The resultant coenzyme A was acetylated back to acetyl coenzyme A in the presence of adenosine-5-triphosphate and acetate, catalysed by acetyl coenzyme A synthetase. The adenosine-5-monophosphate formed in this reaction produced twice the quantity of adenosine-5-diphosphate, in the presence of adenosine-5-triphosphate and myokinase. This was converted by

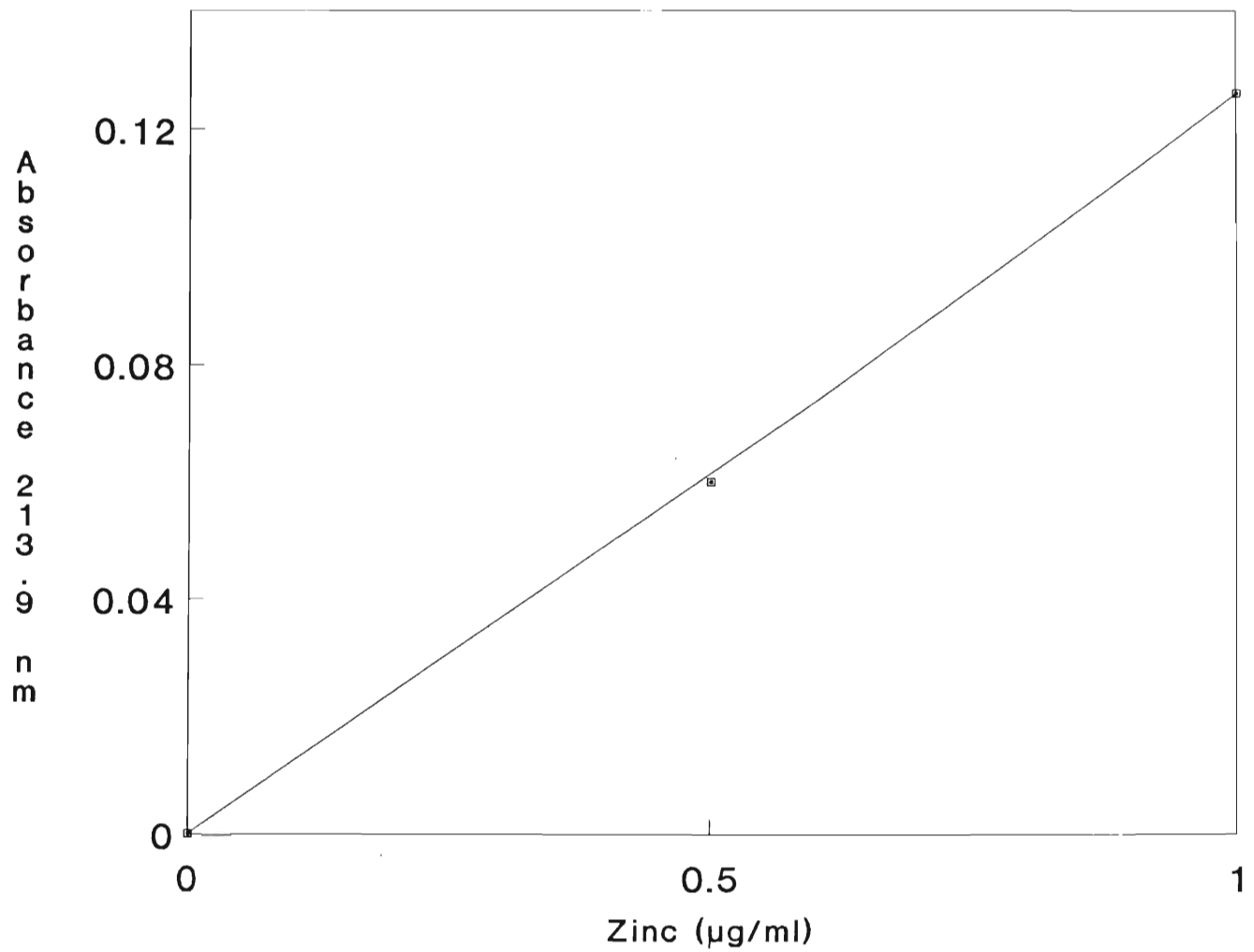

Fig.3 Zinc Standard Curve

phosphoenol pyruvate in the presence of pyruvate kinase, to pyruvate. The pyruvate was reduced to lactate by reduced nicotinamide adenine dinucleotide (NADH) in the presence of lactate dehydrogenase. The quantity of NADH consumed during this reaction was equivalent to half the quantity of carnitine in the seminal plasma.

The seminal plasma was deproteinised as follows:
Half a ml of cold perchloric acid (1 mol/l) was added to an equal volume of seminal plasma, mixed and allowed to stand in an ice-bath for 10 min. After centrifuging for 10 min at 2000 g, 0,5 ml of the supernatant was pipetted into a test tube containing 0,1 ml of 1,75 mol/l tripotassium solution. The suspension was again allowed to stand in an ice-bath for 20 min and centrifuged at 2000 g for 5 min. The supernatant was brought to room temperature for the test.

The test reaction was allowed to proceed in glass cuvettes into which the following solutions were added: 1,5 ml buffer solution; 0,05 ml acetyl CoA; 0,1 ml coenzyme solution; 0,1 ml enzyme suspension; 0,1 ml sample, distilled water (blank) or standard solution and 0,4 ml distilled water. The solutions were mixed and after 5 min the absorbance A_1 was determined, against the blank, on a Beckman spectrophotometer (Model 25), at 20°C at a wavelength of 339 nm. The next reaction was started by the addition of 0,005 ml of carnitine acetyl transferase suspension. After mixing, the solutions were allowed to stand at room temperature for 30 min and the absorbance A_2 was read, and then after a further 10 min, A_3 was measured.

The absorbance difference of the blank =

$$(A_1 - A_2)_{blank} - 3 \times (A_2 - A_3)_{blank}$$

The absorbance difference of the sample was similarly calculated. The absorbance difference of the blank was then subtracted from the absorbance difference of the sample, represented by "A", and this was used to calculate the amount of carnitine present in the seminal plasma.

The dilution factor F was calculated by taking into account the deproteinising procedures and the density (1,035 g/ml) and the fluid portion of seminal plasma (0,98). Since 0,5 ml seminal plasma, 0,5 ml perchloric acid solution, 0,5 ml supernatant after deproteinisation and 0,1 ml tripotassium phosphate solution were used, the dilution factor was:

$$F = \frac{(0,5 \times 1,035 \times 0,98)}{0,5} \times \frac{0,6}{0,5} = 2,42$$

Therefore in accordance with the general equation for reactions in which the quantity of NADH consumed is equivalent to half the quantity of substrate:

$$c = \frac{V \times MW \times F}{E \times d \times v \times 1000 \times 2} \times A \text{ (mg/l)}$$

where V = final volume (ml)

v = sample volume (ml)

MW = molecular weight of substance to be assayed (g/mol)

d = light path length (cm)

c = concentration (mg/ml)

E = absorbance coefficient of NADH at 339 nm = 6,3 ($l \times mmol^{-1} \times cm^{-1}$)

It follows that for L-carnitine:

$$c = \frac{2,255 \times 162,2 \times F}{6,3 \times 1 \times 0,1 \times 1000 \times 2} \times A \ (g/l)$$

The inter-assay coefficient of variation obtained for the determination of carnitine was 4,8% and the intra-assay value was 4,1%.

2.1.2.11 Determination of Citric Acid Concentration

A commercial test kit (Boehringer Mannheim, Germany) was used for the determination of the quantity of citric acid in seminal plasma. Citric acid was converted to oxaloacetate and acetate in a reaction catalysed by the enzyme citrate lyase. In the presence of the enzymes malate dehydrogenase and L-lactate dehydrogenase, oxaloacetate and its decarboxylation product, pyruvate were reduced to L-malate and L-lactate respectively, by reduced nicotinamide-adenine dinucleotide (NADH). The quantity of NADH oxidised in these reactions was stoichiometric with the quantity of citric acid. NADH was determined by means of its absorbance at 340 nm and 25 °C on a Beckman spectrophotometer (Model 25).

Seminal plasma was deproteinised prior to the citric acid determination. One hundred µl of seminal plasma was mixed with 3,9 ml of perchloric acid (0,3 mol/l) and placed in an ice bath for 10 min. Thereafter the solution was centrifuged at 480 g for 10 min and 0,2 ml of the supernatant was added to 1,0 ml of 0,75 mol/l potassium carbonate. The mixture was again allowed to stand in an ice bath for 15 min, and then centrifuged at 480 g for 10 min.

The supernatant (0,2 ml) was used in the assay, together with 1 ml of glycylglycine buffer and 1,8 ml of distilled water. The absorbance of this reaction was read after 5 min (A_1). The next reaction was started by the addition of 0,02 ml citrate lyase and was read after 5 min (A_2). The blank and standard values were obtained by the addition of distilled water and the standard solution respectively, in place of the sample.

To calculate the concentration of citric acid in seminal plasma the absorbance difference of the blank was subtracted from the absorbance difference of the sample. This value (A) was then used in the general equation to calculate concentration.

$$c = \frac{V \times MW}{E \times d \times v \times 1000} \times A \ (g/l)$$

where,

V = final volume (ml)

v = sample volume (ml)

MW = molecular weight of substance to be assayed (g/mol)

d = light path (cm)

c = concentration (g/l)

E = absorbance coefficient of NADH at 340 nm = 6,3 (l x $mmol^{-1}$ x cm^{-1})

It follows that for citric acid (calculated as an anhydrous acid):

$$c = \frac{3.02 \times 192.1}{6,3 \times 1 \times 0,2 \times 1000} \times A \quad (g/l)$$

Since the sample solution had been diluted during preparation the result was multiplied by the dilution factor F = 60 which was obtained from the deproteinisation process described earlier. The specific gravity of the sample material and the liquid fraction were negligable. The solid fraction of the precipitate (potassium perchlorate) was also negligable.

The inter-assay coefficient of variation for this assay was 8,5%, whilst that of the intra-assay was 5,6%.

2.1.3 Hormone Analyses

2.1.3.1 Determination of Follicle Stimulating Hormone Concentration

The quantity of FSH in blood plasma was determined with a commmercial FSH double antibody kit (Diagnostic Products

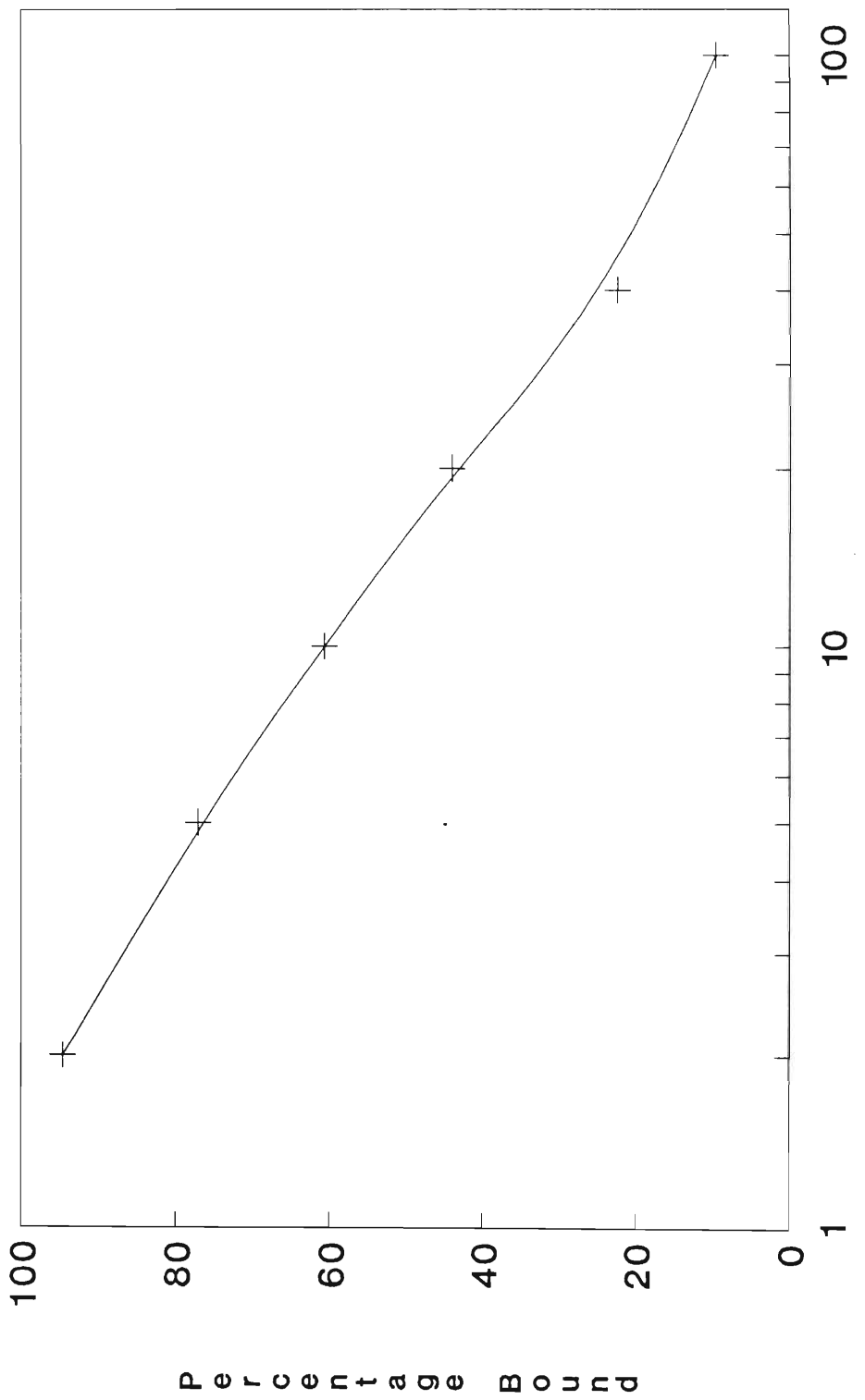

Fig.4 FSH Standard Curve

Corporation, U.S.A).

The principle of the radioimmunoassay depended on the ability of an antibody to bind to its antigen. To quantitate the antigen, the radioactive and nonradioactive forms of the antigen competed for the binding sites on their specific antibodies. As more nonradioactive antigen was added, less radioactive antigen remained bound until an equilibrium between the free and antibody-bound antigen occurred. In the FSH assay the antibody-bound antigen was precipitated by a second antibody (goat anti-rabbit gamma globulin) during centrifugation.

Frozen plasma samples were thawed and vortexed prior to use. Eighteen polypropylene tubes were labelled as T (total counts), NSB (nonspecific binding), A (maximum binding) and B through to G for standards (0, 2, 5, 10, 20, 40, 100 mIU/ml). Two hundred µl of the zero calibrator A was pipetted into the NSB and A tubes, and 200 µl of each of the standards B through to G into the correspondingly labelled tubes. Two hundred µl of each sample was pipetted into appropriately labelled tubes. One hundred µl of FSH antiserum was then added to all tubes except the NSB and T tubes. All tubes were vortexed and incubated for 120 min at 37°C in a water bath. Thereafter 100 µl iodinated FSH was added to the tubes and vortexed. The tubes were again incubated at 37°C for a further 120 min. One ml of cold precipitating solution was then added to all the tubes and the contents thoroughly mixed. The tubes were centrifuged at 3000 g for 15 min in a refrigerated centrifuge. The supernatant was decanted and the precipitate counted for 1 min in a gamma counter (Riastar, Packard). The quantity of FSH was calculated from the counts obtained as

follows: the average counts per min were calculated for each pair of tubes.

The net counts =

average counts/min - average NSB counts/min

Thereafter the binding of each pair of samples was expressed as a percent of maximum binding (MB), with the NSB-corrected counts of the A tubes taken as 100%:

$$\text{percent bound} = \frac{\text{net counts}}{\text{net MB counts}} \times 100$$

Using log paper, the percent bound was plotted on the vertical axis against the concentration for each of the standards, B through G on the horizontal axis (Fig.4). FSH concentrations for the unknowns were then determined from the graph and expressed in mIU/ml.

The intra-assay coefficient of variation was 1,6%.

2.1.3.2 Determination of Luteinising Hormone Concentration

The quantity of LH in blood plasma was determined with a commmercial double antibody kit (Diagnostic Products Corporation, U.S.A). The method used was similar to that for FSH described in 2.1.3.1 with the following exceptions: the standard values were now 3, 10, 20, 40, 100 and 200 mIU/ml (Fig.5), and the two incubation periods were changed to 30 min at 37°C and 60 min at room temperature respectively.

The intra-assay coefficient of variation obtained for this

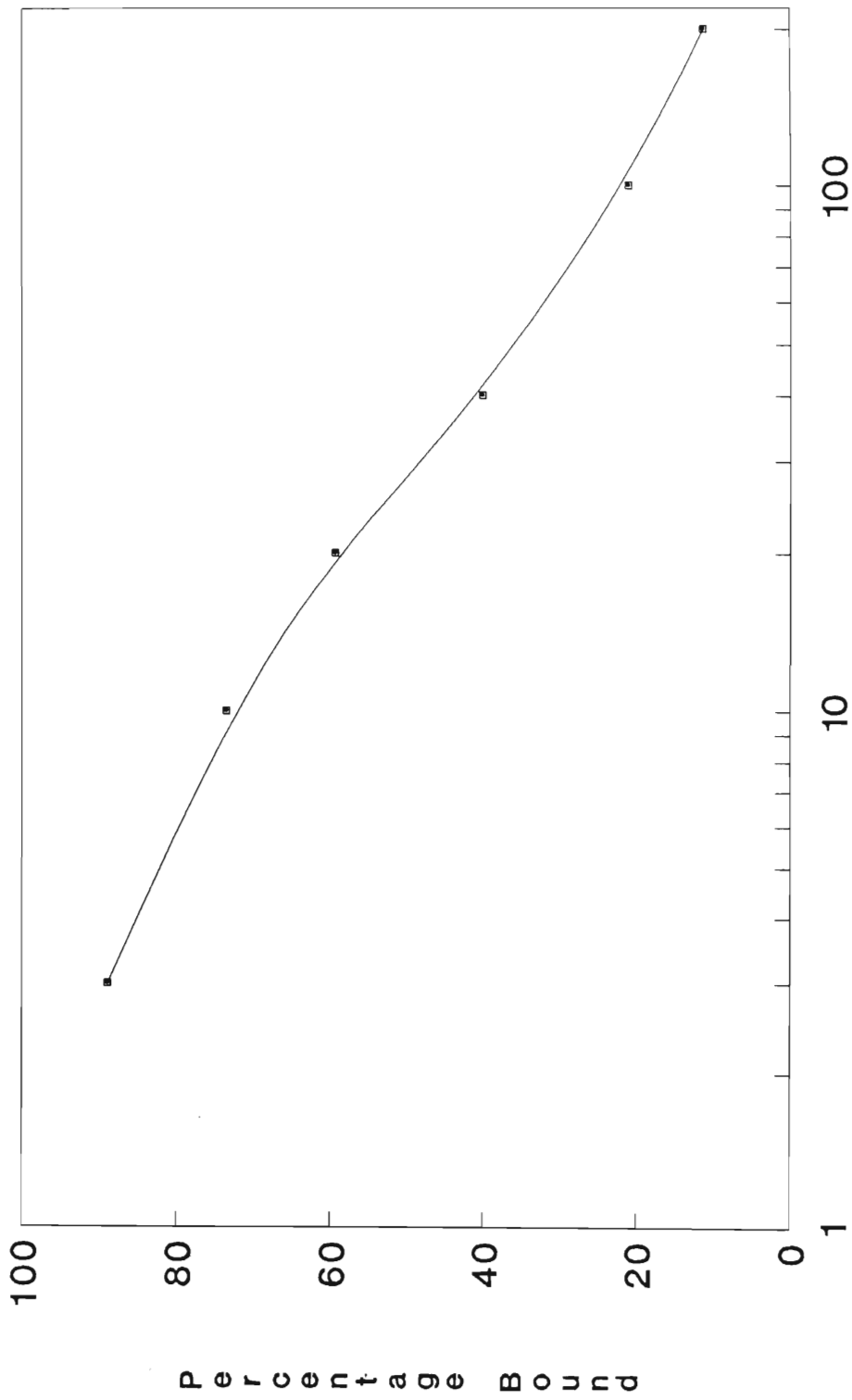

Fig.5 LH Standard Curve

procedure was 5,9%.

2.1.3.3 Determination of Prolactin Concentration

The quantity of prolactin in blood plasma was determined with a commmercial prolactin double antibody kit (Diagnostic Products Corporation, U.S.A). This procedure was also similar to the FSH assay except for the following: the prolactin standards were 5, 10, 20, 50, 100 and 200 mIU/ml (Fig.6). The iodinated prolactin was added immediately after the samples into the assay tubes, and only one incubation period of 3 hours at room temperature was necessary.

A 0,5% intra-assay coefficient of variation was obtained for this protocol.

2.1.3.4 Determination of Testosterone Concentration in Human Plasma

To evaluate the quantity of testosterone in blood plasma, a commercial radioimmunoassay kit was used (ICN Biomedicals Inc, U.S.A.). Each standard or unknown (25ul) was pipetted in duplicate into coated assay tubes which had been brought to room temperature. One ml of labelled testosterone was added to all tubes and the contents vortexed. The tubes were then incubated for 120 min at 37°C. Thereafter the tubes were drained and counted for 1 min on a gamma counter (Riastar, Packard). In this assay the antibody was covalently bound to the inner surface of the polypropylene assay tubes, thus the antibody-bound antigen was also bound to the inner surface of the tube. At the

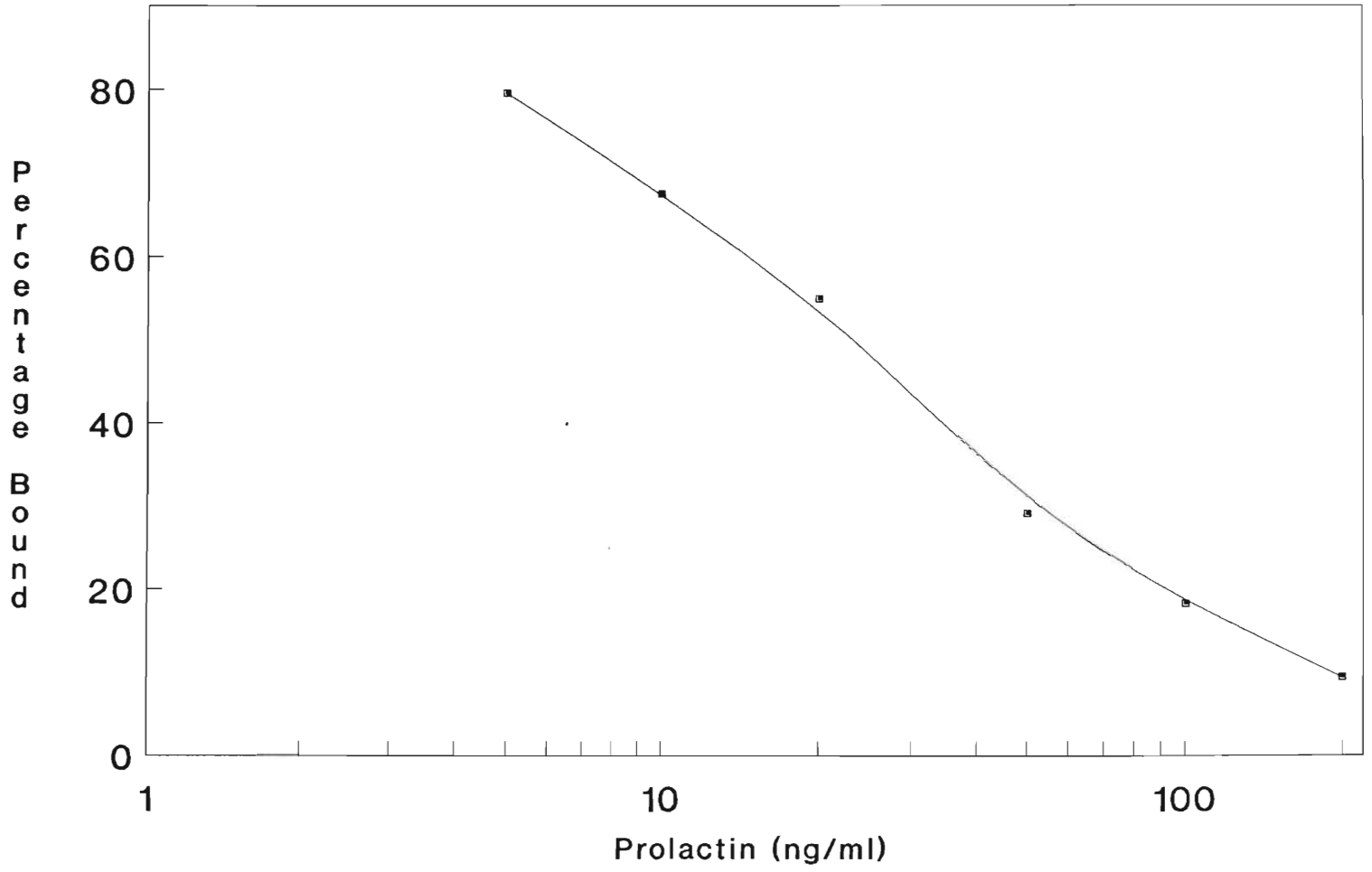

Fig.6 Prolactin Standard Curve

conclusion of the assay, free antigen was decanted, leaving only antibody-bound antigen on the inner surface of the coated tube. The testosterone concentration of each sample was calculated from the counts obtained and from a standard curve drawn from the following values: 0; 0,2; 0,6; 2,0; 6,0; 20,0 ng/ml (Fig.7) as follows:

The average counts per minute were calculated for each sample and standard, and these were divided by the average counts for the zero standard, and then multiplied by 100 to obtain % B/B_0. A graph of % B/B_0 was plotted on log paper against the concentration of standards. Using the standard curve, the testosterone concentration for each sample was determined. The inter-assay coefficient of variation was 7,3%, with an intra-assay value of 3,9%.

2.1.4 Biochemical Analyses of Blood Plasma

2.1.4.1 Determination of Glucose Concentration

Glucose levels in blood plasma were determined using a commercial test kit (Boehringer Mannheim, Germany).

D-Glucose was phosphorylated to glucose-6-phosphate (G-6-P) in the presence of hexokinase and adenosine-5-triphosphate (ATP) with the simultaneous formation of adenosine-5-diphosphate (ADP). In the presence of glucose-6-phosphate dehydrogenase, G-6-P was oxidised by nicotinamide adenine dinucleotide phosphate (NADP) to gluconate-6-phosphate with the formation of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The quantity of NADPH

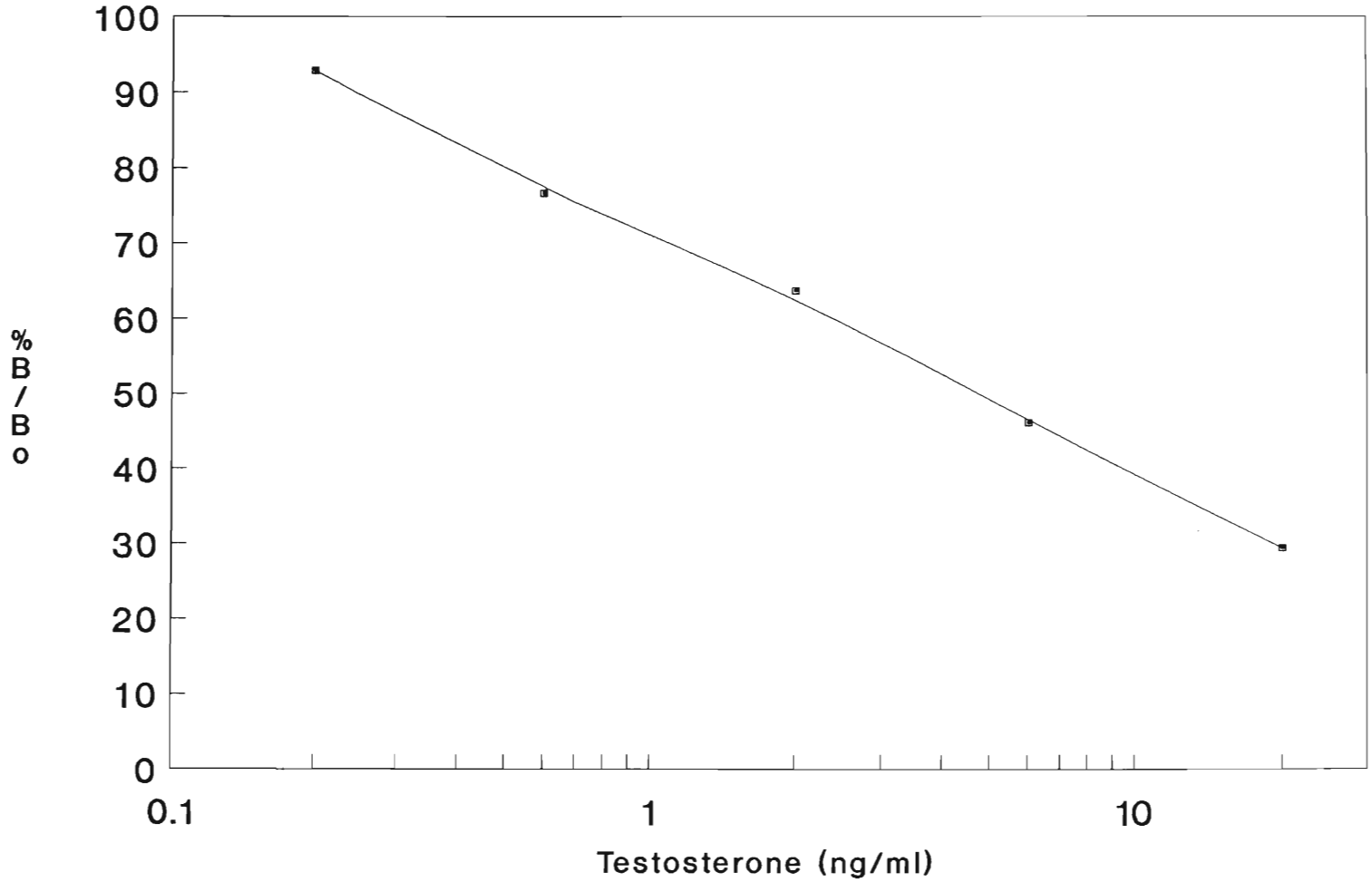

Fig. 7 Testosterone Standard Curve

formed in this reaction was stoichiometric with the quantity of D-glucose. The quantity of NADPH was measured by means of its absorbance at 340 nm on a Beckman spectrophotometer (Model 25). The readings were determined against air at 25°C.

To determine the glucose level in blood plasma, 0,1 ml of sample was mixed with 1 ml of 0,33 mol/l perchloric acid and centrifuged for 10 min at 480 g. The supernatant was used for the assay. One ml of triethanolamine buffer (pH 7,6), consisting of NADP, ATP, magnesium sulphate and stabilisers, was pipetted into the cuvettes marked blank, standard and sample. One hundred µl of the sample was dispensed into the appropriate cuvettes while distilled water was used for the blank and the standard solutions for the standards. The volume in the cuvettes was increased to 3 ml by adding 1,90 ml of distilled water. The solutions were then mixed with a plastic spatula and the absorbance (A_1) was determined after 3 min at 340 nm. The reaction was then started by the addition of 0,02 ml enzyme suspension, consisting of 320 U hexokinase and 160 U glucose-6-phosphate dehydrogenase. The final absorbance (A_2) was read after 10 min. The absorbance difference (A_2-A_1) for the blank, standard and sample was calculated and absorbance difference of the blank was then subtracted from that of the samples and the standard to obtain the absorbance of the glucose (A), which was used for the calculation of the plasma glucose content.

$$c = \frac{V \times MW}{E \times d \times v \times 1000} \times A \ (g/l)$$

where,

V = final volume (ml)

v = sample volume (ml)

MW = molecular weight of glucose (g/mol)

d = light path (cm)

c = concentration (g/l)

E = absorption coefficient of NADP

at 340 nm = 6,3 (1 x $mmol^{-1}$ x cm^{-1})

Therefore for D-glucose:

$$c = \frac{3,02 \times 180,16}{6,3 \times 1 \times 0,1 \times 1000} \times A \ (g/l)$$

Since the sample had been diluted during preparation the result was multiplied by the dilution factor F which was obtained from the sample volume, the volume of the perchloric acid, the specific gravity of the plasma (1,03 g/ml) and the fluid content (0,92 ml) of the plasma:

$$F = \frac{0,1 \times 1,03 \times 0,92 + 1,0}{0,1}$$

$$= 10,95$$

The coefficient of variation obtained for the procedure was 5,3% (inter-assay) and 4,8% (intra-ssay).

2.2 Experimental Diabetes Mellitus

2.2.1 Induction of Diabetes with Streptozotocin in Male Wistar Rats

Fourteen, sexually mature male Wistar rats were divided into two groups, one serving as the control and the other the diabetic group. The animals were housed individually and food and water were supplied ad libitum for the duration of the experiment, which lasted 90 days. Initially all the animals were fasted for ca. 12 hours, after which their body weights and blood glucose levels were determined. The glucose levels were determined with the use of a Glucometer II (Model 5550) and Glucostix (Ames Division , Miles Laboratories Inc. U.S.A).

Diabetes was induced by injecting the animals intra-peritoneally with 65 mg of streptozotocin (Boehringer Mannheim, Germany) per Kg body weight (Anderson et al, 1987, Crowe et al, 1983). Since streptozotocin was dissolved in double distilled water, control animals were given an equivalent volume of double distilled water. Twenty four hours later, the streptozotocin-treated animals demonstrated blood glucose levels greater than 14 mmol/l. This level clearly indicated a diabetic state (Ford & Hamilton, 1984). Daily treatment with insulin (Protophane HM, NOVO Industries, S.A.) commenced 24 hours after streptozotocin administration. Initially, 0,5 U of insulin /Kg body weight was administered, subcutaneously, but since the response to this treatment varied amongst individuals, the dosage had to be altered from time to time to maintain the glucose levels at ca.

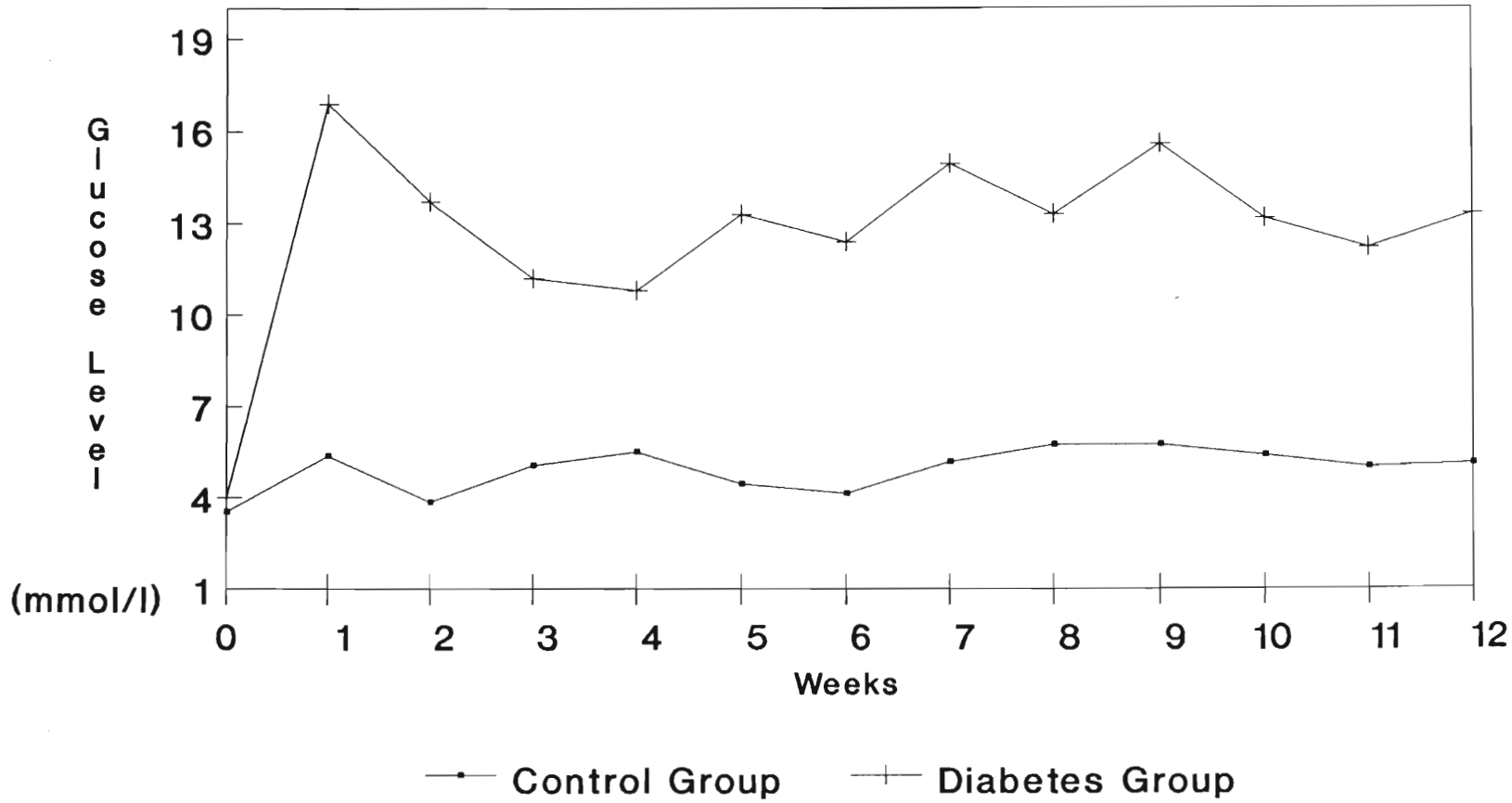

Fig.8 Changes in glucose levels in control and diabetic rats.

14 mmol/l (Fig. 8). If however, the glucose level dropped below 8 mmol/l, insulin treatment was stopped, and these animals were monitored regularly should they require further treatment. The control group were given equivalent volumes of 0,9 % NaCl. Diabetes was periodically verified by measurement of blood glucose levels. The body weights changes were also recorded weekly.

2.2.2 Fertility Tests

Twenty six days after diabetes had been successfully induced, each male from both groups, was housed with a sexually mature normal female for the length of an estrus cycle in a female Wistar rat (5 days). The females were permitted to deliver their litters, after a gestation period of 21 days. The number, weights and abnormalities of the pups produced were noted. Females were again introduced to the males 57 days and 85 days after diabetes was diagnosed, thus covering 2 spermatogenic cycles in the male rat.

2.2.3 Tissue Sampling

At the end of the experiment, the male rats were anaesthetised with thiopentone sodium (Intraval Sodium, M & B Veterinary Medicine). Each rat received an intra-peritoneal injection of 0,25 g/9 Kg body weight. After decapitation, the blood was collected from the neck, in heparinised centrifuge tubes and was centrifuged at 480 g for 10 min. Plasma was extracted and frozen at - 20°C until required.

The right cauda epididymis was masserated in 5 ml of PBS, to extract sperm. The right testis, with the tunica albuginea, and the left epididymis were weighed and then fixed in Bouin-Hollande fixative (Lillie, 1965; Appendix 1) for 48 hours, for histology. The weight of the right seminal vesicle and the prostate gland were recorded. The left testis, without the tunica albuginea, was weighed and immediately frozen for biochemical analysis.

2.2.4 Examination of Epididymal Sperm

2.2.4.1 Assessment of Sperm Motility

One drop of the sperm suspension, obtained from the cauda, was placed on a slide and examined under the 40 x objective of a light microscope. Motile and non-motile sperm in 10 random fields were counted and the number of motile sperm was expressed as a percentage of the total number of sperm counted.

2.2.4.2. Assessment of Sperm Viability

One drop of the sperm suspension was mixed with 1 drop of 1% eosin (dissolved in water) on a slide and allowed to stain for 15 sec. The sperm were then counterstained for 30 sec with 10% nigrosin (dissolved in water). This mixture was smeared onto a slide and left to air dry before counting. One hundred sperm were scored under the 40 x objective of a light microscope, and live sperm, which remained unstained, were expressed as a percentage of the total number of sperm counted.

2.2.4.3 Assessment of Sperm Morphology

To determine the percentage of abnormal sperm, a drop of sperm suspension was smeared onto a microscope slide and left to air dry. The smear was then stained with an acrosomal stain consisting of 2 volumes of 1% fast green, 1 volume of 1% eosin B and 1,7 volumes of ethyl alcohol for ca. 24 hours (Vawda & Davies, 1986). The slides were rinsed in tap water, dehydrated in an alcohol series, then transferred to xylene for 20 minutes and mounted in DPX. One hundred sperm were scored under the oil immersion objective (100 x) of a light microscope, and expressed as the percentage of abnormal sperm according to the criteria of Krzanowska (1976).

2.2.4.4 Determination of Sperm Concentration

Sperm concentration was determined on a counting chamber as described in 2.1.2.5. When calculating the concentration of rat sperm however, the dilution in the formula was 25 since the cauda epididymis was masserated in 5 ml of PBS and again 50 µl of this suspension was diluted with 250 µl of PBS to flood the counting chamber.

2.2.5 Extraction of Testicular DNA, RNA and Proteins.

The left testis which was frozen, after the removal of the tunica albuginea, was used for the analysis of DNA, RNA and protein. Potassium hydroxide separated the RNA from the DNA and proteins.

Perchloric acid was then used to precipitate the DNA and protein fraction (Munro & Fleck, 1966).

The testis was homogenised in 4 ml of cold distilled water and 1 ml 1,2 N perchloric acid was added to the suspension, mixed and allowed to stand at 4°C for 15 min. Thereafter the suspension was centrifuged at 600 g for 2 min. The supernatant was discarded and the pellet washed twice with 3 ml of 0,2 N perchloric acid. The remaining precipitate was resuspended in 2 ml of 0,3 N potassium hydroxide and incubated at 37°C for 1 hour to dissolve the precipitate. Once this was accomplished, the test tubes containing the mixture were cooled on ice, and 2 ml of 0,4 N perchloric acid was added to precipitate the proteins and DNA. After allowing this to stand at 4°C for 15 min, the samples were centrifuged for 2 min at 200 g. The supernatant (RNA fraction) was collected and stored at 5°C until required for use.

The precipitate, which contained the DNA and protein fraction, was further treated with 2 ml of 0,3 N potassium hydroxide, incubated at 37°C for 15 min, cooled and then sonicated, after the addition of 4 ml of distilled water, to facilitate the break up of the precipitate.

2.2.5.1 Protein Analysis

The folin phenol reagent was used to measure the quantity of protein in testicular tissue after an alkaline copper treatment (Lowry et al, 1951).

Fifty µl of the DNA/protein fraction was added to appropriate

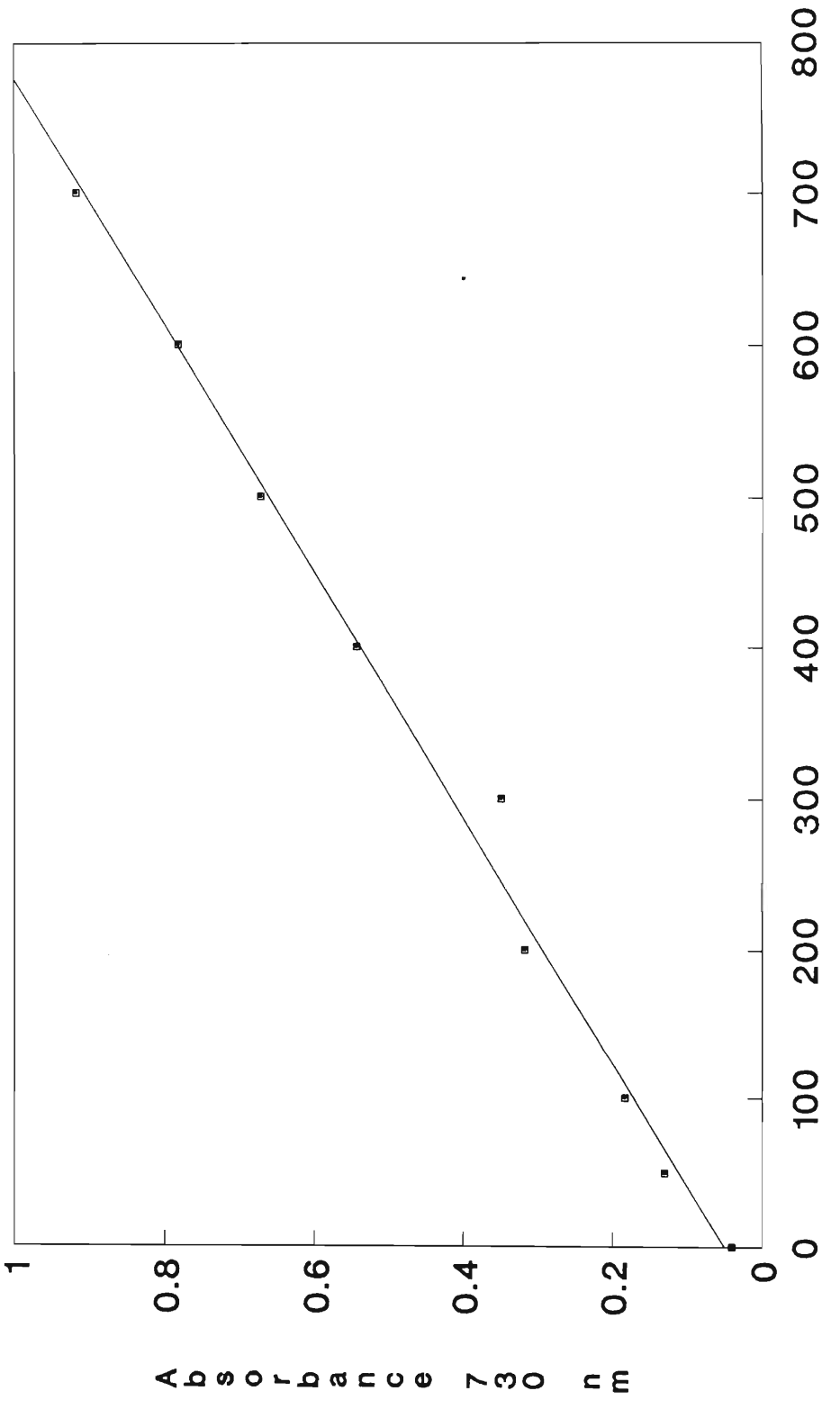

Fig.9 Protein Standard Curve

test tubes together with 1 ml of distilled water and 2 ml of carbonate reagent (Appendix 1). This was allowed to stand at room temperature for 10 min. After adding 0,1 ml of the folin reagent (Appendix 1), the test tubes were again allowed to incubate at room temperature for 30 min. The samples and standards were then read at 730 nm against air on a Beckman Spectrophotometer (Model 25).

The standard curve (Fig.9) was drawn from serial dilutions of a stock solution of 10 mg/10 ml bovine serum albumin (fraction 5, BDH) dissolved in 0,1 N potassium hydroxide.

A regression line was fitted to determine the value of y corresponding to a given x value. This relationship was expressed mathematically by the equation $y = bx + a$, where **a** represents the y-intercept and **b** represents the slope of a straight line graph. The calculated correlation coefficient r value was 0,885 with $y = 0,052 + 0,00122x$. The quantity of protein present in the testis was determined from this graph and expressed as ug of protein per testis.

The intra-assay coefficient of variation was 1,51 %.

2.2.5.2 RNA Analysis

The RNA fraction (0,5 ml) was added to 2,5 ml of 0,2 N perchloric acid and read against air on a spectrophotometer at 260 nm (Burton, 1956).

The RNA standard curve (Fig.10) was constructed from serial dilutions of a stock solution made up of yeast RNA (Boehringer Mannheim, Germany). The stock solution had a concentration of 5 mg of RNA/ 20 ml of 0,2 N perchloric acid. A regression line was

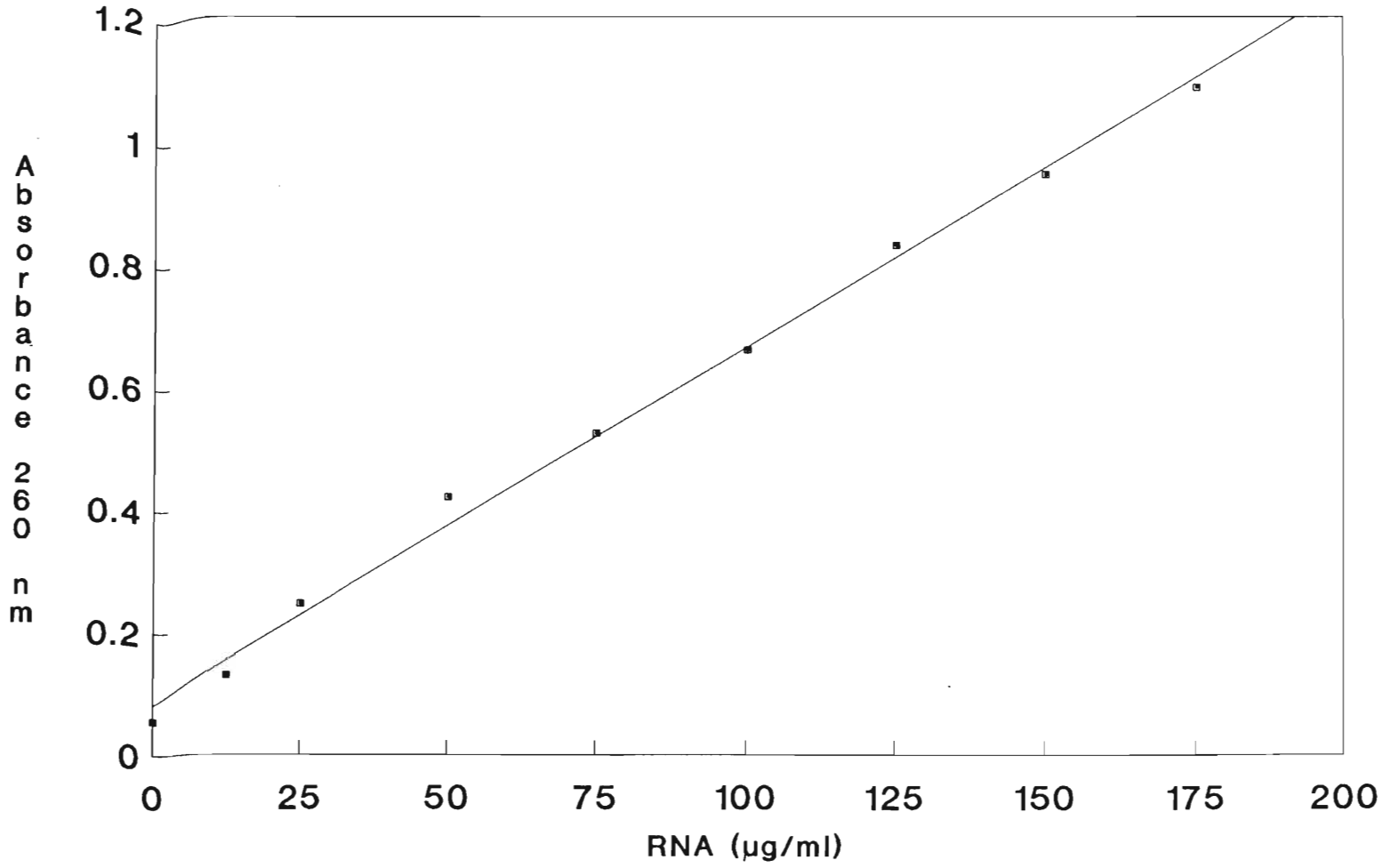

Fig.10 RNA Standard Curve

fitted to determine the standard
curve and the correlation coefficient r value was 0,888 with y = 0,0787 + 0,0058x. The sample values were expressed as ug of RNA per testis.
The intra-assay coefficient of variation was 0,48 %.

2.2.5.3 DNA Analysis

The quantity of DNA per testis was determined by evaluating the colorimetric reaction between DNA and diphenylamine (Burton, 1956).
Half a ml of the DNA/protein fraction was added to 0,5 ml of acetic acid and 2 ml of diphenylamine reagent (Appendix 1). The samples and the standards were allowed to stand overnight at 25-30°C. Thereafter they were read against air on a spectrophotometer at 600 nm. The stock solution of the standard consisted of 0,4 mg calf thymus DNA (Sigma, U.S.A) per ml 0,1 N potassium hydroxide. The working standard was prepared by mixing a measured volume of the stock solution with an equal volume of 70% $HClO_4$. All sample values were determined from the standard curve (Fig.11), which was drawn from serial dilutions of the stock solution. A regression line was fitted to determine the standard curve and the correlation coefficient r value was 0,875 with y = 0,705 + 0,00091x. The sample values were expressed as ug of DNA per testis.
The intra-assay coefficient of variation was 0,98 %.

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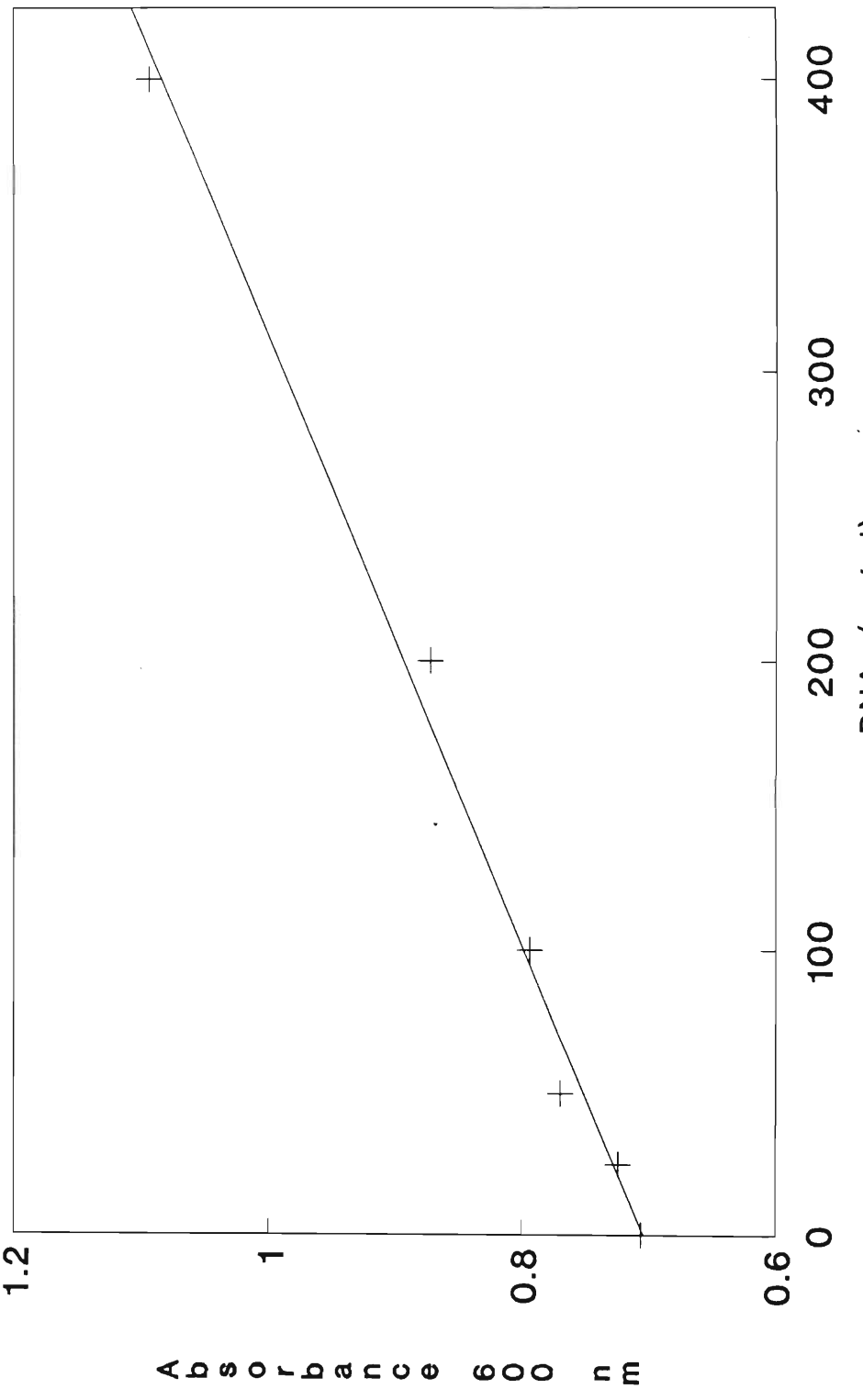

Fig. 11 DNA Standard Curve

2.2.5.4 Determination of testicular cell number and size.

The number and size of the cells contained within the testis were determined according to the method described by Enesco and Leblond (1962). This method was based on the constancy of the quantity of DNA per nucleus, and involved the following calculations:

$$\text{number of cells } (x10^6) = \frac{\text{mg testicular DNA x 1000}}{6,2}$$

where 6,2 is the DNA content (pg) of a diploid nucleus.

$$\text{cell size (ng)} = \frac{\text{testicular weight (g) x 1000}}{\text{number of cells in millions}}$$

2.2.7 Determination of Testosterone Concentration in Rat Plasma

The testosterone concentration in blood plasma was determined using a commercial radioimmunoassay kit (Diagnostic Systems Laboratories Inc. U.S.A). All reagents were allowed to reach room temperature, and thoroughly mixed by gentle inversion before use. The anti-testosterone IgG coated tubes were marked and 50 µl of the standards and samples were added to the appropriate tubes. Immediately thereafter 500 µl of the labelled testosterone was added and mixed. The tubes were incubated for 60 min at 37°C. The liquid in all the tubes, except the total counts, was decanted.

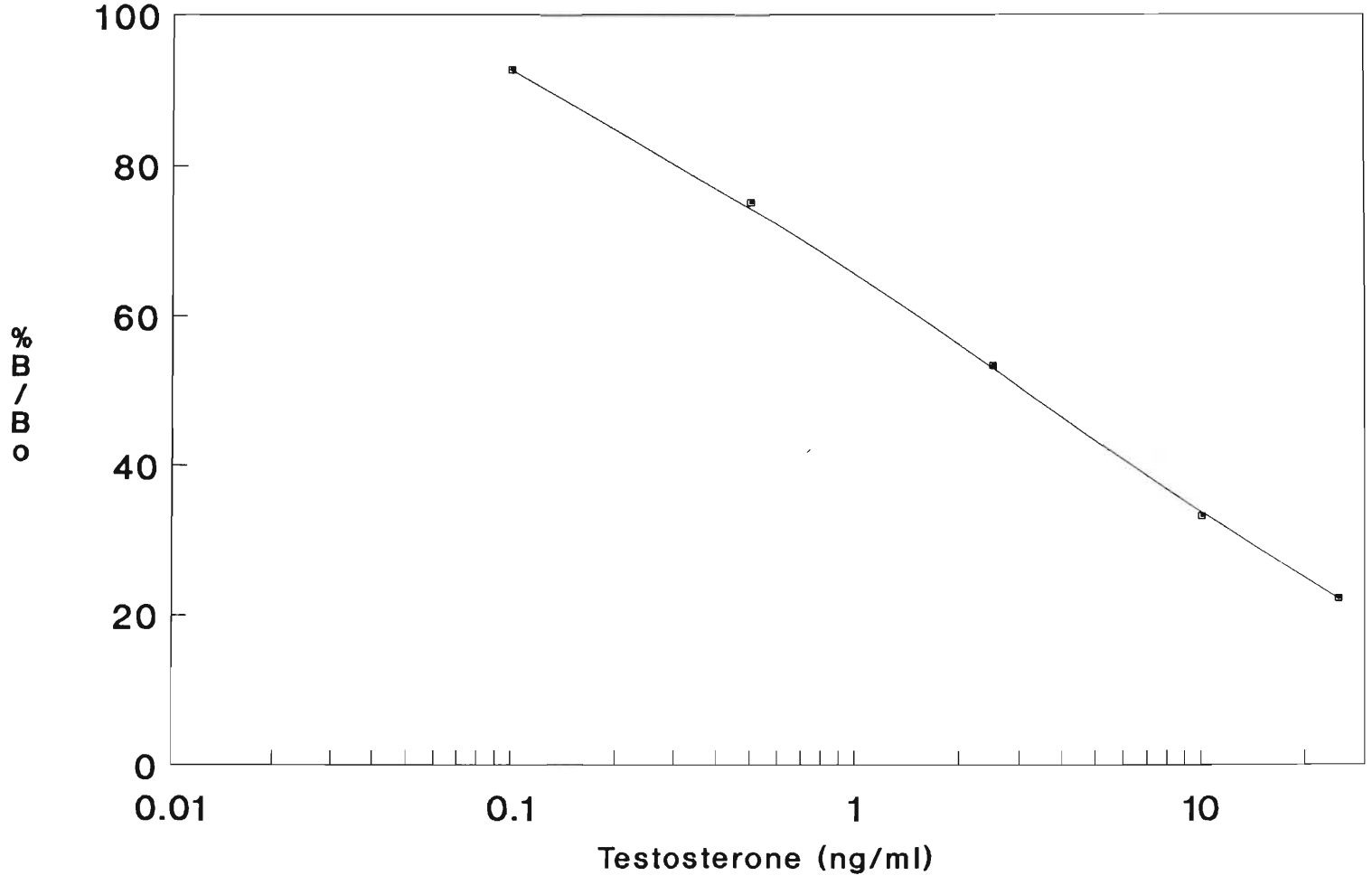

Fig.12 Testosterone Standard Curve

The tubes were counted for 1 min in a gamma counter (Riastar, Packard). The percentage of bound testosterone was calculated as follows:

$$= \frac{\text{average sample counts}}{\text{average total counts}} \times 100$$

From this the percentage B/B_0 was determined:

$$= \frac{\text{average counts}}{\text{average counts of the zero standard}} \times 100$$

B/B_0 was plotted against the testosterone standard concentration on a linear log graph paper. The testosterone concentration of the samples were determined from this standard curve (Fig.12). The intra-assay coefficient of variation was 5,5 %

2.3 STATISTICAL ANALYSIS

The experimental data were subjected to an analysis of variance (ANOVA) when more than 2 groups were compared. The least significant difference (LSD) test was applied to those values that were significantly different with the ANOVA. The students-t test was used when only 2 groups were compared.

2.4 EXPERIMENTAL DESIGN

Blood and semen samples were collected from twenty three diabetic, infertile and control human patients. Various characteristics of the sperm produced by these patients were assessed, and the biochemical status of the seminal plasma was determined. The blood samples were subjected to hormone analyses.

Fourteen male Wistar rats were divided into 2 groups of seven animals each. Diabetes was induced in the experimental group, with a single i.p. dose of 65 mg/kg body weight of streptozotocin. On three occasions, during the experimental period, the males were mated with normal females to determine their reproductive potential. After 90 days the animals were sacrificed and their tissues and organs sampled for analysis.

3. RESULTS

3.1 Human Diabetes

The results of this study indicate that diabetes has an adverse effect on some aspects of testicular function. Sperm motility, viability and testosterone levels were mostly affected (Tables 1 and 4). There was a significant difference in sperm motility between the diabetes and control samples. Sperm from diabetic patients exhibited a 35% decrease in gross motility ($p < 0,0005$), a 13% reduction in progressive motility ($p < 0,025$) and a 49% decrease in rapid motility ($p < 0,05$). There was also a 17% decrease in gross motility in the diabetes group when compared to the infertility group ($p < 0,05$).

The motility of sperm from the infertility group was significanlty different from that of the control samples. Gross motility was reduced by 21% ($p < 0,0005$) and progressive motility decreased by 11% ($p < 0,05$). Rapid motility in this group did not differ significantly from the control group (Table 1).

Sperm viability decreased by 31% in the diabetes group when compared to the control ($p < 0,0005$) and by 25% when compared to the infertility group ($p < 0,0005$). However sperm viability of the infertility samples was also 9% lower than the controls ($p < 0,025$) (Table 1).

Table 1. Seminal pH, volume, sperm number, motility and viability in human diabetics and in patients presenting for infertility.

	Group		
	Control	Infertility	Diabetes
	(mean ± s.e.m.)		
pH	7,79 ± 0,05	7,78 ± 0,06	7,76 ± 0,02
volume (ml)	2,50 ± 0,05	3,11 ± 0,33	2,30 ± 0,10$^{\#}$
Sperm number			
x 10^6/ml	8,89 ± 6,94	79,28 ± 13,76	96,92 ± 4,09
x 10^6/ ejaculate	169,65 ± 16,95	237,36 ± 47,74	187,56 ± 10,45
Motility			
gross (%)	67,36 ± 2,42	53,03 ± 3,13*	44,09 ± 0,68$^{*\#}$
progressive^{+}	4,74 ± 0,18	4,20 ± 0,22*	4,14 ± 0,18*
rapid (%)	1,85 ± 0,39	1,27 ± 0,38	0,94 ± 0,33*
Viability	78,26 ± 2,11	71,30 ± 2,24*	53,85 ± 1,00$^{*\#}$

\# significantly different from the infertility group
* significantly different from the control
\+ on a scale from 1 to 7
n = 23

The number of morphologically normal sperm in diabetics was similar to that of the control group. However, when compared to the control samples, the occurrance of mega headed sperm increased by 40% ($p < 0,05$) in the diabetes group.

Normal sperm morphology in diabetic patients was 49% higher than that of the infertile patients ($p < 0,01$; Table 2).

Table 2. Sperm morphology in diabetics and patients presenting for infertility.

Morphological Criteria (%)	Group		
	Control	Infertility	Diabetes
	(mean ± s.e.m.)		
Normal Sperm	24,09 ± 3,21	13,13 ± 1,93*	25,47 ± 1,63#
Head Abnormalities			
Amorphous	11,65 ± 1,68	16,39 ± 2,46*	12,29 ± 0,30
Mega	1,61 ± 0,24	1,78 ± 0,41	2,67 ± 0,06*#
Small	5,35 ± 0,93	5,57 ± 0,96	3,09 ± 0,15*#
Tapered	51,39 ± 3,75	54,70 ± 3,36	52,31 ± 2,21
Duplicate	0,17 ± 0,10	0,48 ± 0,29	0,27 ± 0,02
Neck Abnormalities	1,96 ± 0,43	2,31 ± 0,81	2,00 ± 0,15
Tail Abnormalities	1,26 ± 0,36	2,57 ± 0,57*	2,00 ± 0,14
Immature Sperm	1,35 ± 0,98	3,52 ± 0,98*	0,53 ± 0,04#

* significantly different from the control
\# significantly different from the infertility group
n = 23

A 46% decrease in the number of morphologically normal sperm in the infertility group was evident when compared to the control group ($p < 0,01$). The infertility samples displayed a 29% increase in the number of amorphous sperm heads ($p < 0,05$), a 51% rise in the number of tail abnormalities ($p < 0,05$), and an increase in the presence of immature sperm in the sample ($p < 0,05$) when compared to control samples. There was no significant difference in the number of tapered and duplicate sperm heads, and neck abnormalities amongst the three groups (Table 2).

Biochemical analyses of the semen indicated that some parameters in the diabetes samples were significantly different from the control and infertility group. The quantity of zinc in the semen of diabetic patients decreased by 30% compared to the infertility samples ($p < 0,05$) but was not significantly lower than the value obtained for the control group. Fructose levels were 23% higher in the diabetes group in comparison to the infertility samples ($p < 0,05$) which in turn were 27% lower than the control samples ($p < 0,025$). The level of acid phosphatase in diabetics was 13% higher than the control value ($p < 0,05$). The carnitine level also increased by 47% in the diabetes samples ($p < 0,025$) and by 38% in the infertility samples ($p < 0,01$) in comparison to the control group. Citric acid content of the diabetes semen was 32% higher than the infertility group ($p < 0,05$), but not significantly different from that of the control group (Table 3).

As anticipated, the level of glucose in blood plasma of the diabetes group was 67% higher than the infertility group ($p < 0,0005$) and 79% higher than in the control group ($p < 0,0005$). There was, however, an increase of 38% in the glucose level of the infertility samples compared to the control samples ($p < 0,025$).

Table 3. Seminal biochemistry in diabetics and patients presenting for infertility.

	Group		
	Control	Infertility	Diabetes
	(mean ± s.e.m.)		
Zinc (mmol/l)	2,61 ± 0,27	2,92 ± 0,27	2,06 ± 0,17#
Fructose (mmol/l)	8,17 ± 0,83	5,96 ± 0,70*	7,78 ± 0,11#
Acid phosphatase (U/ml)	55,89 ± 2,0	60,61 ± 6,38	63,95 ± 1,37*
Carnitine (µmol/l)	276,00 ± 3,96	447,52 ± 73,42*	519,00 ± 13,65*
Citric acid (mmol/l)	38,75 ± 4,40	30,63 ± 5,57	44,69 ± 0,96#

* significantly different from the control group
\# significantly different from the infertility group
n = 23

The level of circulating testosterone in the infertility and diabetes groups was significantly lower than that of the control group by 20% and 16% respectively ($p < 0,01$). The level of FSH was 29% higher in the diabetes samples than in the infertility group ($p < 0,05$), but did not differ significantly from the control group. LH levels were also higher (54%) in the diabetes samples when compared to the infertility samples ($p < 0,05$), but not significantly different from the control values. The quantity of LH in the infertility group was however, 28% lower than the value obtained for the control group ($p < 0,05$). Prolactin levels decreased by 25% in the diabetes group compared to the infertility group ($p < 0,05$), but neither differed significantly from the control group (Table 4).

Table 4. Biochemistry of blood plasma in diabetics and patients presenting for infertility.

	Group		
	Control	Infertility	Diabetes
	(mean ± s.e.m.)		
Glucose (mmol/l)	2,51 ± 0,47	3,88 ± 0,36*	11,80 ± 0,89*#
Testosterone (ng/ml)	5,45 ± 0,26	4,56 ± 0,25*	4,37 ± 0,35*
LH (mIU/ml)	11,42 ± 1,78	8,10 ± 0,80*	17,42 ± 4,33#
FSH (mIU/ml)	9,42 ± 1,00	8,87 ± 1,20	12,41 ± 2,11#
Prolactin (ng/ml)	7,16 ± 1,55	9,35 ± 1,09	6,99 ± 1,11#

* significantly different from the control group
\# significantly different from the infertility group
n = 23

As far as the general characteristics of semen were concerned, there was no significant difference between the pH of the samples obtained from all 3 groups, whilst the volume of the ejaculates from the diabetes group was 26% lower than the infertility group ($p < 0,05$) but not lower than that of the control group (Table 1).

Details of the macroscopic examination of the semen, which included the state of liquifaction, coagulation, colour, viscosity and debris, are listed in Appendix A3.3 and A3.4. It was not possible to determine whether the process of coagulation had occurred in those semen samples that were collected at home. However, all diabetic and control samples indicated some degree of liquifaction, either complete or incomplete. Four infertility samples did not

liquify. Semen produced by some patients were slightly contaminated with urine, as suggested by a yellow colouration of the sample. The changes in the colour, debris content and viscosity of the semen did not have any obvious effect on the various sperm characteristics investigated in those particular patients. The results of the MAR test are also recorded in Appendix A3.4. None of the diabetic patients exhibited a positive MAR test, whilst 1 infertile and 2 control patients indicated the presence of antibodies on the surface of the sperm. An abnormally high number of leucocytes was also not observed in any of the semen samples (Appendix A3.4).

The medical histories of patients are reported in Appendix A3.1 and A3.2. Normal patients did not indicate any history of infertility. Some patients in the infertility group did indicate a history of testicular or prostate dysfunction whilst others could not provide any obvious causes for infertility, other than their presence at the infertility clinic.

Ten of the 23 diabetic patients in this study were receiving insulin whilst the others controlled blood glucose levels with oral hypoglycaemic medication. Two patients were also on antihypertensive medication, 1 of whom was not able to produce a semen sample. The medical history of the other 7 patients who were not able to produce a sample, did not indicate any factor, other than diabetes, which could be responsible for their impotence. Four diabetic patients who complained of erectile

dysfunction were, however able to produce a semen sample for this study.

3.2 Streptozotocin-induced diabetes.

Streptozotocin-treated rats did suffer weight loss during the first week of diabetes, and weight gain was observed thereafter. Although this increase in weight was maintained throughout the experimental period, it was assumed that diabetes did have an adverse effect on body weight. The difference between the mean body weights of the two groups, at the end of the experimental period, was greater than the difference between the mean body weights before streptozotocin administration, hence the adverse effect of diabetes on body mass (Fig. 13).

The total number of pups sired by diabetic rats was lower than that of the control rats (Table 5). The animals were housed with females 26, 57 and 85 days after diabetes induction. The number of pups sired thereafter were 55%, 63% and 58% lower than those sired by control animals. The weights of the pups were not adversely affected, and none of the pups were stillborn or had any gross morphological abnormalities.

Examination of the epididymal sperm indicated that the proportion of motile sperm decreased by 39% in diabetic rats ($p < 0,01$), and the number of sperm per cauda was also reduced significantly by 59% ($p < 0,005$). The number

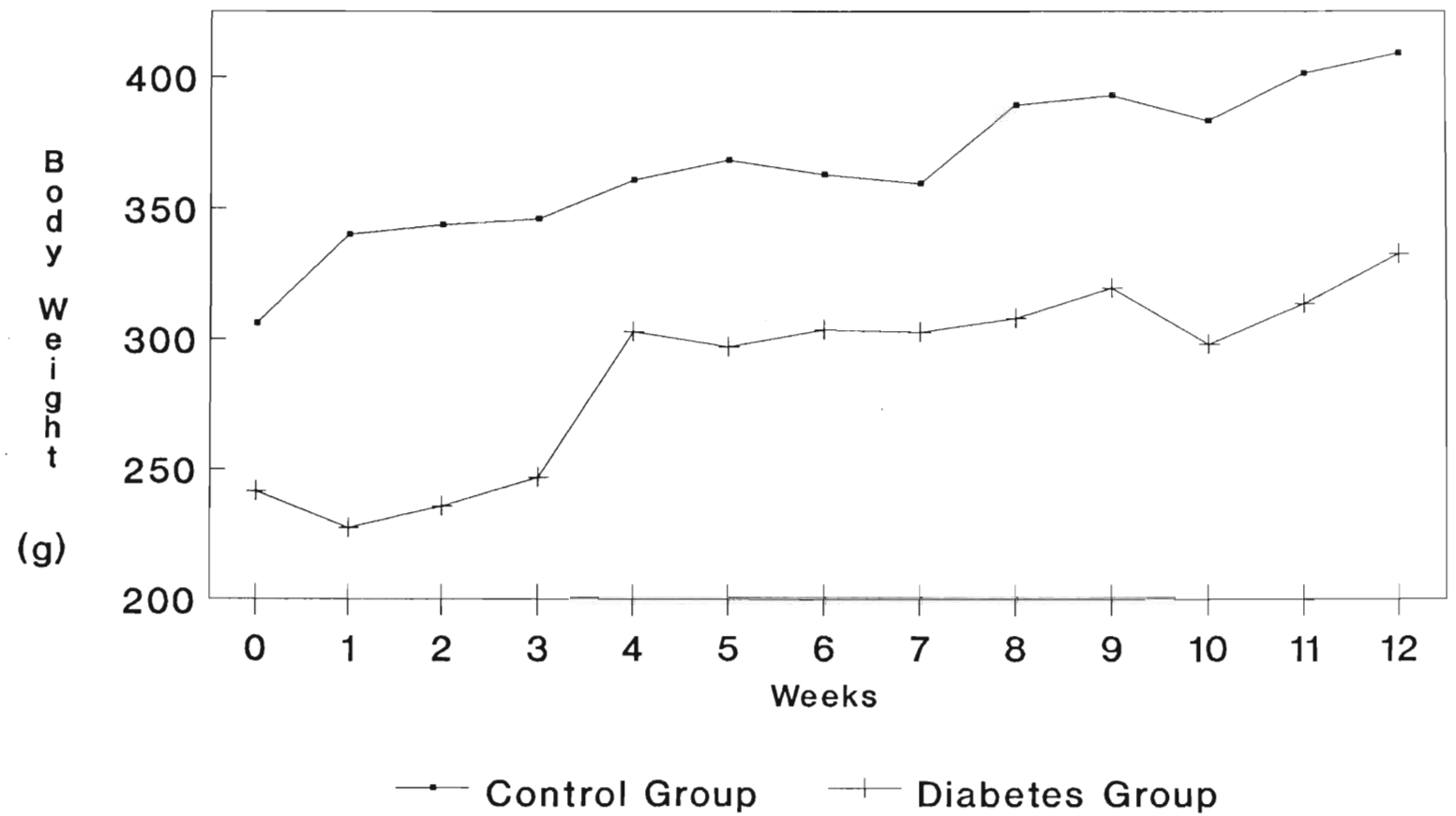

Fig.13 Changes in body weight
in control and diabetic rats.

of viable sperm decreased in the diabetes group by 19% ($p < 0,005$), whilst the proportion of morphologically abnormal sperm increased by 86% ($p < 0,01$; Table 6).

Diabetes did not reduce testicular organ weight significantly. There was also no significant difference in testicular cell size or number (Table 7).

Table 5. Fertility tests in streptozotocin-treated rats.

Group	Duration of Diabetes (days)	Total No. of Pups Sired	No. Stillborn	No. Abnormal	Weight of Pups (g)
		(mean ± s.e.m.)			
Control	0	62	0	0	6,35 ± 0,10
Diabetes	26	28*	0	0	6,30 ± 0,40
Control	0	57	0	0	7,19 ± 0,15
Diabetes	57	21*	0	0	6,70 ± 0,08
Control	0	69	0	0	6,45 ± 0,06
Diabetes	85	29*	0	0	5,99 ± 0,91

* significantly different from the control group
n = 7

Table 6. Epididymal sperm motility, number, viability, and morphology in streptozotocin-treated rats.

Group	Sperm Motility (%)	Sperm No. (million/cauda)	Sperm Viability (%)	Sperm Morphology (% abnormal)
		(mean ± s.e.m.)		
Control	79,13±1,86	162,71±11,34	91,86±1,50	1,86±0,40
Diabetes	48,41±9,63*	66,71±27,46*	74,00±4,24*	13,43±4,20*

* significantly different from the control group
n = 7

Table 7. Testicular weight, cell size and number in streptozotocin-treated rats.

Group	Testis Weight (as a % of body weight)	Cell Number (million)	Cell Size (ng)
	(mean ± s.e.m.)		
Control	0,41 ± 0,02	27,53 ± 1,81	57,85 ± 5,43
Diabetes	0,41 ± 0,03	24,42 ± 3,87	55,80 ± 5,16

n = 7

The weight of the seminal vesicle and epididymis in diabetic rats did not differ significantly from the control. The prostate indicated a weight loss of 44% ($p < 0,025$; Table 8).

The quantity of circulating testosterone and testicular DNA, RNA and protein content in streptozotocin-treated rats did not differ significantly from that of control rats (Table 9).

Table 8. Organ weights in streptozotocin-treated rats expressed as a percentage of final body weight.

Group	Epididymis	Prostate	Seminal Vesicle
		(mean ± s.e.m.)	
Control	0,18 ± 0,01	0,13 ± 0,01	0,22 ± 0,02
Diabetes	0,15 ± 0,01	0,09 ± 0,01*	0,15 ± 0,04

* significantly different from the control group
n = 7

Table 9. Testosterone, nucleic acid and protein levels in streptozotocin-treated rats.

Group	Testosterone (ng/ml)	DNA (as a % of testicular weight)	RNA (as a % of testicular weight)	Protein (as a % of testicular weight)
		(mean ± s.e.m.)		
Control	2,45±0,87	0,012±0,001	0,002±0,001	0,023±0,001
Diabetes	1,95±0,56	0,011±0,001	0,003±0,002	0,028±0,003

n = 7

4. DISCUSSION

The results obtained in this study indicate that diabetes mellitus has adverse effects on the male reproductive system. In human patients, sperm motility and viability were lowered whilst the concentration of seminal carnitine, acid phosphatase and testosterone differed from the control group. Streptozotocin-treated animals demonstrated severe deterioration of sperm quality, loss of accessory organ weight and reduced fecundity.

4.1 Human Diabetes

Semen is a complex mixture of products from the testis, epididymis and accessory glands. Since the constituents of seminal plasma was being produced continuously the period of sexual abstinence had to be standardised to ensure accurate interpretation of results. However some diabetics complained of impotence for long periods of time, therefore sexual abstinence was greater than 3 days in these cases, whilst others could not produce a semen sample for this investigation.

Semen, which is ejaculated in a liquid form, coagulates immediately, only to liquify approximately 30 minutes later (Amelar, 1962). Upon receipt of the semen sample, an attempt was made to establish whether the processes of coagulation or liquifaction had occurred, but this was not possible

since a large number of the samples were not produced at the laboratory. Although these processes are not critical in themselves in determining fertility, they do serve as indicators of the functioning of the seminal vesicles and prostate. Fructose, which is required for the process of coagulation is synthesised and secreted by the seminal vesicles, whilst the enzymes required for liquifaction are produced by the prostate (Amelar, 1962). The absence of liquifaction was observed in 4 infertile patients thus suggesting prostate dysfunction in these patients.

The pH values obtained for all semen samples fell within the normal range of 6,8 and 8,8 which is optimal for sperm survival (Mandal and Bhattacharyya, 1985).

The colour of the semen was also noted. Semen is normally greyish white in colour (Mortimer, 1985), but some samples obtained were yellow, suggesting contamination with urine. Since the volume of semen in these cases was not unusually high and the pH did not differ from the normal range, it is assumed that this contamination was not excessive and therefore did not have an adverse effect on sperm.

Debris present in semen was usually in the form of epithelial cells and streaks of mucus which is suggested to be patches of unliquified semen. A high concentration of debris would be expected to hinder the forward progression of sperm. Two diabetic and 2 control patients exhibited a high debris content in the semen. However, this did not seem

to have an adverse effect on sperm motility, since rapid and progressive sperm motility, in these individuals, was equal to, or above their group average.

Of 15 patients, from the 3 experimental groups, who produced highly viscous semen, 2 diabetic and 2 infertile patients indicated gross sperm motility below 50%, progressive motility ranging from slow and sluggish to none at all and 0% rapid motility. This highly viscous medium could be partly responsible for the reduced sperm motility displayed in these particular cases.

None of the diabetic samples displayed a positive mixed antiglobulin reaction (MAR) test. Therefore it can be assumed that antisperm antibodies were not responsible for poor sperm motility in this group, although a high incidence of antibodies can reduce motility (Etribi et al, 1982). One infertile patient and 2 control patients indicated the presence of antibodies on the surface of the spermatozoa. However sperm motility was not adversely affected in these patients.

Semen was scanned for the presence of an excessive number of leucocytes, which is indicative of an infection in the male reproductive tract. The results of these observations indicated a normal number of leucocytes in all experimental groups, hence any adverse effect on sexual function could not be the result of infection affecting the epididymis or accessory glands.

Since the volume of seminal plasma is not regulated by homeostatic mechanisms, but depends on the secretory output of the accessory glands and the time interval between ejaculations, the concentration of sperm in the seminal plasma was not only expressed as number per ml but also as number of sperm per ejaculate. However, the volume of semen and sperm concentration in the diabetes and infertility groups did not differ significantly from the control group.

Gross motility was adversely affected in the diabetes and infertility groups. Since all immotile spermatozoa are not necessarily dead, the number of live sperm is expected to exceed the number of motile sperm. However, sperm viability was also significantly reduced in both these groups. The number of morphologically normal sperm in the diabetes group suggests that sperm were probably able to develop normally in the testis, but sperm motility was not fully established in the epididymis and the ability of sperm to survive was also reduced. The decrease in the number of morphologically normal sperm, reduced sperm motility and viability in the infertility group, on the other hand, could suggest disturbances in spermatogenesis together with epididymal dysfunction.

A significantly large number of sperm with mega heads, was evident amongst diabetic patients. This could signify an abnormal nuclear condensation process in the epididymis (Calvin & Bedford, 1971). The infertility group indicated a

large percentage of immature sperm, amorphous sperm heads and tail defects. These types of abnormalities are indicative of low fertilising potential.

To find possible explanations for the poorer sperm profiles discribed above, circulating hormone levels and the biochemistry of the seminal plasma have to be considered. FSH levels in the diabetes and infertility groups were not significantly different from that of the control group. This could provide an explanation for the similarity in sperm concentration in the 3 groups. FSH is known to be responsible for the initiation of spermatogenesis, and the levels of FSH detected in these groups were sufficient to stimulate the appropriate number of spermatogonia to divide and produce a normal sperm concentration.

LH levels in the infertility group were lower than those of the control group. Since LH is responsible for the stimulation of testosterone production by the Leydig cells, it is assumed that the reduced LH level was responsible for the low circulating testosterone concentration detected in this group. This in turn could explain the reduction in morphologically normal sperm. If steroidogenesis is impaired as a result of low LH levels, then testosterone levels within the testis must also be low. Therefore normal spermatogenesis could not be maintained.
The low circulating testosterone concentration detected in the diabetes group was not a result of low LH levels. Since the number of morphologically normal sperm produced in this

group was similar to the control group, this suggests that testosterone within the testis was sufficient to support normal spermatogenesis. Low circulating testosterone levels could be a result of thickening of blood vessel walls often found in diabetic patients (Cameron et al, 1985), hence altered capillary function preventing the absorption of the hormone into the blood. If this had occurred, then it should be expected that circulating LH and FSH levels would be significantly higher in the diabetes group than the control group. If the functioning of the testicular capillaries had been compromised then these gonadotrophins would not be able to enter the testis in sufficient quantities to stimulate the production of substances required for their negative feedback control. In this study however, circulating LH and FSH levels in the diabetes group were significantly higher than the infertility group, and not significantly different than the control. On the basis of this finding, one would normally disregard the explanation given above for the low circulating testosterone levels in diabetic patients. However, the high LH and FSH levels found in these patients, although not significantly higher than the control, is difficult to ignore and warrants further study.

It is also important to note that only 10 of the 23 diabetic patients investigated in this study were on insulin therapy. Insulin is known to restore testosterone levels to normal in diabetic rats (Paz & Homonnai, 1979b) by promoting the absorption of glucose by the testis, where it is metabolised as a source of energy, thereby supporting testicular

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function. Four of the patients receiving insulin were not able to produce a semen sample for this study and 3 of these had low testosterone levels, this in turn could be responsible for an erectile and/or ejaculatory disorder. The other 6 patients receiving insulin produced semen samples and their testosterone levels were similar to the average value of the control patients. Therefore it is possible that insulin was responsible for restoring testosterone levels to normal in these patients.

Prolactin levels in the diabetes group was significantly lower than that of the infertility group. Prolactin has been demonstrated to increase the activity of 17-α -hydroxysteroid for the conversion of androstenedione to testosterone (Musto et al, 1972), therefore low prolactin levels will be associated with low testosterone levels. Increased prolactin secretion by the infertility group however, could also be responsible for decreased testosterone production by the Leydig cells. The mechanism by which this hormone mediates these stimulatory and inhibitory effects is unclear (de Krester, 1984).

Since testosterone is known to maintain accessory sex organ structure and function, the low circulating levels of this hormone could have had some effect on the synthesising and secretory capacity of the epididymis, prostate and seminal vesicle.

The concentration of zinc in seminal plasma was lower in the diabetes samples compared to the infertility group. Clademone and co-workers (1979) observed that only a slight increase in seminal zinc was required to increase sperm motility significantly. Testosterone levels also increase with zinc therapy (Netter et al, 1981). Since sperm motility and testosterone concentrations are two obvious factors adversely affected by diabetes, further study should be centred on zinc and the onset of infertility in diabetic patients.

Fructose levels in diabetic patients were similar to those of control patients. This normal fructose level is expected since there would not be a lack of the fructose precursor (i.e. glucose), in a diabetic patient. If a reduction in fructose concentration occurred, then it would probably be due to decreased synthetic and secretory capacity of the seminal vesicles.

Fructose levels were significantly lower in the infertility group. This could probably explain the reduced sperm motility in this group. Sperm utilise fructose as an energy source after ejaculation (Mann & Lutwak-Mann, 1981), hence a reduced fructose level could be partially responsible for reduced sperm motility. The low testosterone levels of this group could prevent the optimum output of fructose by the seminal vesicles. Although Moon and co-workers (1970) suggest that there is no correlation between seminal fructose and plasma testosterone, they do not explain how the seminal vesicle will continue to function optimally in

the presence of lowered testosterone levels. The blood glucose level in the infertility group was also higher than in the control group, hence an abundance of substrate for the production of fructose. Therefore the low fructose concentration in the seminal plasma suggests seminal vesicle dysfunction.

The concentration of seminal acid phosphatase in diabetic patients was significantly higher than in the controls. Since testosterone is known to stimulate acid phosphate release from the prostate (Mann & Lutwak-Mann, 1981), a high concentration of the enzyme should not be expected since these patients have low circulating testosterone levels. This high concentration of acid phoshatase could be due to the stimulatory action of dihydrotestosterone in the prostate. Testosterone stored in the prostate is metabolised to dihydrotestosterone, which is more active than testosterone (Dondero et al, 1972). Even under these conditions an abundant secretion of acid phosphatase would not be expected. It is possible that those patients with abnormally high concentrations of acid phosphatase, may have prostatic cancer. Excessive production of this enzyme by the prostate is often associated with prostatic carcinoma (Mann & Lutwak-Mann, 1981).

The level of citric acid in the infertility and diabetes groups was not significantly different from the control level. This normality could again be explained by dihydrotestosterone activity in the prostate. The diabetes

group did however indicate a significanlty higher concentration of citric acid compared to the infertility group. Adverse effects of high concentrations of citric acid are at present unclear. Since citric acid is utilised as an energy source in the absence of carbohydrates (Mann & Lutwak-Mann, 1981), it is unlikely that, in large quantities, citric acid would be detrimental to sperm.

The level of carnitine in the seminal plasma of diabetic patients was much higher than the value obtained for normal samples. As previously reported, the length of sexual abstinence in diabetic patients was often greater than that of normal subjects in this study. This could explain the excessive accumulation of carnitine in the ejaculate (Soufir et al, 1984). The quantity of carnitine was also significanly higher in the infertility specimens. Since both the diabetes and infertility groups indicated reduced motility, it can be assumed that the concentration of carnitine was responsible for the quiescent nature of the sperm (Hinton et al, 1981). However, the viability studies indicate otherwise. Almost fifty percent of the sperm obtained from diabetics were dead. The above explanation may only be acceptable for the infertility group, where the number of live sperm, although significantly lower than the control value, constituted approximately 70% of the sperm population. Since both the diabetes and infertility groups have indicated reduced sperm viability it could be possible that the abnormally high carnitine levels are responsible for the death of the sperm.

4.2 Streptozotocin-induced Diabetes

It should be noted that although indicating high glucose levels after streptozotocin administration, two rats, in this study, later displayed glucose levels which paralled those of the controls, hence insulin was not administered to them.

Diabetes did have an adverse effect on body weight in streptozotocin-treated rats. Although these animals reflected weight gain similar to the control rats, the difference between the mean of the final body masses was greater than the differences in weight at the begining of the experimental period. The wasting away of muscle and fat, usually displayed by uncontrolled diabetes, was counteracted to a limited degree by insulin therapy.

The abnormally thickened basal membrane of the seminiferous tubule, in diabetic rats (Karl & Griswold, 1980), could explain the decline in sperm number and normal sperm morphology obtained in this part of the study. This structural alteration of the seminiferous tubule could reduce permeability of the tubule wall thereby disallowing the seminiferous epithelium access to essential substances required for the initiation and maintenance of spermatogenesis. A decrease in sperm viability and motility was also observed in diabetic rats, suggesting epididymal dysfunction. This reduction in sperm quality was further reflected in the fertility tests.

There was a significant decrease in the number of pups sired by streptozotocin-treated rats. There were however, no differences in the weights of the pups, nor were there any still births or gross morphological abnormalties. The animals were allowed to mate on three occasions, spanning the entire experimental period, to establish if there was any progressive loss in fertility. Three diabetic individuals did not produce any pups at all, whilst additionally only one did not sire pups during the second and third mating trials. Those with normal glucose levels produced young, whereas only one animal which continually displayed high blood glucose concentrations sired pups on all occasions. Therefore it could possibly be suggested that poor glycaemic control was responsible for their lack of response toward the female and/or diabetes related infertility resulted in the lack of offspring.

Testosterone levels in diabetic rats were not significantly lower than that of the control rats. A possible explanation why this was noted in rats but not in humans, is the action of insulin. Since diabetic rats were being maintained for a long period of time, they had to be treated with insulin to prevent their untimely demise, and insulin is known to restore testosterone levels to normal in diabetic rats (Paz & Homonnai, 1979b). As mentioned before, only 10 of the 23 human diabetic patients investigated were receiving insulin.

The prostate, in streptozotocin-treated rats, indicated a reduction in weight. This weight reduction could imply a decrease in the synthesising and secretory capacity of this organ, which could result in the reduced sperm motility and viability observed in this group. As discussed earlier in 4.1, this accessory organ is responsible for producing and/or secreting a variety of substances that maintain a suitable environment for the survival of the sperm. The weight of the testis, epididymis and seminal vesicle however, was not adversely affected by the induction of diabetes. Insulin received by the diabetic rats throughout the course of the experiment could be responsible for reversing the detrimental effects of diabetes on these organs but this action did not extend to the prostate. The number and size of the cells within the testis also indicated no significant difference between the two experimental groups.

Testicular DNA, RNA and protein contents were similar in both the diabetes and control groups. Santti and Johnsson (1973) reported that in culture, insulin facilitated the synthesis of RNA and protein by the incorporation of their precursors into the prostate. Similarly it can be assumed that insulin together with testosterone was able to maintain normal protein and nucleic acid levels in the testis.

5. CONCLUSION

It appears that the sexual problems encountered by diabetic men are more varied and complex than what reports of impotence and ejaculatory disorders would lead one to believe. Neurologic, vascular and psychologic disturbances have been implicated (Crowe et al, 1983; McCulloch et al, 1986; Carlin, 1988). These are thought to be compounded, in some cases, by medication administered for hypertension (Newman & Marcus, 1985).

The results of this study indicate that diabetes, in some cases, affects the male reproductive system in a manner similar to that occuring in infertile men. The low sperm motility, viability and testosterone levels, and the abnormally high carnitine concentration indicate reproductive dysfunction in the human diabetics. These factors including a reduced number of morphologically normal sperm, lowered seminal fructose and a high LH levels comprised the infertility profile.

The endocrine profile of the diabetic male requires further investigation, perhaps with a larger diabetic population and age matched controls. Although negative age related changes in sperm and hormone profiles have been reported in men over the age of fifty only (Blackman, 1989) and therefore age should not affect the data in the present study to a large extent, it should be borne in mind that the differences between the average age of the control group (27), the

infertile group (34) and the diabetic group (42) is significant. Some controversy does exist regarding age related differences in testosterone levels. Testosterone levels have been reported to decrease in men over the age of fifty (Pirke & Doerr, 1975), whilst other reports indicate no age related difference in circulating testosterone (Harman & Tsitouras, 1980). However age related alterations in seminiferous tubule histology have been demonstrated in men beyond the age of fifty (Blackman, 1989). These changes include the increased thickness of the basement membrane, patchy impairment of spermatic maturation with accompanying immaturity or degeneration of germ cells. Semen analysis in these men have revealed a relative preservation of sperm numbers but decreased sperm motility and increased numbers of abnormal forms.

Variables such as the quality of metabolic control and medication should also be considered in greater detail. As observed in the streptozotocin-treated animals, insulin controls the symptoms of diabetes but not the development of long term complications which could eventually result in infertility.

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7. APPENDIX 1: REAGENTS

7.1 Bouin Hollande Fixative

Stock solution :

25 g copper sulphate

40 g picric acid

100 ml of 40% formaldehyde

1000 ml double distilled water

1,5 ml glacial acetic acid added to 100 ml stock before use

7.2 Folin Reagent

1 ml folin + 3 ml distilled water

7.3 Diphenylamine Reagent

1,5 g diphenylamine

100 ml glacial acetic acid

1,5 ml concentrated sulphuric acid

0,1 ml acetaldehyde added to 20 ml stock before use

7.4 Carbonate Reagent

Solution A

2 g sodium carbonate

400 mg sodium hydroxide

100 ml distilled water

Solution C

1 g copper sulphate

100 ml distilled water

Solution D

2 g sodium or potassium tartrate

100 ml distilled water

1 ml of C added to 1 ml of D before use = B

100 ml of A added to 2 ml of B at the time of use

8. APPENDIX 2: DATA: WISTAR RATS

TABLE A2.1 BLOOD GLUCOSE LEVELS IN STREPTOZOTOCIN-TREATED RATS.

	Weekly readings of blood glucose levels (mmol/l)												
RAT No.	0*	1	2	3	4	5	6	7	8	9	10	11	12
CONTROL GROUP													
R6/90	4.0	4.8	3.6	4.7	5.3	5.4	4.9	3.2	6.0	6.1	5.2	5.6	4.5
R11/90	4.1	4.6	4.7	6.0	6.5	5.6	4.0	7.7	6.7	5.6	5.8	5.2	4.8
R13/90	3.4	5.5	4.5	5.0	4.8	4.2	3.1	4.3	4.4	5.4	4.9	5.1	5.4
R16/90	3.2	5.8	3.4	4.9	5.8	3.9	4.0	4.2	5.0	5.3	5.5	4.6	5.8
R17/90	2.8	6.1	3.5	5.0	4.8	4.1	4.6	4.2	5.8	6.1	5.7	3.5	4.1
R19/90	3.0	5.4	3.3	5.2	5.6	3.6	3.9	4.8	4.9	5.5	4.6	6.0	4.8
R20/90	4.2	5.4	3.9	4.6	5.7	4.3	4.4	7.8	7.4	6.2	6.0	5.0	6.3
DIABETES GROUP													
R2/90	4.0	16.9	13.7	11.2	*4.4	16.8	18.1	16.1	15.9	13.2	15.4	15.1	13.6
R3/90	3.5	13.5	7.3	6.2	5.9	7.0	7.2	5.3	5.5	6.5	5.9	4.6	5.5
R7/90	3.6	22.0	20.7	14.0	18.1	14.0	14.0	20.9	℞	22.0	14.0	14.0	℞
R8/90	3.6	12.7	11.0	14.0	℞	14.0	14.0	21.2	℞	16.7	21.4	14.0	21.8
R9/90	4.5	22.0	17.8	14.0	14.0	14.0	14.0	22.0	18.7	22.0	14.0	℞	18.9
R10/90	4.4	22.0	19.8	14.0	19.1	14.0	14.0	14.0	20.8	22.0	14.0	19.6	14.0
R15/90	4.6	9.3	5.5	4.8	3.4	13.5	5.2	5.1	5.6	6.7	7.4	5.7	6.0

* - fasting level

℞ - tail nicks, during these weeks, were unadvisable and they were allowed to heal before further testing.

TABLE A2.2 BODY WEIGHT OF STREPTOZOTOCIN-TREATED RATS.

	Weekly measurements of body weight (g)										
RAT No.	0*	1	2	3	4	5	6	7	8	9	10
CONTROL GROUP											
R6/90	316	345	346	350	359	360	357	356	386	388	409
R11/90	321	361	357	357	385	390	384	380	417	421	423
R13/90	309	335	343	340	359	372	365	360	388	402	408
R16/90	281	326	331	330	349	350	352	352	363	370	369
R17/90	314	347	342	350	368	373	370	362	395	397	397
R19/90	285	310	327	335	316	329	320	311	339	341	338
R20/90	316	357	360	361	391	405	392	395	438	433	340
DIABETES GROUP											
R2/90	242	227	236	247	328	318	324	325	338	340	242
R3/90	288	309	314	327	338	269	341	338	363	370	334
R7/90	277	254	261	276	278	270	273	274	287	272	294
R8/90	270	205	230	256	268	278	279	277	276	282	282
R9/90	292	242	262	276	274	256	276	280	270	291	271
R10/90	281	261	255	263	258	371	260	255	221	261	243
R15/90	283	321	329	331	377	318	373	370	401	422	420

* - weight on day 1

TABLE A2.3 ORGAN WEIGHTS IN STREPTOZOTOCIN-TREATED RATS

CONTROL GROUP (rat no.)	RIGHT TESTIS (mg)	EPIDIDYMIS (mg)	PROSTATE (mg)	SEMINAL VESICLES (mg)
R6/90	1828	728	653	972
R11/90	1671	722	552	954
R13/90	1707	696	520	1056
R16/90	1796	880	572	518
R17/90	1695	735	602	943
R19/90	1504	600	392	857
R20/90	1428	657	534	902
DIABETES GROUP				
R2/90	1593	636	396	997
R3/90	1585	640	574	635
R7/90	1488	520	274	331
R8/90	823	445	270	576
R9/90	1263	362	133	117
R10/90	944	294	116	103
R15/90	1983	778	534	965

TABLE A2.4 TESTICULAR CELL SIZE AND NUMBER IN STREPTOZOTOCIN-TREATED RATS

CONTROL GROUP (rat no.)	LEFT TESTIS - TUNICA ALBUGINEA (mg)	CELL SIZE (ng)	CELL NUMBER (million)
R6/90	1682	74.49	22.58
R11/90	1591	78.92	20.16
R13/90	1629	50.50	32.26
R16/90	1607	55.36	29.03
R17/90	1611	52.56	30.65
R19/90	1367	47.09	29.03
R20/90	1337	46.06	29.03
DIABETES GROUP			
R2/90	1509	42.53	35.48
R3/90	1497	66.30	22.58
R7/90	1359	44.34	30.65
R8/90	634	78.56	8.07
R9/90	1222	50.52	24.19
R10/90	876	49.38	17.74
R15/90	1837	56.95	32.26

TABLE A2.5 ANALYSIS OF CAUDA EPIDIDYMAL SPERM IN STREPTOZOTOCIN-TREATED RATS

CONTROL GROUP (rat no.)	SPERM MOTILITY (%)	SPERM NUMBER (million /cauda)	SPERM VIABLILITY (%)	SPERM MORPHOLOGY (% abnormal)
R6/90	80.0	139	93	1
R11/90	74.2	138	96	1
R13/90	82.9	199	98	3
R16/90	74.8	131	89	1
R17/90	80.7	206	87	3
R19/90	86.3	159	90	3
R20/90	75.0	167	90	1
DIABETES GROUP				
R2/90	43.6	50	74	8
R3/90	81.3	137	80	3
R7/90	33.7	30	64	20
R8/90	27.0	6	78	35
R9/90	29.5	24	63	11
R10/90	35.5	21	65	13
R15/90	88.3	199	94	4

TABLE A2.6 BIOCHEMICAL AND HORMONE ANALYSES IN STREPTOZOTOCIN-TREATED RATS

CONTROL GROUP (rat no.)	TESTOSTERONE (ng/ml)	DNA (µg/testis)	RNA (µg/testis)	PROTEIN (µg/testis)
R6/90	4.40	140.00	27.50	415.00
R11/90	6.80	125.00	22.50	310.00
R13/90	1.80	200.00	33.75	360.00
R16/90	0.69	180.00	27.50	415.00
R17/90	0.85	190.00	62.50	360.00
R19/90	1.50	180.00	22.50	430.00
R20/90	1.10	180.00	40.00	325.00
DIABETES GROUP				
R2/90	3.80	220.00	22.50	370.00
R3/90	4.00	140.00	24.00	420.00
R7/90	1.20	190.00	20.00	390.00
R8/90	2.00	50.00	103.75	345.00
R9/90	0.13	150.00	12.50	330.00
R10/90	0.69	110.00	8.13	320.00
R15/90	1.80	200.00	38.75	415.00

TABLE A2.7 FERTILITY TESTS IN STREPTOZOTOCIN-TREATED RATS.
(females introduced 26 days after diabetes was induced)

CONTROL GROUP (rat no.)	No. OF PUPS SIRED	ABNORMALITIES	WEIGHT g	STILL BIRTHS
R6/90	9	0	6.00 6.20 5.10 6.00 6.30 6.10 6.40 5.20 5.30	0
R11/90	9	0	5.90 6.40 6.70 6.00 6.50 6.40 6.60 6.20 5.20	0
R13/90	13	0	5.10 6.20 6.00 6.50 6.00 5.80 6.20 6.10 6.70 7.00 6.40 7.10 6.60	0
R16/90	12	0	5.80 5.40 6.70 6.90 7.00 7.00 7.20 6.50 6.70 6.80 7.30 7.20	0

Table A2.7 continued.

RAT No.	No. OF PUPS SIRED	ABNORMALITIES	WEIGHT g	STILL BIRTHS
R17/90	3	0	7.30 8.10 9.00	0
R19/90	4	0	6.40 6.20 6.00 6.00	0
R20/90	12	0	6.20 6.00 4.10 7.10 5.20 6.70 6.20 6.40 6.30 6.30 7.00 6.30	0
DIABETES GROUP				
R2/90	9	0	7.30 7.50 7.30 7.30 7.00 6.80 8.00 6.80 7.50	0
R3/90	7	0	5.70 6.00 5.90 5.70 6.10 5.70 5.60	0
R7/90	12	0	5.90 6.40 5.70 6.20 5.70 6.20	0

Table A2.7 continued.

RAT No.	No. OF PUPS SIRED	ABNORMALITIES	WEIGHT g	STILL BIRTHS
			5.90	
			4.00	
			5.90	
			6.00	
			5.70	
			6.50	
R8/90	0	0	0	0
R9/90	0	0	0	0
R10/90	0	0	0	0
R15/90	0	0	0	0

TABLE A2.8 FERTILITY TESTS IN STREPTOZOTOCIN-TREATED RATS.
(females introduced 57 days after diabetes was induced)

CONTROL GROUP (rat no.)	No. OF PUPS SIRED	ABNORMALITIES	WEIGHT (g)	STILL BIRTHS
R6/90		0	6.50 6.60 6.70 6.70 6.90	0
R11/90	14	0	6.20 6.50 6.60 6.60 6.50 6.20 6.30 6.40 6.20 6.70 6.40 6.50 6.70 6.70	0
R13/90	11	0	6.00 5.90 5.80 6.40 6.20 6.20 6.40 6.30 6.40 6.30 6.00	0
R16/90	9	0	7.00 7.20 6.80 7.00 6.90 6.90 7.20 7.20 6.70	0
R17/90	7	0	7.00 7.50 7.30 7.20	0

Table A2.8 continued.

RAT No.	No. OF PUPS SIRED	ABNORMALITIES	WEIGHT (g)	STILL BIRTHS
			6.90	
			7.00	
			7.20	
R19/90	10	0	6.40	0
			6.70	
			6.30	
			6.70	
			6.80	
			6.90	
			6.80	
			6.50	
			6.80	
			6.90	
R20/90	6	0	6.40	0
			6.00	
			6.40	
			6.90	
			6.50	
			7.00	
DIABETES GROUP				
R2/90	2	0	7.00	0
			7.20	
R3/90	10	0	6.40	0
			6.40	
			6.50	
			6.20	
			6.40	
			6.70	
			7.00	
			6.90	
			6.20	
			6.40	
R7/90	0	0	0.00	0
R8/90	0	0	0.00	0
R9/90	0	0	0.00	0
R10/90	0	0	0.00	0
R15/90	9	0	6.30	0
			7.50	
			6.90	
			7.20	
			6.90	
			6.90	
			6.70	
			6.70	
			6.30	

TABLE A2.9 FERTILITY TESTS IN STREPTOZOTOCIN-TREATED RATS.
(females introduced 85 days after diabetes was introduced)

CONTROL GROUP (rat no.)	No. OF PUPS SIRED	ABNORMALITIES	WEIGHT (g)	STILL BIRTHS
R6/90	8	0	6.70 6.80 6.70 6.90 7.20 7.10 6.70 6.70	0
R11/90	11	0	6.70 6.80 7.30 7.20 6.70 6.50 6.80 6.70 6.30 6.50 6.60	0
R13/90	12	0	6.30 6.20 6.00 6.10 6.20 6.20 6.50 6.20 6.40 6.20 6.00 6.30	0
R16/90	10	0	5.70 6.00 6.10 6.00 5.70 5.90 6.00 6.70 6.60 5.90	0
R17/90	3	0	7.10 7.00	0

Table A2.9 continued.

RAT No.	No. OF PUPS SIRED	ABNORMALITIES	WEIGHT (g)	STILL BIRTHS
R19/90	12	0	6.90 6.00 6.20 6.90 6.60 5.80 6.30 6.60 6.00 6.40 6.20 5.90	0
R20/90	13	0	6.30 7.20 6.40 6.30 6.40 6.50 6.40 6.60 6.90 6.30 6.20 6.60 6.50 6.40	0
DIABETES GROUP				
R2/90	9	0	7.20 6.90 6.80 6.90 6.80 7.10 7.00 7.20 7.00	0
R3/90	9	0	6.40 6.60 6.60 6.90 7.00 6.70	0

Table A2.9 continued.

RAT No.	No. OF PUPS SIRED	ABNORMALITIES	WEIGHT (g)	STILL BIRTHS
			6.50	
			6.50	
			6.30	
R7/90	0	0	0.00	0
R8/90	0	0	0.00	0
R9/90	0	0	0.00	0
R10/90	0	0	0.00	0
R15/90	11	0	6.70	0
			7.00	
			7.20	
			6.80	
			6.50	
			6.80	
			6.70	
			6.90	
			7.00	
			6.90	
			6.70	

9. APPENDIX 3: DATA: HUMAN PATIENTS

TABLE A3.1 MEDICAL HISTORY OF ALL HUMAN PATIENTS

PATIENT No.	AGE (years)	OTHER ILLNESSES AND/OR MEDICATION	EXPOSURE TO HAZARDOUS MATERIALS	USE OF ALCOHOL	CIGARETTES /DAY	GENERAL REMARKS
CONTROL GROUP						
500	30	flu-3 weeks earlier antibiotics	no	no	0	-
501	38	ulcers	no	no	0	-
502	35	-	radio-isotopes	no	0	-
503	22	flu-3 weeks earlier	no	no	0	-
504	24	flu-1 month earlier antibiotics	no	yes	0	-
505	29	varico-celectomy	formalin xylene etc	occ	0	-
506	25	flu-2 weeks earlier antibiotics	xylene etc	yes	6	-
507	23	flu-1 week earlier antibiotics antihistamines urticaria	no	no	0	-
508	22	spinal surgery 5 months earlier	no	no	0	-
509	23	flu-2 weeks earlier antibiotics	formalin	yes	6	-
510	28	circumcission	no	yes	10	-
589	43	flu-1 month earlier	xylene etc	occ	0	-

Table A3.1 continued

PATIENT No.	AGE (years)	OTHER ILLNESSES AND/OR MEDICATION	EXPOSURE TO HAZARDOUS MATERIALS	USE OF ALCOHOL	CIGARETTES /DAY	GENERAL REMARKS
590	29	-	no	no	10	-
591	28	herpes zoster 5 months earlier	thinners acetone	no	0	-
592	26	-	no	occ	10	-
593	28	cold antibiotics	no	occ	12	-
594	21	flu-3 weeks earlier antibiotics	no	no	10	-
595	37	flu-1 month earlier	no	occ	5	-
596	29	flu antibiotics	no	occ	15	-
598	28	-	no	occ	3	-
599	22	-	no	occ	10	-
602	24	-	no	occ	10	-
603	29	-	no	no	2	-
DIABETES GROUP						
1	57	flu-2 weeks earlier antibiotics renal calculi	no	occ	10	impotence no semen produced
3	27	flu-2 months earlier antibiotics	no	no	0	-
4	30	hernia	no	no	0	-
5	37	hypertensive	timber dust	no	0	infertile

Table A3.1 continued.

PATIENT No.	AGE (years)	OTHER ILLNESSES AND/OR MEDICATION	EXPOSURE TO HAZARDOUS MATERIALS	USE OF ALCOHOL	CIGARETTES /DAY	GENERAL REMARKS
8	52	arthritis	no	no	0	impotence no semen produced
11	34	flu	no	no	2	no semen produced
14	33	flu-3 weeks earlier antibiotics	no	no	0	-
17	40	circumcission	no	no	10	-
18	52	-	no	no	0	loss of libido no semen produced
20	58	spinal and heart problems flu-1 week earlier	no	seldom	0	impotent diabetes prevalent in family
27	37	-	no	occ	0	no semen produced
30	30	varico-celectomy	no	no	6	infertile prior to varicocele family history of
31	36	right foot amputated hypertensive - dyafide, tagamet &	no	no	2	paternal history of diabetes infertile no semen produced
32	56	-	no	no	0	impotent
34	55	-	no	no	0	diabetes prevalent in family

Table A3.1 continued.

PATIENT No.	AGE (years)	OTHER ILLNESSES AND/OR MEDICATION	EXPOSURE TO HAZARDOUS MATERIALS	USE OF ALCOHOL	CIGARETTES /DAY	GENERAL REMARKS
35	47	flu-1 month earlier by pass surgery	no	no	0	impotence loss of libido
36	61	flu-1 month earlier	no	no	5	impotence loss of libido
38	58	elephantiasis in right foot	no	occ	5	no semen produced
43	26	-	no	no	0	oligo - spermia
44	47	flu- 1 month earlier tuberculosis 6 years ago	no	occ	12	no semen produced
46	55	flu-1 month earlier	no	no	8	-
47	44	paracetamol	no	no	0	diabetes prevalent in family
49	47	-	no	occ	30	family history of diabetes and hyper-tension
INFERTILITY GROUP						
511	37		gases in paper mill	yes	0	-
512	33	-	no	yes	5	-
513	41	-	no	occ	0	oligozoo spermia

Table A3.1 continued.

PATIENT No.	AGE (years)	OTHER ILLNESSES AND/OR MEDICATION	EXPOSURE TO HAZARDOUS MATERIALS	USE OF ALCOHOL	CIGARETTES /DAY	GENERAL REMARKS
514	34	flu	no	yes	0	-
515	40	-	no	yes	0	-
516	34	flu	no	yes	0	-
517	30	-	no	occ	0	-
518	24	tick bite fever 6 years earlier	no	no	0 0	-
519	36	gynaecomastia hernia	no	yes	0	-
520	30	-	no	no	0	-
522	37	flu-1 month earlier antibiotics polio when 15 months of age	no	yes	0	-
523	38	-	no	rarely	0	-
524	30	-	white spirits	no	0	-
525	33	cold	no	no	0	left testical affected by mumps 22 years earlier
526	46	cold appendectomy	no	occ	5	-
527	23	-	no	no	0	-
528	36	german-measles hernia	no	yes	0	-

Table A3.1 continued.

PATIENT No.	AGE (years)	OTHER ILLNESSES AND/OR MEDICATION	EXPOSURE TO HAZARDOUS MATERIALS	USE OF ALCOHOL	CIGARETTES /DAY	GENERAL REMARKS
		bilharzia gastric-flu gynaecomastia				
529	33	-	no	no	0	-
530	38	-	no	no	0	-
531	39	cold chronic sinusitus treated with flagyl tritet celestamine	no	occ	0	prostate infection treated
532	27	meningitis 25 years earlier	no	no	0	-
533	38	kidney stone passed 5 years ago	no	yes	0	varicocele treated
534	30	appendectomy 3 weeks earlier	no	yes	3	testicular operation 3 years earlier (not specified)

TABLE A3.2 MEDICAL HISTORY OF DIABETIC PATIENTS

PATIENT No.	DURATION OF DIABETES (years)	TREATMENT	TYPE
1	20	insulin	I
3	18	insulin	I
4	5	insulin	I
5	5	daonil, mega & carbuten	II
8	5	daonil	II
11	1	tolinase	II
14	10	insulin	I
17	10	insulin	I
18	30	insulin	I
20	27	daonil & metaformin	I
27	9	daonil	II
30	1	insulin	I
31	25	insulin, carbuten	I
32	25	tolinase & daonil	II
34	15	daonil & metaformin	II
35	15	tolinase	II
36	3	daonil	II
38	7	tolinase	II
43	5	insulin	I
44	13	insulin	I
46	1	diet	II
47	1	metaformin	II
49	5	daonil	II

Insulin - controls level of glucose in the blood by promoting its absorption into tissue cells.
Daonil - oral hypoglycaemic
Mega - antihypertensive
Carbuten - antihypertensive
Tolinase - oral hypoglycaemic

TABLE A3.3 GENERAL CHARACTERISTICS OF HUMAN SEMEN

PATIENT No.	COAGULATION	LIQUIFACTION	COLOUR	DEBRIS
CONTROL GROUP				
500	complete	complete	yellow	none
501	complete	complete	normal	none
502	complete	complete	normal	none
503	complete	complete	normal	slight
504	complete	complete	normal	v. heavy
505	complete	complete	normal	slight
506	complete	complete	normal	none
507	complete	complete	normal	slight
508	absent	complete	yellow	none
509	complete	complete	normal	none
510	complete	complete	normal	none
589	complete	incomplete	normal	medium
590	complete	complete	normal	slight
591	complete	complete	normal	none
592	complete	complete	normal	none
593	complete	complete	normal	slight
594	complete	complete	normal	none
595	complete	complete	normal	slight
596	complete	complete	yellow	heavy
598	complete	complete	normal	none
599	complete	complete	normal	slight
602	complete	complete	normal	slight
603	complete	complete	normal	none
DIABETES GROUP				
1	n/a	n/a	n/a	n/a
3	*	complete	normal	none
4	absent	complete	normal	none
5	complete	complete	yellow	none
8	n/a	n/a	n/a	n/a
11	n/a	n/a	n/a	n/a
14	n/a	n/a	n/a	n/a
17	complete	complete	normal	none
18	n/a	n/a	n/a	n/a
20	n/a	complete	yellow	heavy
27	n/a	n/a	n/a	n/a
30	complete	incomplete	yellow	medium
31	n/a	n/a	n/a	n/a
32	*	complete	normal	none
34	*	complete	normal	none
35	*	complete	normal	none
36	*	complete	yellow	none
38	n/a	n/a	n/a	n/a
43	complete	complete	normal	none
44	n/a	n/a	n/a	n/a

Table A3.3 continued.

PATIENT No.	COAGULATION	LIQUIFACTION	COLOUR	DEBRIS
46	complete	complete	white	none
47	complete	incomplete	yellow	heavy
49	complete	incomplete	normal	none
INFERTILITY GROUP				
511	*	complete	normal	none
512	complete	incomplete	normal	slight
513	complete	complete	normal	none
514	complete	absent	normal	none
515	*	complete	normal	none
516	complete	absent	normal	medium
517	*	complete	normal	none
518	*	complete	normal	none
519	complete	incomplete	normal	none
520	*	complete	normal	none
522	complete	incomplete	normal	none
523	*	complete	normal	none
524	complete	absent	normal	none
525	*	complete	yellow	none
526	*	complete	yellow	none
527	*	complete	normal	none
528	*	complete	normal	none
529	*	complete	normal	slight
530	complete	absent	normal	medium
531	*	complete	normal	none
532	*	complete	normal	none
533	complete	incomplete	normal	none
534	complete	incomplete	normal	slight

* - unknown
n/a - patient could not produce semen sample

TABLE A3.4 GENERAL CHARACTERISTICS OF HUMAN SEMEN

PATIENT No.	VISCOSITY	VOLUME (ml)	pH	MAR TEST	INFECTION
CONTROL GROUP					
500	high	3.00	7.71	negative	none
501	normal	1.00	8.06	negative	none
502	normal	1.50	7.59	negative	none
503	normal	5.00	7.62	negative	none
504	high	2.00	7.58	negative	none
505	normal	4.00	7.57	doubtful	none
506	normal	2.50	7.63	negative	none
507	normal	4.00	7.53	negative	none
508	normal	6.00	7.55	negative	none
509	normal	2.50	7.53	negative	none
510	normal	1.50	7.90	positive	none
589	high	1.50	8.34	negative	none
590	normal	1.50	7.83	positive	none
591	high	2.00	8.15	negative	none
592	high	2.50	8.27	negative	none
593	normal	1.00	7.83	negative	none
594	normal	1.50	7.83	negative	none
595	normal	2.50	7.93	negative	none
596	normal	2.50	7.71	negative	none
598	normal	2.50	7.67	negative	none
599	normal	4.00	7.62	negative	none
602	normal	2.00	7.85	negative	none
603	normal	1.00	7.80	negative	none
DIABETES GROUP					
1	n/a	n/a	n/a	n/a	n/a
3	normal	3.50	7.78	negative	none
4	normal	1.50	7.90	doubtful	none
5	normal	2.50	7.80	negative	none
8	n/a	n/a	n/a	n/a	n/a
11	n/a	n/a	n/a	n/a	n/a
14	normal	3.50	7.45	negative	none
17	normal	2.50	7.62	negative	none
18	n/a	n/a	n/a	n/a	none
20	normal	1.5	8.03	doubtful	none
27	n/a	n/a	n/a	n/a	n/a
30	high	3.0	8.06	negative	none
31	n/a	n/a	n/a	n/a	n/a
32	normal	1.00	7.38	negative	none
34	normal	1.50	7.71	negative	none
35	normal	3.00	7.67	negative	none
36	normal	2.00	8.16	n/a	none
38	n/a	n/a	n/a	n/a	n/a
43	normal	3.00	7.47	negative	none

Table A3.4 continued

PATIENT No.	VISCOSITY	VOLUME (ml)	pH	MAR TEST	INFECTION
44	n/a	n/a	n/a	n/a	n/a
46	normal	1.50	7.66	negative	none
47	normal	3.50	7.69	doubtful	none
49	high	1.00	8.06	negative	none
INFERTILITY GROUP					
511	normal	2.00	7.80	negative	none
512	high	3.00	7.73	doubtful	none
513	normal	2.00	8.20	positive	none
514	high	2.50	7.92	negative	none
515	normal	3.50	7.74	negative	none
516	high	3.50	8.19	negative	none
517	normal	3.00	7.62	doubtful	none
518	normal	4.00	7.83	negative	none
519	normal	1.00	7.92	negative	none
520	normal	1.00	7.96	negative	none
522	high	5.00	7.09	negative	none
523	normal	5.00	7.58	negative	none
524	high	2.50	8.10	negative	none
525	normal	4.00	7.54	negative	none
526	normal	3.00	7.35	negative	none
527	normal	2.00	7.33	negative	none
528	normal	1.50	7.77	negative	none
529	normal	4.50	7.86	negative	none
530	high	3.00	7.84	negative	none
531	normal	1.50	7.89	negative	none
532	normal	8.00	7.66	negative	none
533	high	3.50	7.89	negative	none
534	high	2.50	8.10	negative	none

n/a - patient could not produce semen sample

TABLE A3.5 SPERM MOTILITY.

PATIENT No.	TOTAL MOTILITY (%)	PROGRESSIVE MOTILITY	RAPID MOTILITY (%)
CONTROL GROUP			
500	59.00	good	0.70
501	52.00	excellent	0.90
502	63.00	slow/sluggish	0.60
503	52.00	slow/sluggish	0.20
504	50.60	excellent	2.90
505	81.00	good	0.60
506	90.00	slow/sluggish	3.60
507	52.00	slow/sluggish	2.60
508	66.00	good	0.36
509	64.00	slow/sluggish	0.61
510	59.00	excellent	2.16
589	74.50	good	0.23
590	75.00	slow/sluggish	2.60
591	66.00	slow/sluggish	1.10
592	79.00	slow/sluggish	0.00
593	68.00	slow/sluggish	1.90
594	63.30	slow/sluggish	1.40
595	72.00	good	1.50
596	51.00	slow/sluggish	1.10
598	75.10	excellent	6.80
599	81.80	excellent	4.50
602	84.00	excellent	6.20
603	71.00	slow/sluggish	0.00
DIABETES GROUP			
1	n/a	n/a	n/a
3	11.30	slow/sluggish	0.00
4	64.00	slow/sluggish	3.80
5	39.00	slow/sluggish	1.70
8	n/a	n/a	n/a
11	n/a	n/a	n/a
14	43.00	occurs occasionally	0.40
17	56.00	good	0.75
18	n/a	n/a	n/a
20	50.40	slow/sluggish	1.50
27	n/a	n/a	n/a
30	32.00	slow/sluggish	0.00
31	n/a	n/a	n/a
32	41.00	slow/sluggish	0.00
34	39.00	slow/sluggish	0.27
35	44.00	slow/sluggish	0.23
36	n/a	n/a	n/a

Table A3.5 continued.

PATIENT No.	TOTAL MOTILITY (%)	PROGRESSIVE MOTILITY	RAPID MOTILITY (%)
38	n/a	n/a	n/a
43	66.00	good	1.22
44	n/a	n/a	n/a
46	44.00	good	0.94
47	52.60	good	2.40
49	35.00	occurs occasionally	0.00
INFERTILITY GROUP			
511	53.70	slow/sluggish	0.74
512	35.00	slow/sluggish	0.00
513	61.70	slow/sluggish	0.16
514	67.00	slow/sluggish	2.70
515	43.00	slow/sluggish	0.40
516	51.00	slow/sluggish	0.00
517	76.00	good	6.30
518	58.00	good	1.00
519	63.00	good	0.32
520	53.00	good	1.80
522	30.30	no forward motility	0.00
523	24.00	slow/sluggish	0.00
524	52.40	slow/sluggish	0.18
525	51.40	good	1.80
526	54.70	good	2.10
527	51.00	slow/sluggish	0.24
528	26.60	slow/sluggish	0.00
529	75.00	excellent	4.70
530	67.00	no forward motility	0.00
531	52.00	slow/sluggish	0.17
532	79.00	good	5.30
533	40.00	slow/sluggish	1.00
534	55.00	slow/sluggish	0.40

n/a - patient could not produce semen sample

TABLE A3.6 SPERM NUMBER AND VIABILITY.

PATIENT No.	SPERM NUMBER (million/ml)	SPERM NUMBER (million/ejaculate)	SPERM VIABILITY (%)
CONTROL GROUP			
500	56.50	169.50	64
501	149.00	149.00	67
502	47.50	71.25	70
503	59.00	295.00	55
504	52.40	104.80	76
505	63.00	252.00	83
506	79.90	199.75	93
507	59.10	236.40	70
508	16.80	100.80	70
509	63.10	157.75	74
510	54.00	81.00	67
589	80.00	120.00	81
590	91.00	136.50	92
591	70.75	141.50	89
592	123.38	308.45	86
593	169.00	169.00	81
594	95.00	142.50	86
595	91.30	91.30	87
596	65.50	163.75	70
598	81.90	204.75	81
599	98.40	393.60	85
602	65.60	131.20	89
603	82.25	82.25	84
DIABETES GROUP			
1	n/a	n/a	n/a
3	88.00	132.00	12
4	68.00	102.00	67
5	45.50	113.75	62
8	n/a	n/a	n/a
11	n/a	n/a	n/a
14	58.00	203.00	63
17	191.00	477.50	73
18	n/a	n/a	n/a
20	96.50	144.75	53
27	n/a	n/a	n/a
30	22.00	66.00	53
31	n/a	n/a	n/a
32	70.00	70.00	65
34	179.00	268.50	53
35	125.00	375.00	59
36	7.40	14.80	28
38	n/a	n/a	n/a

Table A3.6 continued.

PATIENT No.	SPERM NUMBER (million/ml)	(million/ejaculate)	SPERM VIABILITY (%)
43	52.00	156.00	70
44	n/a	n/a	n/a
46	384.00	576.00	50
47	19.80	66.50	60
49	47.60	47.60	40
INFERTILITY GROUP			
511	16.00	32.00	60
512	74.00	222.00	75
513	14.10	28.20	63
514	117.00	292.50	73
515	98.00	343.00	67
516	3.40	11.90	54
517	155.00	465.00	87
518	64.00	256.00	66
519	66.00	66.00	63
520	21.00	21.00	71
522	8.50	42.50	85
523	20.00	100.00	47
524	222.00	555.00	68
525	50.40	151.20	78
526	221.00	663.00	67
527	175.00	350.00	70
528	140.00	210.00	83
529	56.00	252.00	80
530	18.00	54.00	83
531	60.00	90.00	61
532	112.00	896.00	89
533	78.00	273.00	78
534	34.00	85.00	72

n/a - patient could not produce semen sample

TABLE A3.7 SPERM MORPHOLOGY

PATIENT No.	NORMAL SPERM (%)	ABNORMALITIES (%) HEAD amorphous	mega	small	tapers	duplicate	NECK	TAIL	OTHERS immature sperm
CONTROL GROUP									
500	36	7	2	17	28	0	0	0	0
501	37	14	4	13	28	1	2	1	0
502	57	7	1	7	26	0	2	0	0
503	23	3	1	0	51	1	0	0	21
504	53	4	1	12	22	0	5	2	0
505	6	12	2	3	74	0	1	2	0
506	24	9	3	8	30	0	7	3	1
507	35	9	2	7	42	0	2	2	0
508	35	7	1	5	41	2	4	6	0
509	37	3	0	12	41	0	4	2	0
510	42	2	2	4	49	0	1	0	0
589	17	5	1	2	72	0	1	1	2
590	8	11	0	2	79	0	0	0	0
591	6	17	2	1	72	0	1	1	0
592	6	25	0	2	67	0	0	0	0
593	13	16	1	1	69	0	0	0	0
594	23	15	2	4	45	0	4	6	0
595	9	10	3	3	75	0	0	0	0
596	4	39	2	7	38	0	2	1	7
598	23	12	1	4	57	0	2	1	0
599	30	14	0	5	51	0	0	0	0
602	18	12	2	2	59	0	6	1	0
603	12	15	4	2	66	0	1	0	0
DIABETES GROUP									
1	n.a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	61	15	2	1	16	2	0	3	0
4	40	4	2	1	52	0	0	1	0
5	40	9	0	6	41	0	0	4	0
8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
11	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
14	27	11	5	5	50	0	0	2	0
17	27	6	3	2	60	0	1	1	0
18	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
20	15	15	3	0	60	0	2	3	2
27	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
30	17	15	1	6	56	0	5	0	6
31	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
32	34	10	2	3	50	0	0	1	0
34	5	22	10	6	50	0	5	2	0
35	35	17	6	4	33	0	5	0	0
36	15	7	2	3	71	0	2	0	0

Table A3.7 continued.

PATIENT No.	NORMAL SPERM (%)	ABNORMALITIES (%) HEAD					NECK	TAIL	OTHERS
		amorphous	mega	small	tapers	duplicate			immature sperm
38	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
43	22	8	0	5	54	1	5	5	0
44	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
46	30	7	1	0	50	1	5	6	0
47	11	27	1	3	56	0	0	2	0
49	3	11	2	1	83	0	0	0	0
INFERTILITY GROUP									
511	35	15	2	4	26	1	6	7	4
512	10	14	2	11	61	0	1	1	0
513	18	14	1	2	57	0	4	3	1
514	31	5	2	5	50	0	0	0	7
515	13	16	4	10	52	0	2	3	0
516	6	58	0	3	23	6	0	0	4
517	28	20	0	2	50	0	0	0	0
518	14	27	0	9	27	0	18	1	4
519	15	5	0	3	65	1	2	5	4
520	3	9	0	0	78	3	3	3	1
522	4	23	0	4	55	0	0	0	14
523	7	25	0	10	51	0	3	4	0
524	12	5	0	3	82	0	0	0	0
525	16	7	1	5	61	0	0	5	5
526	20	23	5	0	52	0	0	0	0
527	15	33	0	6	39	0	0	2	5
528	3	8	5	3	79	0	0	2	0
529	8	13	2	3	71	0	0	3	0
530	3	10	5	4	75	0	1	1	0
531	3	11	3	20	40	0	2	3	18
532	23	9	1	3	48	0	5	11	5
533	9	14	6	12	51	0	2	5	1
534	6	13	2	6	65	0	0	0	8

n/a - patient could not produce semen sample

TABLE A3.8 HORMONE LEVELS IN PLASMA

PATIENT No.	TESTOSTERONE (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	PROLACTIN (ng/ml)
CONTROL GROUP				
500	5.00	9.60	8.80	10.00
501	3.90	8.70	5.00	4.10
502	4.80	10.00	5.00	5.00
503	4.90	47.50	5.60	18.80
504	6.60	7.80	3.50	5.60
505	6.40	10.00	13.50	7.40
506	7.63	10.60	10.00	7.10
507	4.35	11.25	4.30	8.10
508	4.35	11.90	7.10	18.80
509	4.87	13.10	4.30	7.10
510	5.89	10.60	4.90	0.76
589	3.10	7.00	16.00	33.00
590	7.20	10.00	11.00	3.20
591	5.40	10.20	9.90	10.00
592	6.00	10.30	18.00	3.50
593	4.80	7.00	9.90	1.40
594	3.70	10.25	13.00	3.00
595	5.20	10.55	15.50	5.00
596	6.00	20.30	11.00	1.80
598	7.00	3.00	8.50	3.20
599	5.40	3.60	2.50	1.60
602	4.80	10.10	5.20	4.20
603	8.00	9.20	20.00	2.00
DIABETES GROUP				
1	2.50	10.00	23.10	1.20
3	5.49	10.00	7.50	10.00
4	5.38	29.90	8.10	20.00
5	2.70	43.80	8.90	7.70
8	2.70	15.10	10.80	4.90
11	4.15	8.70	9.00	2.90
14	6.65	15.10	12.70	18.80
17	5.89	10.00	9.60	0.76
18	2.80	10.80	6.40	14.00
20	2.73	9.10	7.50	10.00
27	6.65	13.80	13.10	4.50
30	7.63	15.00	8.50	11.50
31	0.54	104.20	54.10	9.40
32	4.70	10.60	8.80	3.80
34	4.87	10.60	12.70	4.40
35	5.89	6.30	9.60	8.70
36	4.50	25.00	18.10	3.80
38	2.82	9.60	9.00	1.20

Table A3.8 continued.

PATIENT No.	TESTOSTERONE (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)	PROLACTIN (ng/ml)
43	4.67	11.20	3.30	8.70
44	5.49	8.70	8.80	3.80
46	3.13	9.60	9.60	3.80
47	4.35	7.70	19.20	1.20
49	4.35	5.90	7.10	5.60
INFERTILITY GROUP				
511	3.64	10.60	9.50	5.20
512	6.00	15.00	6.80	18.50
513	3.95	4.00	3.70	13.00
514	5.08	9.10	5.60	4.50
515	3.64	6.30	3.90	6.40
516	5.38	3.60	11.00	12.00
517	5.38	19.40	3.50	8.10
518	6.00	11.90	10.80	18.10
519	2.41	5.20	4.60	17.00
520	3.95	7.00	18.00	5.00
522	4.36	4.60	11.00	4.00
523	3.54	4.20	8.00	18.50
524	5.38	11.90	8.80	7.70
525	3.54	10.00	29.00	7.20
526	3.64	8.00	8.80	9.40
527	3.13	4.60	2.30	7.40
528	4.15	5.20	5.20	15.50
529	6.00	7.00	5.40	2.40
530	3.10	7.40	6.60	12.25
531	6.00	8.00	3.70	6.80
532	5.20	7.40	8.70	6.40
533	4.40	5.80	15.00	7.80
534	7.00	10.05	14.00	2.00

TABLE A3.9 BIOCHEMICAL ANALYSES IN BLOOD AND SEMINAL PLASMA

PATIENT No.	GLUCOSE (plasma) (mmol/l)	FRUCTOSE (mmol/l)	ACID PHOSPHATASE (U/ml)	ZINC (mmol/l)	CARNITINE (µmol/l)	CITRIC ACID (mmol/l)
CONTROL GROUP						
500	5.30	7.30	53.57	2.94	346.00	57.95
501	5.60	4.00	54.50	1.74	*	23.90
502	4.70	10.40	59.00	5.84	144.00	73.33
503	5.30	12.50	44.75	2.08	342.00	37.03
504	4.90	4.40	92.00	3.84	558.00	68.31
505	5.20	7.30	55.00	4.85	745.00	16.82
506	4.30	3.60	52.70	2.98	129.00	16.97
507	4.10	13.30	52.00	2.23	296.00	37.82
508	4.00	14.20	58.00	1.44	111.00	48.03
509	5.30	4.00	56.00	3.24	163.00	27.47
510	3.80	1.10	49.00	2.20	288.00	63.13
589	0.50	9.17	35.00	0.83	130.00	1.29
590	0.40	13.30	56.25	2.17	167.00	43.72
591	0.50	6.25	53.00	1.99	173.00	10.21
592	0.50	7.94	59.00	1.62	111.00	46.16
593	0.30	13.30	59.50	2.48	309.00	41.27
594	0.40	10.00	58.75	1.28	204.00	19.84
595	0.40	1.63	52.75	4.22	433.00	67.30
596	0.50	10.00	57.50	1.28	415.00	23.44
598	0.40	4.20	58.25	3.82	167.00	54.21
599	0.50	11.67	56.50	1.99	254.00	22.15
602	0.40	9.17	58.00	1.28	402.00	70.17
603	0.40	9.17	54.50	3.67	198.00	20.71
DIABETES GROUP						
1	6.20	n/a	n/a	n/a	n/a	n/a
3	12.80	1.70	53.25	1.25	288.00	69.74
4	17.90	9.60	52.50	1.87	85.00	107.27
5	13.40	6.30	52.00	1.48	290.00	31.49
8	9.60	n/a	n/a	n/a	n/a	n/a
11	14.40	n/a	n/a	n/a	n/a	n/a
14	13.20	8.30	57.75	2.66	593.00	104.40
17	17.40	8.30	43.25	0.44	808.00	26.60
18	15.10	n/a	n/a	n/a	n/a	n/a
20	15.70	9.60	50.00	1.39	405.00	23.87
27	3.50	n/a	n/a	n/a	n/a	n/a
30	16.48	12.50	45.25	1.62	187.00	9.78
31	10.60	n/a	n/a	n/a	n/a	n/a
32	10.40	1.10	112.00	1.81	*	31.78
34	9.00	6.40	98.00	4.31	364.00	38.11
35	16.60	15.80	48.25	1.42	635.00	47.89
36	9.90	12.50	42.50	0.67	199.00	60.40

Table A3.9 continued

PATIENT No.	GLUCOSE (plasma) (mmol/l)	FRUCTOSE (mmol/l)	ACID PHOSPHATASE (U/ml)	ZINC (mmol/l)	CARNITINE (µmol/l)	CITRIC ACID (mmol/l)
38	8.80	n/a	n/a	n/a	n/a	n/a
43	13.80	11.30	82.00	0.34	1490.00	14.98
44	2.70	n/a	n/a	n/a	n/a	n/a
46	14.20	0.30	52.50	4.37	760.00	25.74
47	7.00	7.90	88.00	2.92	643.00	33.65
49	12.80	6.40	82.00	4.33	*	*
INFERTILITY GROUP						
511	5.50	7.60	46.75	2.17	270.00	52.34
512	4.60	3.60	56.50	5.78	296.00	72.33
513	4.40	4.00	46.00	2.88	298.00	35.95
514	5.50	4.40	49.00	1.29	200.00	42.78
515	4.90	5.80	82.00	2.57	331.00	63.85
516	4.50	2.80	37.00	1.53	111.00	17.26
517	6.00	8.30	49.00	3.32	386.00	27.47
518	4.40	9.20	51.75	2.51	4.00	58.38
519	4.20	10.40	60.00	2.39	560.00	11.36
520	2.80	9.20	48.00	1.16	111.00	31.06
522	4.70	4.40	51.25	1.99	35.00	10.35
523	4.40	6.40	43.00	3.00	218.00	3.31
524	2.80	0.03	43.25	1.97	157.00	7.77
525	4.30	10.40	104.00	4.68	451.00	13.66
526	1.10	4.00	104.00	3.78	767.00	54.93
527	0.50	3.20	94.00	3.89	458.00	3.60
528	0.40	5.40	166.50	5.97	1357.00	96.06
531	3.20	0.28	41.00	3.81	1016.00	7.05
532	4.80	10.00	35.00	2.40	371.00	58.53
533	4.70	10.83	45.25	2.55	855.00	4.03
534	4.80	0.56	45.25	2.43	917.00	6.47

* - insufficient semen
n/a - patient could not produce semen sample